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Agronomic performance of Conkesta E3® soybean to increasing doses of 2,4-D herbicide

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Abstract

Soybean is a dicotyledonous plant from the Fabaceae family, originally from East Asia, and today, Brazil is the largest global producer of this legume. However, over the past few decades, various challenges in weed control have led to significant economic losses for soybean producers. As a response, new technologies have been introduced to address this issue. Among these innovations, multiple resistance biotechnology stands out, enabling producers to use glyphosate, 2,4-D, and glufosinate-ammonium in soybean crop. The present study, conducted at IFSULDEMINAS Campus Inconfidentes, was developed in order to evaluate the performance of soybean cultivars carrying this new technology when subjected to increasing doses of 2,4-D, commercially known as Enlist® Colex-D. The experiment was carried out with two commercial soybean cultivars (B5710 CE and 98R30 CE), with a randomized complete block design (RCBD) in a 2x9 factorial scheme, involving two cultivars and nine different herbicide dosages per cultivar, totaling 18 treatments and four replications. The herbicide doses were (g ha^{-1}): absent, 456, 912, 1368, 1824, 2280, 2736, 3192 and 3648. The variables assessed included phytotoxicity, chlorophyll index, plant height, thousand-grain weight, and grain yield. Increasing herbicide doses gradually induced phytotoxicity, but statistically, there was no significant impact on the final grain yield of the crop in both cultivars.

Keywords: *Glycine max*. Phytotoxicity. Biotechnology. Auxinic.

Introduction

Soybean (*Glycine max* (L.) Merrill) crop in Brazil began in the 1970s, driven by the global expansion of the oil industry, which significantly increased the demand for soybeans (KLEIN, LUNA, 2020). Over the years, the crop of this legume has grown substantially, bringing about a major agricultural revolution and contributing to food security (JARDINE, 2021). Soybeans are recognized as one of the most important protein sources, used to produce a wide array of products, including foods, pasta, beverages, dietary supplements, soy milk, animal feed, meal, cosmetics, soap, and biodiesel among other derivatives crucial for humanity (DILAWARI *et al.*, 2022).

In recent harvests, Brazil has emerged as the world's largest soybean producer, surpassing the United States. During the 2022/2023 season, Brazilian soybean production increased by 20.6 % compared to the previous season, reaching a record 151.4 million metric tons (CONAB, 2023). Alongside this growth, advancements in research and management technologies have significantly grain yield and sustainability. However, the intensification of crop has also presented new challenges, such as the proliferation of weeds. These weeds complicate management practices and may result in severe grain yield losses, potentially reducing yields by up to 100 % in extreme cases (MONTEIRO, SANTOS, 2022).

The legalization and adoption of genetically modified (GM) soybeans in Brazil began in 2003, marking a pivotal shift in weed management practices (ADEGASA *et al.*, 2022). This innovation enabled the use of broad-spectrum herbicides such as glyphosate, which simplified invasive species control and reduced reliance on selective herbicides (FADIN *et al.*, 2018). However, prolonged reliance on this system has led to the emergence of herbicide-resistant weeds, necessitating the implementation of integrated management practices to ensure the long-term sustainability of agricultural systems (GAZZIERO *et al.*, 2023).

In response to the growing prevalence of glyphosate-resistant weeds, companies have invested in research to develop cultivars resistant to multiple herbicidal modes of action through genetic engineering. After nearly two decades of glyphosate dependency in soybean crop, cultivars with resistance to multiple active ingredients have been developed, such as the DAS44406-06 event, marketed as Enlist E3™. This variety is tolerant to glyphosate, glufosinate-ammonium, and 2,4-D choline salt (Corteva Agriscience, Michigan, United States). The DAS44406-06 event (Dow AgroSciences LLC, Auburn, United States) aims to provide farmers with alternative herbicide options for weed control (PAPINENI *et al.*, 2017).

