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ORIGINAL ARTICLE

Conyza spp. control and selectivity of 2,4D in ENLIST® soybean[1]

Controle de *Conyza* spp. e seletividade do 2,4-D em soja ENLIST®

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HIGHLIGHTS:
Sequential glufosinate-ammonium applications enhance Conyza *spp. control in dense infestations.*
Sequential applications boost control, regardless of herbicide combinations with 2,4-D and glyphosate.
The sequential application of glufosinate-ammonium results in high soybean grain yields.

ABSTRACT: The introduction of 2,4-D-tolerant soybeans (ENLIST®) offers a new potential for herbicide application. Therefore, the present study aimed to (a) evaluate the effect of combinations of post-emergent herbicides on the control of *Conyza* spp. and (b) assess the selectivity of the post-emergent herbicide association in soybeans, ensuring effective application without compromising the health of the crop. A field experiment with 2,4-D-tolerant soybeans utilized a randomized complete block design with 14 treatments across four replications. Treatments included various combinations of 2,4-D with glyphosate, chlorimuron-ethyl, cloransulam-methyl, imazethapyr, bentazon, and imazamox. These were applied either as a single treatment or sequentially 15 days after treatment (DAT) using glufosinate-ammonium alongside two controls without herbicide application. The results showed less than 15% control across all treatments. At 21 DAT, only the treatments applied sequentially showed significant control, with effectiveness exceeding 80% against *Conyza* spp. In contrast, the single applications of the herbicide combinations with 2,4-D were ineffective for post-emergence control of *Conyza* spp.

Key words: 2,4-D-tolerant soybeans, horseweed, post-emergent herbicide combinations

RESUMO: A introdução da soja tolerante a 2,4-D (ENLIST®) oferece um novo potencial para a aplicação de herbicidas. Dessa forma, o presente trabalho teve como objetivo: (a) avaliar o efeito das combinações de herbicidas pós-emergentes no controle de *Conyza* spp., e (b) a seletividade da associação de herbicidas pós-emergentes em soja, garantindo uma aplicação eficaz sem comprometer a saúde da cultura. Um experimento de campo com soja tolerante ao 2,4-D utilizou um delineamento experimental de blocos casualizados, com 14 tratamentos em quatro repetições. Os tratamentos incluíram várias combinações de 2,4-D com glifosato, chlorimuron, cloransulam, imazethapyr, bentazona e imazamox. Estes foram aplicados como um único tratamento ou sequencialmente 15 dias após o tratamento (DAT) usando glufosinato de amônio, juntamente com dois controles sem aplicação de herbicida. Os resultados mostraram menos de 15% de fitotoxicidade em todos os tratamentos. Aos 21 DAT, apenas os tratamentos aplicados sequencialmente mostraram controle significativo, com eficácia superior a 80% contra *Conyza* spp. Em contraste, as aplicações únicas das combinações de herbicidas com 2,4-D foram ineficazes para o controle pós-emergência de *Conyza* spp.

Palavras-chave: soja tolerante a 2,4-D, buva, combinações de herbicidas pós-emergentes

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Introduction

Brazil stands out worldwide in the production and export of soybeans, with a forecast of a significant 20.6% increase in production for the 2022/2023 crop (CONAB, 2023). It is essential to implement technologies in the production system to achieve these levels of production, including the control of weeds in soybean crops, which can reduce yield and cause direct damage, such as competition for resources, and indirect damage, such as the unfeasibility of cultivation/harvest (Carvalho et al., 2022).

In southern Mato Grosso do Sul, the effectiveness of chemical control of *Conyza* spp. is compromised by the multiple resistance of these species, resulting from prolonged use of herbicides that inhibit acetolactate synthase (ALS) and 5-enolpyruvylshikimate-3-phosphate synthase (EPSPs) (Adegas et al., 2022). In response, the launch of new transgenic events using 2,4-D in 2021 provided alternatives for controlling biotypes resistant to various mechanisms of action, including glyphosate and ALS, in difficult-to-control weeds (Kumar et al., 2020).

The ENLIST® technology package includes soybeans tolerant to 2,4-D (auxin mimicker), glyphosate (EPSP inhibitors), and glufosinate-ammonium (GS inhibitors), which significantly expands weed management options in agricultural systems that accommodate these herbicides (Jones et al., 2019). However, in the scientific literature, it is noted that the combination of 2,4-D and glyphosate applied to soybeans with the ENLIST® technology can initially cause phytotoxic effects, resulting in chlorosis and leaf curling (Foles et al., 2023).

Thus, the use of 2,4-D along with these herbicides, all registered for post-emergence use in soybeans, can result in effective control of *Conyza* spp., provided that selectivity to the crop is maintained. Therefore, the present study aimed to (a) evaluate the effect of combinations of post-emergent herbicides on the control of *Conyza* spp. and (b) assess the selectivity of the post-emergent herbicide association in soybeans, ensuring effective application without compromising the health of the crop.

