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Herbicides in soybean development: productive aspects and physiological quality of seeds

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ABSTRACT: The objective was to evaluate the production components of soybean and the physiological quality of seeds according to the application of subdoses of herbicides at the reproductive stage of healthy plants. The treatments consisted of the application of glyphosate (122.5 g acid equivalent [ae] ha^{-1}), chlorimuron (1.75 g active ingredient [ai] ha^{-1}), 2,4-D (134 g ae ha^{-1}), fomesafen (60 g ai ha^{-1}), glufosinate (55 g ai ha^{-1}), paraquat (35 g ai ha^{-1}), and control (no application). The chlorophyll index, agronomic performance, and physiological quality of seeds were evaluated. Glyphosate, chlorimuron, fomesafen, or glufosinate resulted in an increase in soybean plant height. Chlorimuron increased yield compared to the control. Paraquat or glufosinate, despite the injury, did not reduce yield, but the application of these herbicides is not recommended to stimulate plant development, given the potential for injury. The 2,4-D was the only one that caused a reduction in the physiological quality of soybean seeds.

Key words: glyphosate; 2,4-D; fomesafen; glufosinate; yield

Herbicidas no desenvolvimento da soja: aspectos produtivos e qualidade fisiológica de sementes

RESUMO: Objetivou-se avaliar os componentes de produção da soja e a qualidade fisiológica das sementes após a aplicação de subdoses de herbicidas na fase reprodutiva de plantas saudáveis. Os tratamentos consistiram-se na aplicação de glifosato (122,5 g de equivalente ácido [ea] ha^{-1}), clorimurom (1,75 g de ingrediente ativo [ia] ha^{-1}), 2,4-D (134 g ea ha^{-1}), fomesafem (60 g ia ha^{-1}), glufosinato (55 g ia ha^{-1}), paraquate (35 g ia ha^{-1}) e controle (sem aplicação). Foram avaliados o índice de clorofila, o desempenho agronômico e a qualidade fisiológica das sementes. Glifosato, clorimurom, fomesafem ou glufosinato resultaram em um aumento na altura das plantas de soja. O clorimurom aumentou a produtividade em relação ao controle. Paraquate ou glufosinato, apesar da injúria, não reduziram a produtividade, mas a aplicação desses herbicidas não é recomendada para estimular o desenvolvimento da planta, dado o potencial de injúria. O 2,4-D foi o único que causou redução na qualidade fisiológica das sementes de soja.

Palavras-chave: glifosato; 2,4-D; fomesafem; glufosinato; produtividade

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Introduction

The beneficial effect of subdoses of toxic substances has been known for a long time and this phenomenon is called hormesis (Calabrese & Blain, 2009). In agriculture, studies have been carried out to assess possible hormesis effects on plants with the use of subdoses of herbicides, which in turn can be advantageous if a desirable phenotypic change is achieved (Jalal et al., 2021).

Herbicides are compounds that inhibit plant metabolic pathways or physiological processes through interaction with proteins, or specific pathways and photosynthesis inhibitors, resulting in plant death or growth arrest (Krähmer et al., 2021). On the other hand, synthetic auxins promote auxin-mediated effects, resulting in disordered plant growth (Silva et al., 2016). As all herbicides act on pathways or processes crucial to plants, such as inhibitors or stimulants, possibly low doses of an herbicide can modify growth, development, or composition in a way that could be beneficial to plants in some circumstances.

The hormesis effect can provoke different stimulatory responses, which depend on the chemical, the target plant, and how it acts on plant physiology and morphology and of that plant. Glyphosate subdoses stimulate plant growth (Velini et al., 2008; Silva et al., 2016; Moraes et al., 2020), sucrose accumulation, and yield in sugarcane (Pincelli-Souza et al., 2020). Furthermore, the use of subdoses herbicides provides crop protection by eliciting defenses against plant pathogens (Nelson, 2008).

With the exception of glyphosate as a sugarcane ripener and synthetic auxins in fruit production, the beneficial effects of herbicide subdoses, are little used commercially. In addition, the secondary effects of subdoses of herbicides can be commercially advantageous and profitable, however, little used and still unknown to Brazilian agriculture.

The hypothesis of this study is that the application of subdoses of herbicides at the reproductive stage of soybean causes beneficial effects on development, altering the production aspects and physiological quality of the seeds. The objective of this study was to evaluate the productive components of soybean and the physiological quality of seeds according to the application of subdoses of herbicides at the reproductive stage of the healthy plants.