Among the herbicides recommended for soybeans cultivars with multiple resistance technology, 2,4-D a synthetic auxin stands out due to its ability to mimic the action of natural auxins. This compound exerts a more pronounced effect than endogenous auxins, inducing metabolic and biochemical changes in plants (STERLING, HALL, 1997). Its efficacy is particularly high against dicotyledonous species (PETERSON *et al.*, 2016). When applied at high concentrations, 2,4-D can inhibit cell division and plant growth, particularly in meristematic tissues where photosynthetic products accumulate (AYDIN *et al.*, 2020).

Given the challenges associated to weed control in soybean crop, the introduction of new resistance mechanisms raises important questions among producers and experts. Therefore, this study was developed in order to evaluate the effects of applying different doses of 2,4-D on soybeans with multiple resistance biotechnology, focusing on agronomic traits and yield crop.

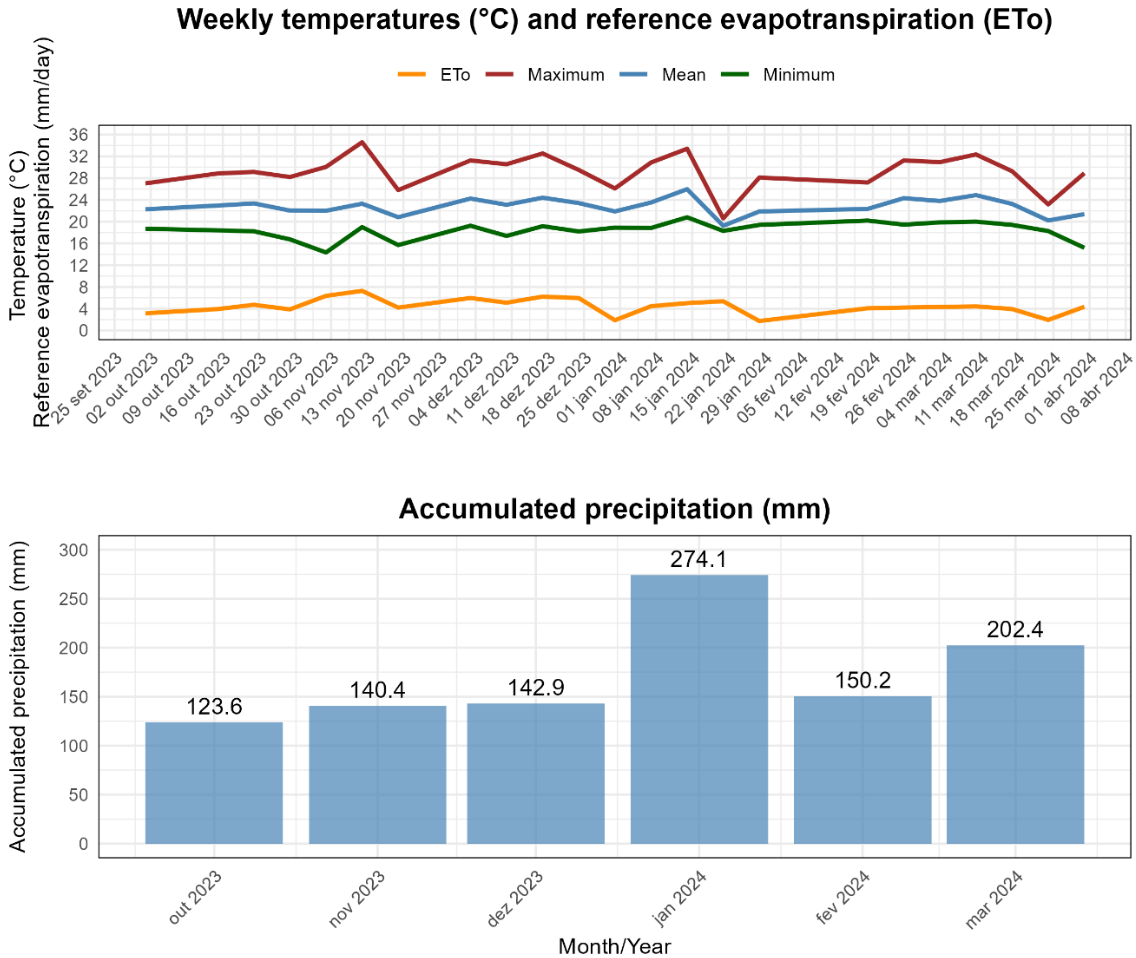
Material and methods

The experiment was carried out during the 2023/2024 growing season at the experimental field of the Fazenda-Escola, part of the Federal Institute of Education, Science, and Technology of South Minas Gerais (IFSULDEMINAS) - *Campus* Inconfidentes (22°18'21" S, 46°20'08" W). The municipality of Inconfidentes is situated at an altitude of 869 m and experiences a humid subtropical climate, characterized by mild summers and the absence of a dry season (ALVARES *et al.*, 2014). According to data from the rain gauge installed at *Campus* Inconfidentes, total precipitation during the growing season reached 831.50 mm (Figure 1).

The experimental area was prepared using conventional tillage methods, including plowing followed by harrowing. Fertilization at sowing and topdressing stages was performed based on soil chemical analysis results (Table 1).

A randomized complete block design (RCBD) in a 2 × 9 factorial scheme was employed, consisting of two soybean cultivars and nine different doses of 2,4-D herbicide with four replications, totaling 72 plots. The doses of 2,4-D were (g ha^{-1}): absent, 456, 912, 1368, 1824, 2280, 2736, 3192 and 3648. The row spacing was 0.50 m, and each plot consisted of four rows, each 4 m long, covering an area of 8 m^2. The central two rows of each plot were used for evaluations. The Conkesta E3® soybeans cultivars selected for the study were B5710 CE and 98R30, both indeterminate growth types with late maturity (145 days).