Material and Methods

The experiment was conducted in the field, starting in October and lasting until the end of February, at the Experimental Farm of Agricultural Sciences (FAECA) of the Federal University of Grande Dourados – UFGD, in Dourados, in the state of Mato Grosso do Sul, Brazil, at 21° 57' S and 46° 51' W. Soybean sowing was performed on October 8, 2022, in areas infested with horseweed (*Conyza* spp.). The treatments were applied on November 9, 2022. The climate of the region is tropical Am-type according to the Köppen climate classification, with significant rainfall most months of the year (Fietz et al., 2017).

The experimental units consisted of 3 × 5 m plots, with a total area of 15 m^2 per plot, with six soybean rows in each plot. Soybean cultivar B5595CE was sown using a seed drill with a spacing of 0.45 m between rows and 14 seeds per meter, aiming to obtain a final stand population of approximately 310,000 plants ha^{-1}. Soil samples were taken from the experimental area before the installation of the experiment in an Oxisol (United States, 2014) that corresponds to a Latossolo Vermelho Distroférrico in the Brazilian Soil Classification System, whose chemical and physical characteristics were determined according to methodologies recommended by Teixeira et al. (2017) and are presented in Table 1.

The area where the experiment was conducted has a history of *Conyza* spp. infestation; however, a population survey of *Conyza* spp. was performed before the implementation of the experiment (Silva et al., 2023) through the square inventory method, which consists of randomly dropping a square of 1 m^2 over the chosen area, aiming to identify the existence of other species and their respective infestation densities. Likewise, in the first evaluation of the controls, a phytosociological survey was conducted again using the square inventory method to analyze the composition and density of the weed flora present in the experimental area, with a result of 14 plants (*Conyza* spp.) per 0.16 m^2 with a height of 20 cm. According to the BBCH classification scale (Hess et al., 1997), the plants were in the phenological stage (23/30).

The soybean seeds were treated before planting with the fungicide and insecticide Standak® Top (25 g L^{-1} pyraclostrobin + 225 g L^{-1} thiophanate methyl + 250 g L^{-1} fipronil), using the recommended dose of 200 mL of the commercial product for 100 kg of soybean seeds. The crop was monitored, and applications (maintenance) of fungicides and insecticides were performed when necessary. Insecticides (Talisman® - bifenthrin 50 g L^{-1} + carbosulfan 150 g L^{-1}, Fipronil 800 WG – 50 g a.i. ha^{-1}, and Bold® - acetamiprid 37.5 g a.i. ha^{-1} + fenpropathrin 56.25 g a.i. ha^{-1}) and fungicides (Orkestra® SC – fluxapyroxad 58.45 g a.i. ha^{-1} + pyraclostrobin 116.55 g a.i. ha^{-1}, and Viovan® - picoxystrobin 60 g a.i. ha^{-1} + prothioconazole 70 g a.i. ha^{-1}) were used. The first application was on October 22, and fipronil (240 g a.i. ha^{-1}) was applied to control the cucurbit beetle. The second application occurred on December 8, with the application of the fungicide Orkestra® SC (fluxapyroxad 58.45 g a.i. ha^{-1} +, pyraclostrobin 116.55 g a.i. ha^{-1}) and the insecticide Bold® (acetamiprid 37.5 g a.i. ha^{-1} + fenpropathrin 56.25 g a.i. ha^{-1}). The third application was on December 20, applying the fungicide Viovan® (picoxystrobin 60 g a.i. ha^{-1} + prothioconazole 70 g a.i. ha^{-1}). The fourth application took place on January 7, with talisman insecticide (bifenthrin 30 g a.i. ha^{-1} + carbosulfan 90 g a.i. ha^{-1}). The fifth application was performed on January 11 with the fungicide Orkestra® SC (fluxapyroxad 58.45 g a.i. ha^{-1} + pyraclostrobin 116.55 g a.i, ha^{-1}).

Table 1. Soil chemical analysis and physical characteristics of the soil of experimental site before experiment

Ca^{2+}	Mg	H+Al	SB	CEC	Al	K	P	BS	pH
$cmol_c$ dm^{-3}						mg kg^{-1}		%	$CaCl_2$
4.56	2.08	7.08	6.74	13.82	0.12	18	40.73	48.8	5.08

CEC - Effective cation exchange capacity; SB - Sum of bases; pH determination using the $CaCl_2$ method; BS - Base saturation

The experimental design was a randomized complete block design, with four replicates, comprising 12 herbicide treatments registered for soybean cultivation (AGROFIT, 2024) and two controls, one with weeding and the other without, totaling 14 treatments, as shown in Table 2.