Materials and Methods

Site description

The experiment was conducted in a soybean production field, in the municipality of Mangueirinha, state of Paraná (PR), Brazil (25° 56’ 42” S, 52° 11’ 16” W), 849 m altitude, on a flat relief, in the 2017/18 growing season. The average annual rainfall in the municipality is 1,897 mm, well distributed, without the occurrence of a dry season. The climate is Cfb (mesothermal humid subtropical), according to the Köppen classification (Aparecido et al., 2016).

The experimental area has been cultivated with annual crops in a no-till system for about 30 years. Before planting the soybean crop, the area was grown with oat and ryegrass intercropping. The soil, in the 0-20 cm layer, is composed of 22% sand, 24% silt and 54% clay, and presented, according to the chemical analysis, $CaCl_2$ = 4.4; H + Al = 6.76 $cmol_c\ dm^{-3}$; Al = 0.6 $cmol_c\ dm^{-3}$; Ca = 4.3 $cmol_c\ dm^{-3}$; Mg = 2.0 $cmol_c\ dm^{-3}$; K = 0.28 $cmol_c\ dm^{-3}$; P (Mehlich) = 3.7 mg dm^{-3}; and, OM = 39.6 g dm^{-3}, respectively.

Soybean was sown on November 6, 2018, in a no-till system, with seeds of the Pioneer 95Y51 soybean cultivar (glyphosate-tolerant), which has an indeterminate growth habit, 5.5 maturity group, 115-day cycle. Seeds were treated with pyraclostrobin/thiophanate/fipronil (Standak® Top) + plant growth regulator (cytokinin/gibberellin/indole butyric acid – Stimulate®), and inoculated with selected strains of *Bradyrhizobium japonicum* and *Bradyrhyzobium elkani*. The spacing was 0.45 m between rows and a population of 278,000 plants ha^{-1}. Basal fertilization was 350 kg ha^{-1} NPK 2-28-20 fertilizer and 38 kg ha^{-1} K_2O as topdressing, 15 days after soybean sowing. For post-emergence weed control in soybean (V3), the herbicide glyphosate - potassium salt (Zapp QI 620®) was applied at a dose of 1,000 g acid equivalent (ae) ha^{-1}.

Experimental design and application of treatments

The experiment was carried out in a randomized block design with 4 replications and 7 treatments. The treatments consisted of the application of glyphosate - potassium salt (122.5 g ae ha^{-1} - Zapp QI 620®), chlorimuron (1.75 g active ingredient [ai] ha^{-1} - Classic®), 2,4-D - dimethylamine salt (134 g ae ha^{-1} – 2,4-D Nortox®), fomesafen (60 g ai ha^{-1} - Flex®), glufosinate (55 g ai ha^{-1} - Finale®), paraquat (35 g ai ha^{-1} - Gramoxone® 200) and control (no application). Herbicides were applied when soybean plants were at the R3 stage, using a CO_2 pressurized backpack sprayer equipped with six AIXR 110.015 nozzles, at a pressure of 2 kgf cm^{-2} and a speed of 3.6 km h^{-1}, providing an application volume of 150 L ha^{-1}.

The area of the experimental unit was 10.8 m² (1.8 × 6.0 m), with 4 seed rows with 6 meters in length spaced by 0.45 m per experimental unit, totaling 388.8 m² of the experimental area. The 2 central rows of each plot were considered as useful areas, disregarding 1.75 m from each end, leaving 2 rows with 2.5 m each (2.25 m^2 useful area).

Physiological and yield components traits

Chlorophyll a, b and total chlorophyll contents were determined with an automated chlorophyll meter (ClorofiLOG - Falker®, Porto Alegre, Brazil). This device measures the light absorption by the leaf in wavelength, where the chlorophyll content is determined through the absorption indices at different frequencies and the chlorophyll index is highly correlated with the actual chlorophyll content (Rigon et al., 2012). In each plot, the chlorophyll index readings were made on a composite leaf, in the upper third, third node from the apex to the base, of 5 plants marked in the two central rows of each experimental unit, in R5 stage soybean, at 7 days after herbicides application (DAA).

At the R8 stage, 50 DAA, plants of the useful area of each plot were harvested and evaluated for: first pod height, number of nodes in the main stem, number of branches, number of grains per pod, number of pods per plant, plant height (cm), thousand-grain weight (g), yield (kg ha^{-1}) with values corrected for 13% moisture.