Figure 1. Accumulated precipitation and weekly average temperature in the experimental area during the conduct of the experiment at IFSULDEMINAS, *Campus* Inconfidentes, Inconfidentes-MG, Brazil.



Source: authors (2025)

Sowing was achieved on October 30th, 2023, at a final population density of 240,000 seeds ha^{-1} (12 seeds m^{-1}). Ten doses of a liquid inoculant containing *Bradyrhizobium* were applied directly to the seeds in the planting furrow. Harvesting of the grain was carried out manually on March 22nd, 2024.

At the V2 phenological stage, when the plants had two fully developed trifoliate leaves, weed management was performed using an application of 912 g a.i. ha^{-1} of glyphosate, following the method described by Frene *et al.* (2018). At this stage, a significant emergence of weeds was observed. This management approach ensured that the study focused on the potential effects of post-emergence application of 2,4-D on soybean.

The application of Enlist® Colex-D (Corteva Agriscience, Michigan, United States) occurred

Table 1. Soil chemical analysis of the experimental area at IFSULDEMINAS, *Campus* Inconfidentes.

pH	P	K	Al	Ca	Mg	H+Al	CTC	V%	O.M.
6.06	30.00	45.00	0.00	3.3	0.69	2.67	6.72	60.32	2.59

P; K= mg/dm³; Al; Ca; Mg; H+Al = Cmol/dm³; V% = %; Organic matter (O.M.) = dag/dm

Source: Soil Fertility Laboratory IFSULDEMINAS Campus Inconfidentes (2023) at the V5 growth stage, using a spray volume of 200 L ha^{-1}. An 18-liter electric backpack sprayer (Yamaho) equipped with a flat-fan nozzle (0.2 L min^{-1}, yellow, fine-to-medium droplets) was employed. To prevent drift between plots, physical barriers of white plastic sheeting (300 microns) were installed around the plot edges. During herbicide application, environmental parameters such as wind speed, relative humidity, and temperature were recorded using a Thermo-Hygro-Anemometer-Luxmeter (Model THAL-300, Instruthem). Crop protection management was conducted as needed to ensure optimal soybean growth.