The treatments were applied using a CO_2-pressurized knapsack sprayer equipped with a four-nozzle spray boom model TT 11002, spaced 0.50 m apart, positioned at 0.5 m height concerning the plant surface, spray volume 200 L ha$^{-1,}$ and working pressure of 250 kPa. The first application of the treatments occurred between soybean stages V2 and V4. At the time of application, the environmental conditions - relative humidity, temperature and wind speed were measured, being 79%, 24 °C, and 3.5 km h$^{-1,}$ respectively. Fifteen hours after the first application, some treatments received a sequential application of glufosinate-ammonium. The environmental conditions at the time of application regarding relative air humidity, temperature, and wind speed were 76%, 26 °C, and 2.9 km h^{-1}, respectively. The weekly values of accumulated rainfall and average, maximum, and minimum temperatures in Dourados, Mato Grosso do Sul, Brazil, collected at the EMBRAPA-UFGD rainfall station, are presented in Figure 1.

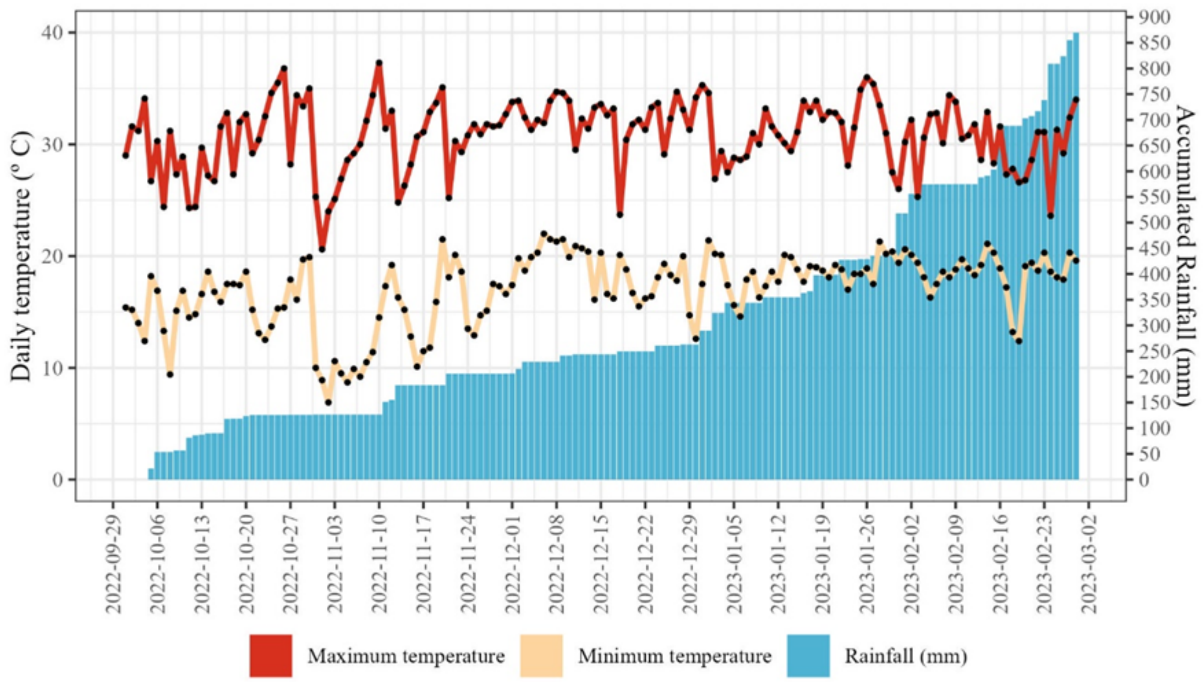
Visual evaluations of *Conyza* spp. control and soybean phytotoxicity were performed at 7, 14, 21, 28, and 35 days after the first application of the treatments (DAT). The ALAM (1974) visual scale was followed, which assigns a score of 0% for the absence of symptoms caused by the herbicide and 100% for the death of the weed. For the characterization of the phytotoxicity symptom, 0% was the absence of damage, and 80–100% indicated total plant destruction (plant death) (Palharani et al., 2023; Silva et al., 2023).

At the end of the soybean cycle, after the plants showed more than 95% leaf senescence, soybean desiccation was performed with diquat (400 g ha^{-1}), after which the three central rows of the observation area of the plots were harvested, discarding 0.5 m from the ends and borders of

Table 2. Dose of herbicides used in post-emergence application

T**	Herbicides*	Rate (g a.i. ha^{-1})	15 DAT	Dose (g a.i. ha^{-1})
T1	2,4-D + Glyphosate	1020 + 1250		
T2	2,4-D + Glyphosate	1020 + 1250	Ammonium Glufosinate	500
T3	2,4-D + Chlorimuron + Glyphosate	1020 + 15 +1250		
T4	2,4-D + Chlorimuron + Glyphosate	1020 + 15 + 1250	Ammonium Glufosinate	500
T5	2,4-D + Cloransulam + Glyphosate	1020 + 33,6 + 1250		
T6	2,4-D + Cloransulam + Glyphosate	1020 + 33,6 + 1250	Ammonium Glufosinate	500
T7	2,4-D + Imazethapyr + Glyphosate	1020 + 100 +1250		
T8	2,4-D + Imazethapyr + Glyphosate	1020 + 100 + 1250	Ammonium Glufosinate	500
T9	2,4-D + Bentazon + Glyphosate	1020 + 720 + 1250		
T10	2,4-D + Bentazon + Glyphosate	1020 + 720 + 1250	Ammonium Glufosinate	500
T11	2,4-D + Imazamox + Glyphosate	1020 + 42 + 1250		
T12	2,4-D + Imazamox + Glyphosate	1020 + 42 + 1250	Ammonium Glufosinate	500
T13	Control without weeding	-	-	-
T14	Weeded control	-	-	-