Seedling analysis

Seed physiological quality was evaluated in Didactic Laboratory of Seed Analysis, Universidade Tecnológica Federal do Paraná, Pato Branco Campus, through by germination test (%), accelerated aging (%), germination speed index (GSI), germination speed (days), shoot length (cm), root length (cm), shoot fresh matter (g), root fresh mass (g), shoot dry matter (g) and root dry matter (g) of seedlings.

To determine the germination percentage, 50 seeds were placed in germination paper rolls moistened to 2.5 times the weight of the dry paper, with four replicates. Seeds were incubated in a germination chamber at 25 °C constant for eight days. The evaluations were performed, at five and eight days after incubation, considering germinated the seeds that emitted the primary root (≥ 2 mm), according to criteria established by the Rules for Seed Analysis (Brasil, 2009).

Accelerated aging was analyzed with 200 seeds distributed on a screen in a gerbox, containing 40 mL distilled water, which were kept in BOD at 41 °C for 48 hours. Afterwards, these seeds were evaluated 5 days after sowing (Marcos Filho, 2015), considering its germination.

The GSI and the germination speed (days) were evaluated with four replicates of 50 seeds, kept at 25 °C constant for eight days. Daily counts were carried out for eight days in seeds with root protrusion greater than 2 mm in length, with results expressed as described by Nakagawa (1999).

The length (cm) of shoots and roots of seedlings was performed from four replicates of 15 seedlings, placed in a row in the upper third of the paper. These rolls remained in a germinator at 25 °C for eight days, when the shoot and root were separated and, with the aid of a millimeter ruler, the length of both structures was determined. These same seedlings were weighed on a precision scale to determine the fresh mass (g) of the shoot and root, placed in paper bags and taken to a forced air oven, at 60 °C, for 72 hours, and weighed again to obtain the dry mass (g) of the shoot and root (Marcos Filho, 2015).

Statistical analysis

The results were tested for normality and homoscedasticity, and then tested by analysis of variance by F-test (p ≤ 0.05) using the Statistical Analysis System Windows 9.2 (SAS, 2010). The data were normal, so the transformation was not necessary. When the null hypothesis was rejected, Tukey mean comparison tests (LSD) p ≤ 0.05 were performed for the treatments.

Results and Discussion

The analysis of variance, by F-test, indicated differences between treatments for the variables first pod height, number of branches, plant height, dry mass and yield. For the other variables (chlorophyll a, b and total chlorophyll, number of nodes in the main stem, number of grains per pod, number of pods per plant) no significant effect was detected after herbicides application (Table 1). For the variables related to the seed physiological quality, the analysis of variance evidenced a significant effect of the treatments on the variables GSI, germination speed and shoot fresh mass; for other variables (germination test, accelerated aging, shoot length, root length, root fresh mass, shoot dry matter and root dry matter (g) of seedlings) there was no significant effect (Table 2).

For first pod height, there was a difference between glyphosate (21.78 cm) and 2,4-D (17.09 cm); however, none of them differed from the control (21.22 cm). For number of branches, the application of 2,4-D (4.39) caused an increase compared to the control (2.5), but with no difference compared to the other herbicides. The application of glyphosate (93.66 cm), chlorimuron (94.00 cm), fomesafen (93.59 cm), and glufosinate (96.38 cm) resulted in an increase in plant height compared to the control (88.53 cm). While the

Table 1. Significance level by F-test for variables related to agronomic performance¹ and chlorophyll indices².

Variable	FPH	NNS	NB	NGP	NPP	PH	SM	Yield	Cl *a*	Cl *b*	T Cl
Block	**	ns	ns	ns	ns	**	ns	ns	*	ns	ns
Treatment	*	ns	*	ns	ns	**	**	*	ns	ns	ns
CV (%)	8.93	4.67	22.1	17.0	10.0	2.01	2.09	7.88	3.11	11.3	5.19

* Significant by F-test (p ≤ 0.05); ** significant by F-test (p ≤ 0.01); [ns] non-significant.
¹ First pod height (FPH), number of nodes in the main stem (NNS), number of branches (NB), number of grains per pod (NGP), number of pods per plant (NPP), plant height (PH), 1,000-seed mass (SM).
² Chlorophyll (Cl), total (T).

Table 2. Significance level by F-test for variables related to seed quality¹.