Phenotypic evaluations

The following agronomic traits were assessed:

i. Phytotoxicity (PHYT): phytotoxicity was evaluated 7 days after herbicide application (DAA), corresponding to the time when necrotic spots caused by product intoxication typically reach their maximum area (FRENE *et al.*, 2018; KALSING *et al.*, 2018). The evaluation considered symptoms on a scale of 0 to 9 scale based on the modified European Weed Research Council (EWRC, 1964) assessment scale (FRANS, 1972).

ii. Chlorophyll Content (CHL): measured at the beginning of flowering (R1 growth stage) using an SPAD-502 Plus chlorophyll meter. Ten plants were randomly selected from each effective plot for this assessment.

iii. Plant Height (PH): measured at the beginning of grain filling (R5 growth stage) using a tape measure to determine the distance from the soil level to the apical meristem of ten randomly selected plants from each effective plot.

iv. Grain Yield (GY): evaluated after manual harvest of the effective plot rows. Grain weight was recorded in kilograms, standardized to 13 % moisture content, and converted to yield per hectare (kg ha^{-1}).

v. Thousand Grain Weight (TGW): determined manually after the harvest by selecting a sample of 1,000 grains from each plot and measuring their mass using a precision scale.

Statistical analysis of phenotypic data

Phenotypic data were subjected to descriptive and exploratory analyses, as well as tests to verify the assumptions of normality (SHAPIRO, WILK, 1965), homogeneity (BARTLETT, 1937), and independence of errors (DURBIN, WATSON, 1950). Subsequently, data were analyzed using analysis of variance (ANOVA). When significant differences were detected, mean values were grouped using the Scott-Knott test (1974) at a 5 % probability level.

Interactions among agronomic traits were analyzed through linear regression, and significant equations ($p < 0.05$) were fitted. All statistical analyses were conducted using the R computational environment (R Development Core Team, 2021) with the AgroR library (SHIMIZU, MARUBAYASHI, GONCALVES, 2023).

Results and discussion

The analysis of variance (ANOVA) summary revealed no significant interaction between cultivar and dose for any of the variables evaluated. For the dose factor, significant results were observed only for the variables phytotoxicity (PHYT) and chlorophyll content (CHL). Conversely, for the cultivar factor, significant differences were noted for CHL, plant height (PH), and thousand-grain weight (TGW). No significant effects were found for grain yield (GY) and PHYT (Table 2).

The coefficients of variation (CV%) for PHYT, CHL, PH, and TGW were below 10 %, indicating high experimental precision in data collection for these variables (PIMENTEL GOMES, 2022). However, CV of 18.18 % was found to GY, which, while relatively high compared to the other variables, is still indicative of good experimental quality. The higher CV for GY can be attributed to its nature, being influenced by numerous environmental and management factors (LEI *et al.*, 2017; GUPTA *et al.*, 2022).

Given the absence of cultivar x dose interaction, it might be concluded that the application of different doses of 2,4-D choline resulted in similar responses among soybean cultivars. A study conducted in Argentina with seven soybean cultivars exposed to full doses and sequential applications of 2,4-D found good crop recovery and positive outcomes for the evaluated variables (FRENE *et al.*, 2018). These findings are aligned with the current study, further demonstrating that soybean cultivars with this biotechnology exhibit consistent behavior under similar 2,4-D choline treatments.

The mean values obtained for each cultivar (B5710 CE and 98R30 CE) for CHL, PH, and TGW were grouped and compared (Table 3).

Over the years, the development of new soybean cultivars has exhibited significant diversification, particularly in genotype x environment interactions. This interaction directly affects the performance of various plant traits, ultimately influencing grain yield (ABDALA *et al.*, 2024). The soybean cultivars used in this study have notable genetic diversity, particularly in traits like CHL, PH, and TGW.