*Addition of mineral oil (0.5% v/v); All treatments registered for soybean cultivation (AGROFIT, 2024); T** (Treatments); a.i. – Active ingredient; DAT - Days after the first application of the treatments



Source: Embrapa-UFGD Pluviometric Station

Figure 1. Historical daily series of accumulated rainfall and minimum and maximum temperatures in the municipality of Dourados, Mato Grosso do Sul, Brazil for the period from October 1, 2022 to February 28, 2023

the experimental units. Tests were performed for the grain moisture content of each plot. Tests were also performed to evaluate the 1000-grain mass.

Generalized additive models for location, scale, and shape (GAMLSS) (Stasinopoulos et al., 2018; Palharani et al., 2023) were used to perform the deviance analysis. The beta distribution was used for the control variables of *Conyza* spp. and percent phytotoxicity, followed by the logit link function for the location (related to the mean) and scale (related to the dispersion) parameters. For the location parameter, the factors Block, Treatment, DAT, and the Treatment-DAT interaction were considered fixed effects. In addition, the plot, formed by combining blocks with treatment, was entered as a random effect.

For yield analysis, GAMLSS was used with Gamma distribution and log linkage function for the location parameter. For moisture (%), being a proportional scale variable, GAMLSS was adjusted with beta distribution followed by the logit link function of the location parameter. In the deviance analysis, for both variables, treatment was considered a fixed effect and block a random effect. The Shapiro-Wilk test was used to verify the adequacy of the normal distribution to the model residuals. The F-test of the deviance analysis was used to verify the significance of the factors inserted as a fixed effect. The Tukey test was applied to compare the treatment levels. The logistic model was used to adjust the response variables as a function of the days after treatment (DAT). In all tests, a 5% significance level was adopted. All statistical analyses were performed in R software (R Core Team, 2021) with support from the GAMLSS (Rigby & Stasinopoulos, 2005).

Results and Discussion

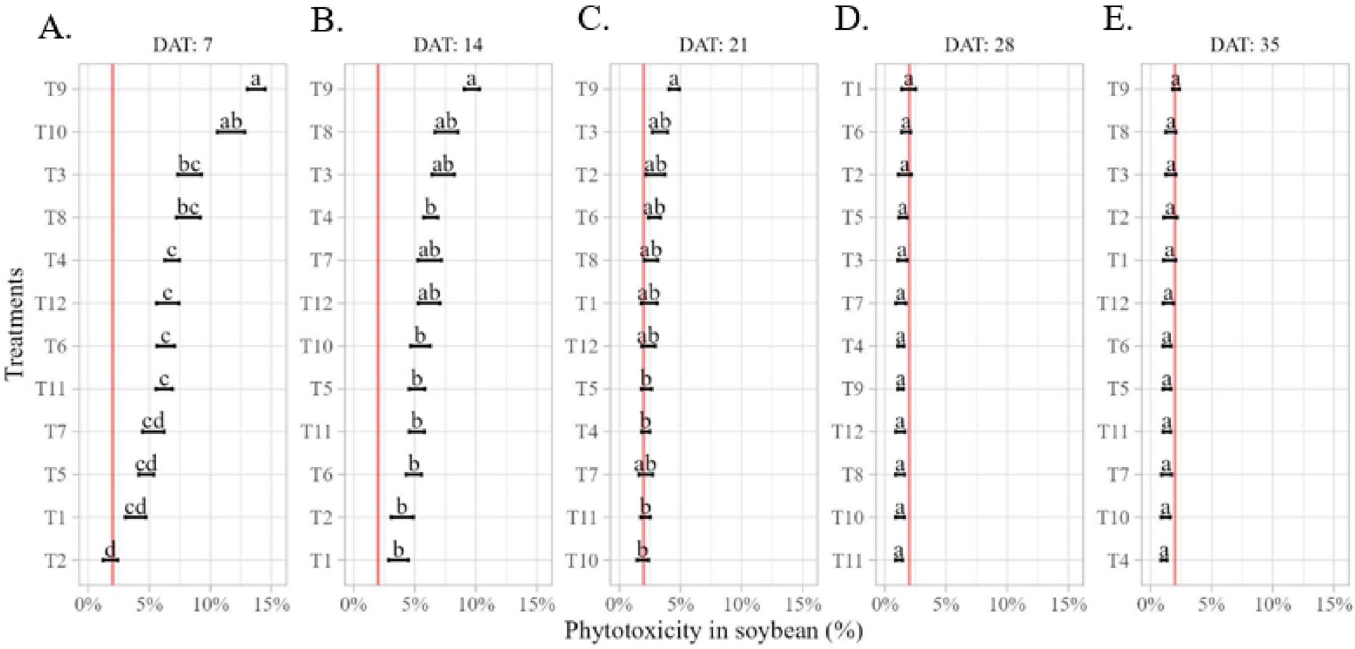
For both variables, *Conyza* spp. control and soybean phytotoxicity, treatment (T), DAT (D), and the T × DAT interaction were significant at 0.05 probability by the F-test of the deviance analysis. The Shapiro-Wilk normality test presented p-values of 0.54 and 0.16 for phytotoxicity and *Conyza* spp. control, respectively, representing a p-value greater than 0.05, indicating that the normal distribution adequately models the residues produced by GAMLSS regression. Regarding the coefficient of variation, for phytotoxicity, a value of 42.68% is observed; this high value is justified because the soybean plants suffered greatly from the initial weed competition, resulting in greater plant variability. For the *Conyza* spp. control, the coefficient of variation was 12.84%, resulting in low variability due to the greater homogeneity of infestation (Table 3).