Variable	GER	AA	GSI	GS	SL	RL	SG-M	RG-M	SD-M	RD-M
Block	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Treatment	ns	ns	**	**	ns	ns	ns	*	ns	ns
CV %	2.94	4.57	1.97	5.21	6.62	8.65	9.93	25.5	32.3	28.5

* Significant by F-test (p ≤ 0.05); ** significant by F-test (p ≤ 0.01); [ns] non-significant.
¹ Germination (GER), accelerated aging (AA), germination speed index (GSI), germination speed (GS), shoot length (SL), root length (RL), shoot fresh mass (SG-M), root fresh mass (RG-M), shoot dry mass (SD-M), and root dry mass (RD-M) of seedlings.

application of paraquat (91.16 cm) did not cause a difference in plant height compared to the control; the application of 2,4-D resulted in the lowest plant height (82.5 cm). Regarding the yield of soybean plants, no differences were detected between the herbicide treatments, except for the application of chlorimuron, which increased the yield compared to the control (Table 3).

Considering the quality of soybean seeds, a negative effect was found for the application of 2,4-D, with a reduction in GSI and germination speed compared to the control and to the other herbicides. For root fresh mass, the value was lower for the application of 2,4-D compared to fomesafen, but none of the herbicides differed from the control (Table 4).

As for some undesirable effects of herbicide application, leaf burning, and necrosis were observed for the application of glufosinate (55 g ai ha^{-1}) and paraquat (35 g ai ha^{-1}). Both are contact herbicides, and non-selective for soybean, even in subdoses they caused injuries to soybean plants. Johnson et al. (2012) observed that the post-emergence application of doses between 16 and 302 g ai ha^{-1} glufosinate in soybean resulted in injury (burning and necrosis) in the plants, even at the lowest doses, with a decline in soybean yield with the application of the highest dose.

In the present study, glufosinate, despite the injury, did not reduce soybean yield compared to the control, and only increased plant height. In other words, the post-emergence application of this herbicide is not recommended to stimulate the development of plants, due to the low or no increase.

The post-emergence application of paraquat in soybean is also not recommended, given the damage to plants and the non-increase in any parameter. According to Henry et al. (2004), the application of 31 g ai ha^{-1} paraquat, lower than that tested in the present study, caused 50% damage to soybean plants.

The application of glyphosate (122.5 g ae ha^{-1}) and fomesafen (60 g ai ha^{-1}), although superior to some herbicides for some parameters (GSI, GC and RF-M), in general, did not differ from the control. These two herbicides only increased plant height, with no effect on yield. The use of non-lethal subdoses of glyphosate can stimulate growth, photosynthesis, and other parameters (Brito et al., 2018). Velini et al. (2008), reported stimulated growth of non-transgenic corn and soybeans aftr the application of subdoses of glyphosate; however, the same effect was not observed for glyphosate-tolerant transgenic cultivar (Velini et al., 2008; Meseldžija et al., 2020).

Table 4. Germination speed index (GSI), germination speed (GS), root fresh mass (RF-M, g) of soybean seedlings treated with different herbicides.

Treatments	Dose[1] (g ai ha^{-1})	GSI (days)	GS (days)	RF-M (g)
Glyphosate	122.5	24.39 a	2.07 a	2.95 ab
Chlorimuron	1.75	24.13 a	2.10 a	3.62 ab
2,4-D	134	22.13 b	1.91 b	2.81 b
Fomesafen	60	24.47 a	2.06 a	4.12 a
Glufosinate	55	24.65 a	2.04 a	3.68 ab
Paraquat	35	24.18 a	2.08 a	3.88 ab
Control	-	24.44 a	2.06 a	3.61 ab

[1] g ae ha^{-1} for glyphosate and 2,4-D.
Means followed by the same letter in the column do not differ by Tukey test at 5% probability.

The stimulation of plant growth, resulting from the application of glyphosate subdoses, is accompanied by the accumulation of shikimate. However, shikimate is not observed in tolerant transgenic cultivars, as it is derived from the inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPs) (Velini et al., 2008). Glyphosate-tolerant transgenic soybean (Roundup Ready®) carries a gene encoding glyphosate insensitive EPSPs, so there is no inhibition of this enzyme, and no shikimate is detected (Bonini et al., 2020), which is correlated to the lack of stimulation of glyphosate subdoses on the growth of tolerant transgenic plants.

Fomesafen is selective for soybeans, therefore, the investigation of doses above those tested in this study is feasible. Studies evaluating fomesafen (PPO inhibitor herbicide) as a growth regulator are scarce, when evaluated, generally without effect (Williams & Nelson, 2014; Beam et al., 2018). Even in the control of weeds in soybean, this herbicide has been little used.