For CHL, cultivar B5710 reached the highest chlorophyll content (43.41). This difference is likely due to genetic diversity, with one genotype exhibiting more active chlorophyll than the other. For instance, quantitative trait loci (QTL) mapping studies have identified genetic loci directly associated with chlorophyll content in soybean leaves (WANG *et al.*, 2020). Yu *et al.* (2020) identified major loci controlling photosynthesis, which can be harnessed in breeding programs to enhance photosynthetic efficiency and environmental adaptability. Begović *et al.* (2023) investigated the effects of selective herbicides for broadleaf weed control in soybean, revealing distinct photosynthetic adaptation mechanisms among cultivars. For example, the Ika cultivar responded more strongly during advanced stages like leaf development and early flowering, whereas Zora responded earlier, during the cotyledon stage.

Regarding PH, cultivar B5710 was also taller, with an average height of 113.59 cm, approximately 5 cm more than 98R30. Plant height is a critical factor in soybean crop, impacting both yield and harvest efficiency. It is also essential for selecting cultivars suitable for specific regions (REZENDE, CARVALHO, 2007). Currently, there is a trend toward developing shorter plants to mitigate lodging issues, which may cause significant losses for producers. According to Balbinot Junior (2012), vegetative

Table 2. Summary of variance analysis for phytotoxicity (PHYT), chlorophyll (CHL), plant height (PH), thousand-grain weight (TGW), and grain yield (GY) for soybean cultivars subjected to different doses of 2,4-D herbicide.

				F value		
Cultivar (C)	1	1.89^{ns}	48.41*	17.77*	124.71*	0.01^{ns}
Dose (D)	8	3133.76*	5.54*	0.40^{ns}	1.06^{ns}	1.86^{ns}
Block	3	3.08×10^{-28}^{ns}	1.93^{ns}	16.67*	3.42*	1.13^{ns}
C x D	8	0.83^{ns}	0.86^{ns}	1.04^{ns}	0.26^{ns}	0.70^{ns}
Residual	51	-	-	-	-	-
CV (%)	-	5.23	0.88	0.99	0.95	1.94

DF: degrees of freedom. *Significant at 5 % probability using the F-test; ^{ns} not significant at 5 % probability using the F-test.

Source: authors (2025)

Table 3. Mean values of chlorophyll (CHL), plant height (PH), and thousand-grain weight (TGW) for soybean cultivars subjected to different doses of 2,4-D.

Cultivar	CHL	PH (cm)	TGW (g)
B5710	43.41a	113.59a	165.06b
98R30	41.15b	108.56b	187.77a
Average	42.28	111.07	176.41

Means followed by the same lowercase letter within the column are not significantly different by the Scott and Knott (1974) test at 5 % probability.

Source: authors (2025)

growth is generally more pronounced in colder regions, necessitating specific management practices to control it.

For TGW, the cultivar 98R30 significantly outperformed B5710, with a thousand-grain weight of 188 g compared to 165 g for B5710. TGW is a vital trait in soybean, reflecting seed quality, nutrient reserves, grain filling, and cultivar selection. Thomas and Costa (2010) noted that grain weight, characteristic of each cultivar, may vary depending on environmental conditions and management practices. The increase in seed weight may be linked to greater protein accumulation due to enhanced amino acid synthesis facilitated by nitrogen availability (NISHIOKA, OKUMURA, 2008).

When 0 to 1,368 g ha^{-1} of Enlist Colex-D was applied, the mean phytotoxicity score was 1, indicating no damage. However, at 1,824 g ha^{-1}, slight phytotoxicity (score 2) was observed; and at 2,280 g ha^{-1}, symptoms were light (score 3), with rare and minor lesions. These results align with Kalsing *et al.* (2018), who reported mild phytotoxicity (up to 3 %) in soybean when subjected to 1,950 g ha^{-1} of 2,4-D combined with 2,050 g ha^{-1} of glyphosate. Similarly, Monteiro *et al.* (2024) found only minor injuries (<3 %) when applying 1,020 g ha^{-1} of 2,4-D with 1,250 g ha^{-1} of glyphosate.