At 7 DAT, the treatments 2,4-D + bentazon + glyphosate (T9) and 2,4-D + bentazon + glyphosate + glufosinate-ammonium (T10) sequential did not differ from each other in terms of statistical analysis, obtaining percentages of phytotoxicity higher than 12%. These differed from the sequential treatment with 2,4-D + glyphosate + glufosinate-ammonium (T2), which showed phytotoxicity lower than 3% (Figure 2A). The treatments 2,4-D + glyphosate (T1), 2,4-D + chlorimuron-ethyl + glyphosate with or without sequential (T3 and T4), 2,4-D + cloransulam-methyl + glyphosate with or without sequential (T5 and T6), 2,4-D + imazethapyr + glyphosate with or without sequential, and 2,4-D + imazamox + glyphosate with or without glufosinate-ammonium sequential (T11 and T12) showed intermediate phytotoxicity percentages,

Table 3. Results of the fit of the generalized additive models for location, scale, and shape (GAMLSS) models to the variables related to the percentage

Variable	F test			SH	CV
	Treatment (T)	DAT (D)	T x D		
Phytotoxicity	13.42**	610.37**	4.07**	0.54	42.68%
Conyza spp.	9.74**	19.32**	29.52**	0.16	12.84%

**Significant at p ≤ 0.01 by the F test of the deviance analysis; SH - p value of the normality test Shapiro-Wilk; CV - Coefficient of variation; DAT - Days after the first application of the treatments



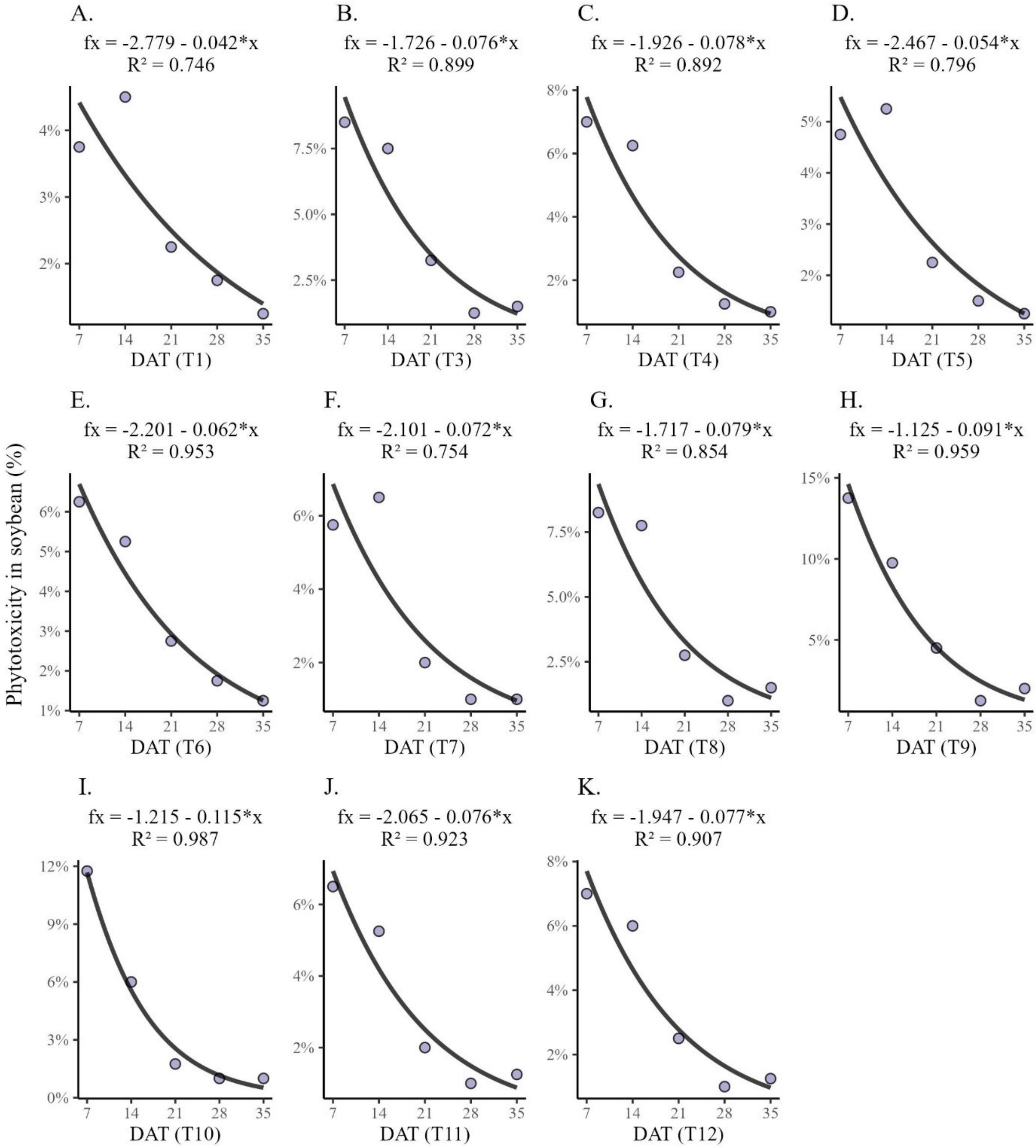
* For details of the treatments, see Table 2. The red line refers to the percentage of 2%. Treatments with the same letters do not differ from each other using the Tukey test (p > 0.05)