Other studies evaluated lactofen (an herbicide with the same mode of action as fomesafen) with the aim of reducing growth, with some promising results being observed, with doses close to those recommended for weed control (Novakoski et al., 2020; Martins et al., 2020). Or even the post-emergence application of fomesafen (393 g ai ha^{-1}), which caused injury to soybean plants, with reduced growth, but

Table 3. First pod height (FPH, cm), number of branches (NB), plant height (PH), 1,000-seed mass (SM), and yield of soybean plants treated with different herbicides.

Treatments	Dose[1] (g ai ha^{-1})	FPH (cm)	NB	PH (cm)	SM (g)	Yield (kg ha^{-1})
Glyphosate	122.5	21.78 b	2.91 ab	93.66 ab	205.12 ab	4,903 ab
Chlorimuron	1.75	21.11 ab	3.63 ab	94.00 ab	205.77 ab	5,464 a
2,4-D	134	17.09 a	4.39 a	82.50 d	215.11 a	5,223 ab
Fomesafen	60	21.94 b	2.79 ab	93.59 ab	202,45 b	4,710 ab
Glufosinate	55	19.97 ab	3.09 ab	96.38 a	206,70 ab	4,780 ab
Paraquat	35	19.66 ab	2.94 ab	91.16 bc	203,04 b	4,745 ab
Control	-	21.22 ab	2.50 b	88.53 c	200,01 b	4,483 b

[1] g ae ha^{-1} for glyphosate and 2,4-D.
Means followed by the same letter in the column do not differ by Tukey test at 5% probability.

no reduction in yield (Priess et al., 2020). Fomesafen can be indicated as a growth reducer, aiming to reduce the occurrence of lodging, for example, and not as a growth promoter.

Chlorimuron is a selective herbicide for soybeans, an inhibitor of the enzyme acetolactate synthase (ALS), and is recommended for application up to a dose of 20 g ai ha^{-1}. The use of this substance proved to be promising, as it resulted in an increase in plant height and soybean yield compared to the control, without application, being the only herbicide to cause such an effect. Silva et al. (2020) observed similar results, with an increase in dry mass and height of soybean plants after chlorimuron application of 0.4 g ai ha^{-1}. However, studies that report an increase in yield through the use of subdoses of chlorimuron are scarce, which reinforces the relevance of the results of the present study.

Another herbicide that showed interesting results was 2,4-D (134 g ae ha^{-1}), which increased the 1,000-seed mass compared to the control. It was also superior to other herbicides in yield, but without differing from the control, without application. Silva et al. (2019) observed an increase in the number of pods, nodes, and grain mass of soybean plants with the application of 2,4-D (22.5 g ae ha^{-1}), at the V4 stage, indicating the effect of hormesis. Nevertheless, the authors also observed reductions in grain mass from the dose of 30 g ae ha^{-1}, at the V4 stage. Given the result obtained in the present study and that observed by Silva et al. (2019), it is understood that the application of 2,4-D negatively interferes with the quality of soybean seeds, and therefore, caution is recommended when using this herbicide in seed production fields of this crop.

Also, regarding the application of 2,4-D, despite some promising effects, there is a risk of undesirable effects for soybeans, as already highlighted. On the other hand, it is noteworthy that 2,4-D tolerant transgenic soybean cultivars (Enlist™ E3 soybean) are already available, which have a high tolerance to this herbicide (Kalsing et al., 2018; Silva et al., 2021). However, in these studies, the effects of increment on soybean plants by the application of the herbicide were not evaluated, only the herbicide selectivity. This opens up the possibility of investigating the effect of 2,4-D hormesis in soybean, without the aforementioned risks of reduced yield.

Conclusions

The application of glyphosate, chlorimuron, fomesafen or glufosinate resulted in an increase in the height of soybean plants. Only the application of chlorimuron increased soybean yield compared to the control. The application of paraquat or glufosinate, despite the injury, did not reduce soybean yield compared to the control, but the application of these herbicides to stimulate plant development is not recommended, given the potential for injury and low or none increase in plant performance. The 2,4-D herbicide was the only one that caused a reduction in the physiological quality of soybean seeds.

Compliance with Ethical Standards

Author contributions: Conceptualization: DC[1], DC[2], CPJ; Data curation: DC[1], DC[2], CPJ; Formal analysis: DC[1], CPJ; Investigation: DC[1], CPJ, DADC, APDCR; Supervision: CPJ, JRF; Writing–original draft: AFMS, DC[1]; Writing–review & editing: DC[1], DC[2], DADC, APDCR, CPJ, AFMS, JRF.

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