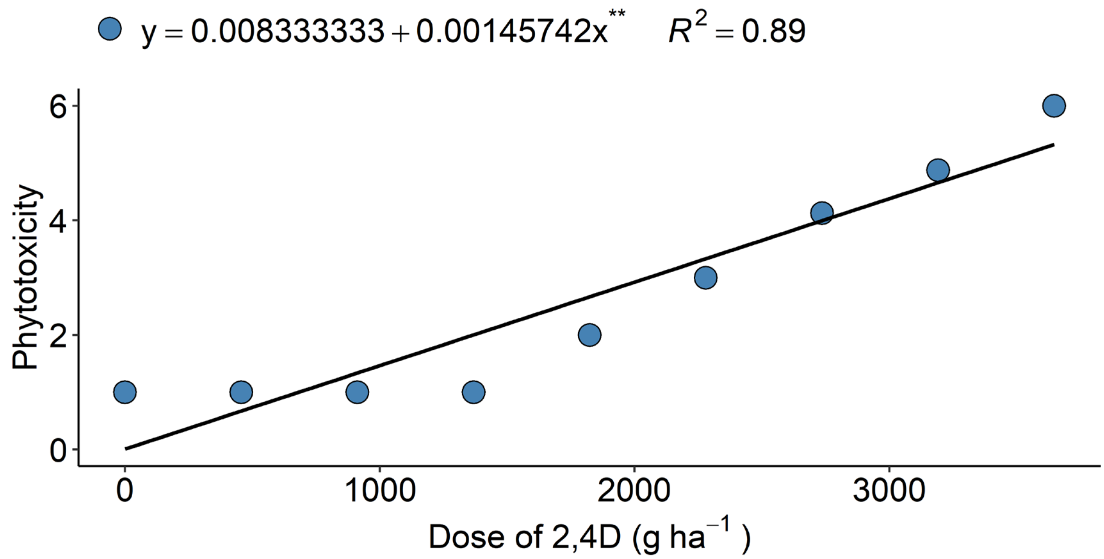
At 2736 g ha^{-1}, phytotoxicity increased, with an average score of 4 (moderate). Phytotoxicity gradually increased up to 3648 g ha^{-1}, as demonstrated by the linear regression analysis (Figure 2).

Therefore, at commercial product doses exceeding 1,824 g ha^{-1}, early symptoms of phytotoxicity become evident. These symptoms intensify significantly at doses above 2,736 g ha^{-1}. However, it is noteworthy that soybean yield remains unaffected, regardless of the applied dose.

As herbicide doses increased, phytotoxicity scores also rose linearly, reaching a score of 6 (classified as "very strong") at the highest dose of 3,648 g ha^{-1}. Symptoms observed included leaf chlorosis, pronounced white/gray spots, leaf edge curling, and occasional wilting. Nevertheless, approximately 15 days after assessment, all visible symptoms had completely disappeared, with no adverse impact on final yield crop compared to lower doses. These findings align with the work of Frene *et al.* (2018), who reported 23 % phytotoxicity following the application of 2,280 g ha^{-1} of 2,4-D and 2,280 g ha^{-1} of glyphosate on various Enlist soybean cultivars. Remarkably, full crop recovery occurred within 14 days, with no negative effects on final grain yield.

For chlorophyll content (CHL), significant differences were observed as a function of applied doses (Figure 3). According to the linear regression, chlorophyll levels decreased as herbicide doses increased. The highest chlorophyll levels were recorded in the untreated

Figure 2. Linear regression of the effect of 2,4-D choline salt doses on phytotoxicity in soybean crop.