Figure 2. Phytotoxicity in soybean (%) as a function of different herbicide combinations at 7 (A), 14 (B), 21(C), 28 (D), and 35 (E) days after the first application of the treatments (DAT)

lower than 9%, and did not differ from each other in terms of statistical analysis, as shown in Figure 2E.

After 14 days of treatment (DAT), the combination of 2,4-D + bentazon + glyphosate (T9) showed the highest phytotoxicity, approximately 10%, differing statistically from other treatments with phytotoxicity below 7%. The treatments 2,4-D + chlorimuron-ethyl + glyphosate (T3), 2,4-D + imazethapyr + glyphosate (T7), and 2,4-D + imazamox + glyphosate (T11) did not differ from each other, with phytotoxicity below 8% (Figure 2B). At 21 DAT (Figure 2C), the treatment 2,4-D + bentazon + glyphosate (T9) presented phytotoxicity close to 10%, differing from the other treatments, which showed percentages lower than 4%. At 28 and 35 DAT, there were no significant differences between treatments (Figure 2D and E).

Regarding the evolution of phytotoxicity over the evaluation days, there was a gradual decrease, representing an involution of the phytotoxic effects, regardless of the treatment. The treatments T1 (R^2 = 0.746; Figure 3A), T3 (R^2 = 0.899; Figure



*For details of the treatments, see Table 2. The points are the mean values, and the smoothed lines (Significant at p ≤ 0.05 by t test) represent the fit of the logistic model, 1/[1+exp(-fx)]