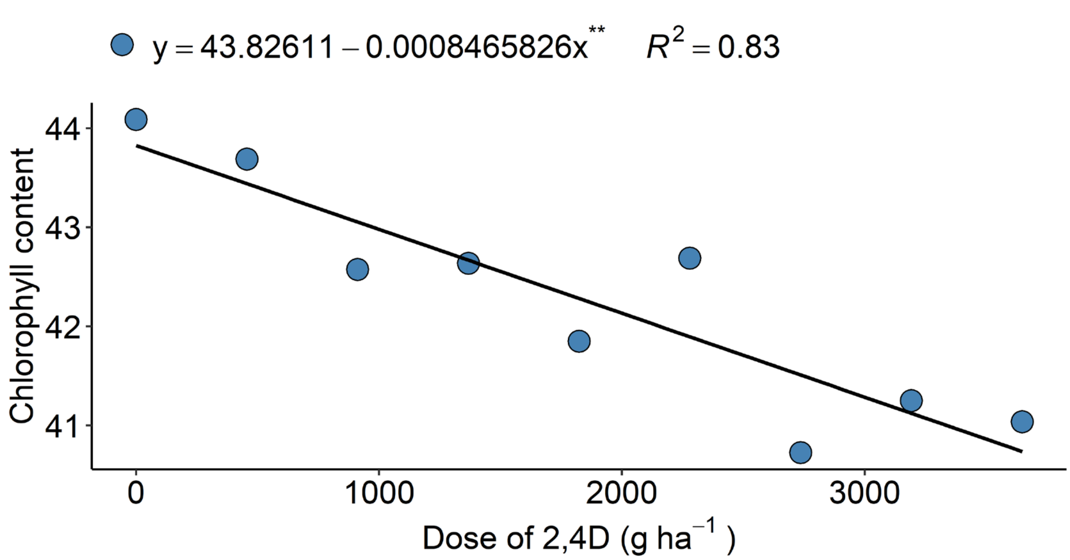
Source: authors (2025)

checkplots (0 L ha^{-1}), while the lowest levels occurred at 2,736 g ha^{-1}.

The 2,4-D molecule affects multiple hormonals signaling pathways, including those involving abscisic acid and ethylene, potentially generating reactive oxygen species (ROS) (SONG, 2014). ROS may damage cellular structures, including chloroplast membranes, thereby directly impairing photosynthetic processes (SONG, 2014). Oxidative stress can destabilize membranes, induce harmful mutations, and disrupt essential metabolic processes, compromising chloroplast functionality (EINHARDT *et al.*, 2022). Moreover, chlorophyll degradation may result from secondary effects of oxidative stress, further reducing the plant's photosynthetic efficiency (KESAWAT *et al.*, 2023).

Despite the presence of the aad-12 gene from *Delftia acidovorans* in soybean cultivars, when

Figure 3. Linear regression of the effect of 2,4-D choline salt doses on chlorophyll content in soybean crop.



Source: authors (2025)

expressed in plants, degrades 2,4-D into inactive 2,4-dichlorophenol (DCP), high concentrations of 2,4-D still caused slight reductions in chlorophyll levels (GRIFFIN *et al*., 2013). The tolerance of Enlist® soybean cultivars to 2,4-D is primarily is due to the insertion of the aad-12 gene, originally isolated from *Delftia acidovorans*. This gene encodes the enzyme Aryloxyalkanoate Dioxygenase-12 (AAD-12), which catalyzes the rapid cleavage of the 2,4-D molecule, converting it into 2,4-dichlorophenol (DCP), a compound with low or no herbicidal activity. Through this metabolic pathway, the herbicide is detoxified before it can accumulate and act as a synthetic auxin, preventing the expression of severe phytotoxic symptoms in the crop. However, at very high concentrations of 2,4-D, transient and mild effects, such as slight reductions in chlorophyll content, may still be observed until the detoxification process is completed (TORRA *et al*., 2024). This mechanism explains the effective field-level tolerance of Enlist® soybean, as well as the temporary physiological changes recorded in our study.

In contrast, Monteiro *et al.* (2024) found no statistical difference in chlorophyll indices between treatments using 1,020 g ha^{-1} of 2,4-D and 1,250 g ha^{-1} of glyphosate. However, glyphosate-tolerant soybean (RR) exhibited reduced chlorophyll levels as glyphosate doses increased, though grain yield was unaffected (ALBRECHT *et al.*, 2018).

When examining grain yield as a function of herbicide doses, it becomes evident that, despite high levels of phytotoxicity (7 DAA) and changes in chlorophyll levels, there were no significant statistical differences in grain yield between the two cultivars tested. Both cultivars exhibited consistent performance under high-dose treatments (Figure 4).

These results are consistent with other studies, such as Frene *et al.* (2018), where 13–23 % phytotoxicity did not impact final grain yield. Similarly, Miller and Norsworthy (2016) reported no yield reductions in soybean cultivars with multiple resistance biotechnology following applications of 2,4-D choline, glyphosate, and glufosinate under different chemical management programs. The tolerance of soybean to post-emergence applications of 2,4-D choline, glyphosate, and glufosinate ensures that these herbicides do not compromise crop performance or final grain yield (MONTEIRO *et al.*, 2024).

Additionally, Castro *et al.* (2018) observed similar results in glyphosate-tolerant RR soybean. Despite increased phytotoxicity at higher glyphosate doses, no significant agronomic or grain yield losses were reported.

Conclusions

The application of increasing doses of 2,4-D choline salt to soybean cultivars with multiple resistance biotechnology results in visible phytotoxicity and a reduction in chlorophyll content, particularly at doses exceeding 1,824 g ha^{-1}, without compromising the final yield of the crop in both evaluated cultivars.

Cultivars B5710 CE and 98R30 CE exhibit similar responses to the doses of 2,4-D, demonstrating notable tolerance to the herbicide even under conditions of severe phytotoxicity, thereby corroborating the effectiveness of multiple resistance biotechnology.

Despite observed physiological alterations, soybean cultivars retain its agronomic performance and productive potential, reinforcing its suitability for weed management systems involving 2,4-D choline salt.

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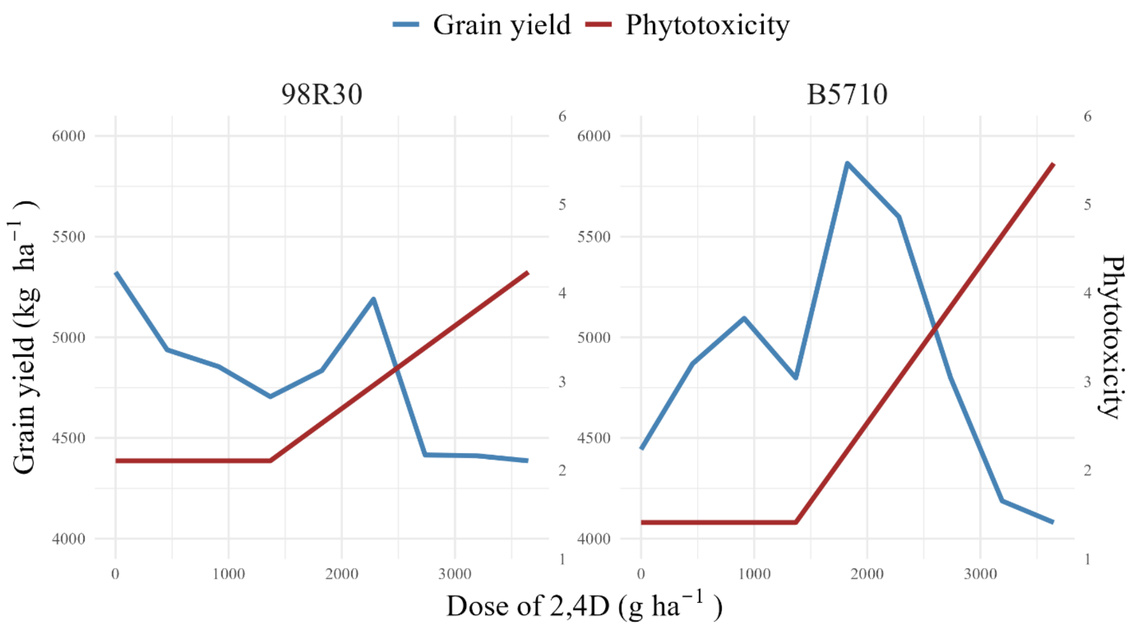
Figure 4. Grain yield and phytotoxicity of the 98R30 CE and B5710 CE cultivars as a function of 2,4-D choline salt herbicide (Colex-D) doses.



Source: authors (2025)

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