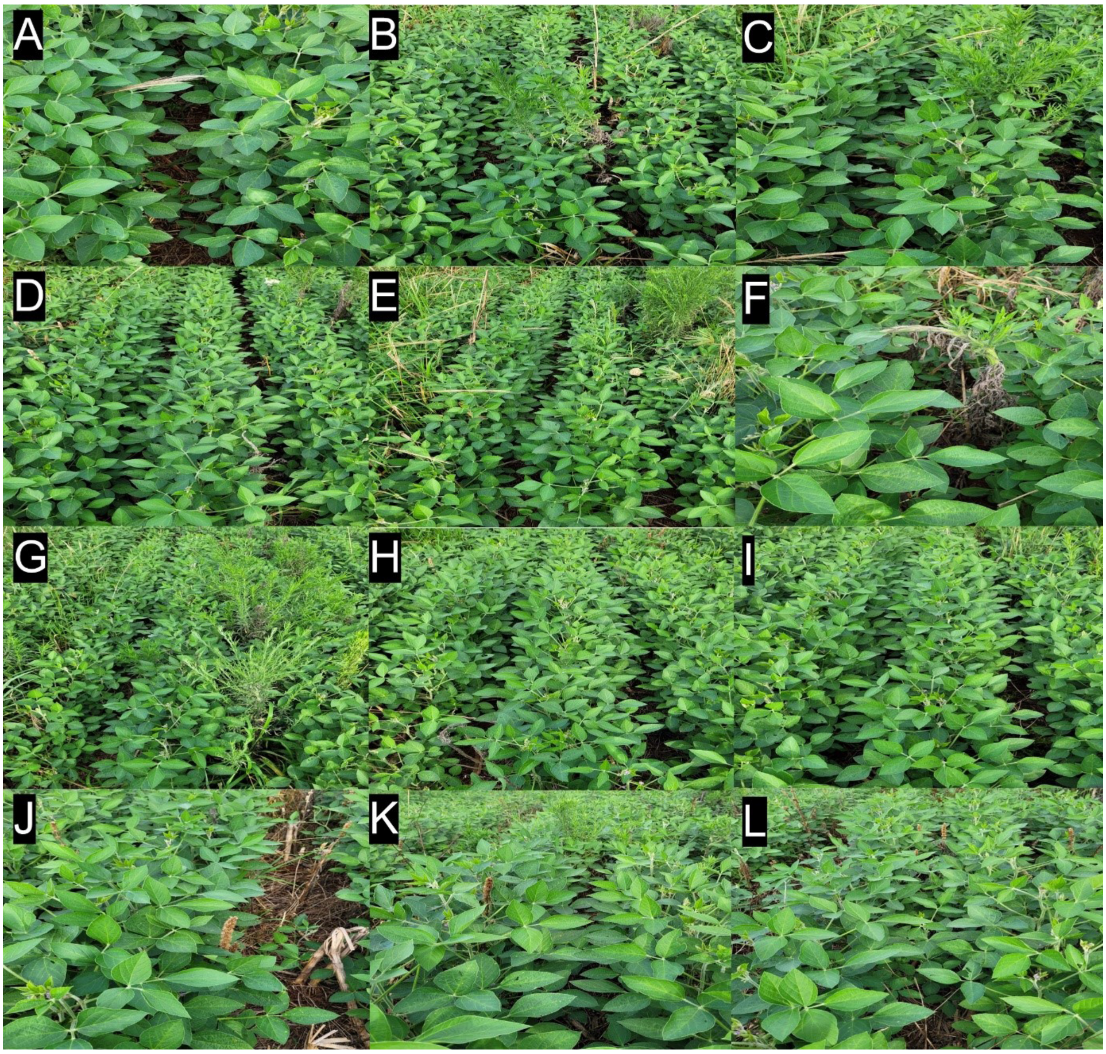
Figure 3. Generalized additive models for location, scale, and shape (GAMLSS) regression with beta distribution and adjusted logit linkage function for phytotoxicity in soybean (%) in relation to days after the first application of the treatments (DAT)

3B), T4 (R^2 = 0.892; Figure 3C), T5 (R^2 = 0.796; Figure 3D), T6 (R^2 = 0.953; Figure 3E), T7 (R^2 = 0.754; Figure 3F), T8 (R^2 = 0.854; Figure 3G), T9 (R^2 = 0.959; Figure 3H), T10 (R^2 = 0.987; Figure 3I), T11 (R^2 = 0.923; Figure 3J), and T12 (R^2 = 0.907; Figure 3K) showed significant results. Moreover, at 35 DAT, no treatment exhibited phytotoxicity above 2% (Figure 3). The adjustment for treatment T2 was not included in Figure 3 due to its low coefficient of determination (R^2 = 0.421), with the predictive equation being -3.084 – 0.032x.

Figure 4 illustrates the effects of various herbicide treatments on soybeans 35 DAT, highlighting the levels of phytotoxicity and the effectiveness of weed control. Treatments T1 (Figure 4A) and T2 (Figure 4B) using 2,4-D + Glyphosate show minimal phytotoxicity with healthy plant growth. Treatments T3 (Figure 4C) and T4 (Figure 4D), which include chlorimuron-ethyl in addition to 2,4-D and glyphosate, exhibit slight stress. T5 (Figure 4E) and T6 (Figure 4F), which combine cloransulam-methyl with 2,4-D and glyphosate, also demonstrate effective weed control with very light phytotoxicity, similar to the results observed in T7 (Figure 4G) and T8 (Figure 4H), where imazethapyr is used instead of cloransulam-methyl.

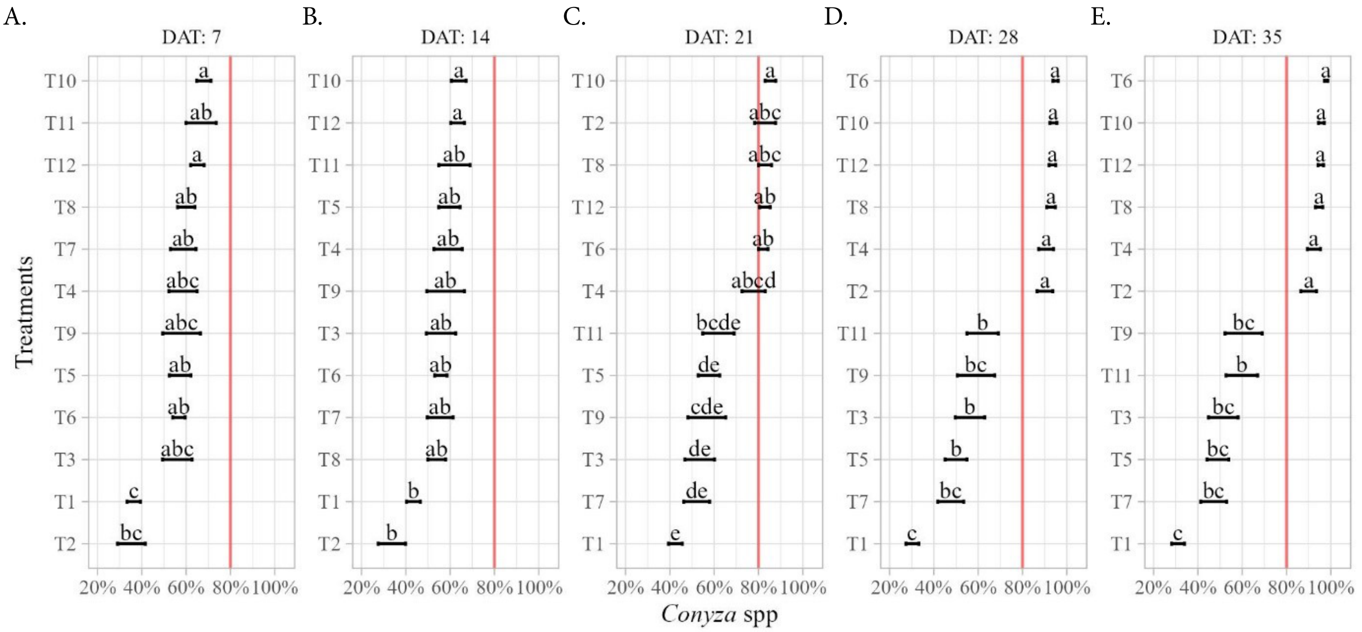
Treatments T9 (Figure 4I) and T10 (Figure 4J), both incorporating bentazon, show good weed suppression with minimal signs of phytotoxicity. T11 (Figure 4K) and T12 (Figure 4L), using imazamox, continue this trend of excellent weed control and minimal soybean damage. In contrast, the untreated control plot (T13, Figure 4M) shows a high density and size of *Conyza* spp. plants. The weeded control (T14, Figure 4N) presents a clean field free from *Conyza* spp.

At 7 DAT and 14 DAT, none of the treatments showed control greater than 80%. The treatments 2,4-D + bentazon + glyphosate + sequential glufosinate-ammonium (T10) and 2,4-D + imazamox + glyphosate + sequential glufosinate-ammonium (T12) were significantly different from the treatments 2,4-D + glyphosate with or without the use of glufosinate-ammonium (T1 and T2) (Figure 5A and 5B). At 21 days after treatment (DAT), all treatments with sequential application of glufosinate-ammonium exceeded 80% of the control. This included the treatments 2,4-D + glyphosate (T1), 2,4-D + chlorimuron-ethyl + glyphosate (T3), 2,4-D + cloransulam-methyl + glyphosate (T5), 2,4-D + imazethapyr + glyphosate (T7), 2,4-D + bentazon + glyphosate (T9), and 2,4-D + imazamox + glyphosate (T11). They did not show



* For details of the treatments, see Table 2.

Figure 4. Visual assessment of phytotoxicity in soybeans following the application of different treatments. The images show the effects observed in the last evaluation, at 35 days after the first application of the treatments (DAT) for treatments A (T1), B (T2), C (T3), D (T4), E (T5), F (T6), G (T7), H (T8), I (T9), J (T10), K (T11), L (T12), M (T13), and N (T14)



*For details of the treatments, see Table 2. The red line refers to the percentage of 2%. Treatments with the same letters do not differ from each other using the Tukey test (p > 0.05)

Figure 5. Control (%) of *Conyza* spp. as a function of different herbicide combinations at 7 (A), 14 (B), 21(C), 28 (D) and 35 (E) days after the first application of the treatments (DAT)

significant differences between them, but they differed statistically from the 2,4-D + glyphosate treatment (Figure 5C).

At 28 and 35 days after treatment (DAT), treatments with sequential application of glufosinate-ammonium, including 2,4-D + glyphosate (T12), 2,4-D + chlorimuron-ethyl + glyphosate (T2), 2,4-D + cloransulam-methyl + glyphosate (T6), 2,4-D + imazethapyr + glyphosate (T8), 2,4-D + bentazon + glyphosate (T10), and 2,4-D + imazamox + glyphosate (T12), showed no significant differences between them, with control superior to 90%. However, they differed statistically from treatments without sequential application of glufosinate-ammonium, 2,4-D + glyphosate (T1), 2,4-D + chlorimuron-ethyl + glyphosate (T3), 2,4-D + cloransulam-methyl + glyphosate (T5), 2,4-D + imazethapyr + glyphosate (T7), 2,4-D + bentazon + glyphosate (T9), and 2,4-D + imazamox + glyphosate (T11), which exhibited less than 70% control (Figure 5D and 5E).

All treatments without sequential glufosinate-ammonium showed a gradual reduction in the percentages of control over the DATs, except for the 2,4-D + bentazon + glyphosate (T10) treatment. None had a percentage higher than 80%.

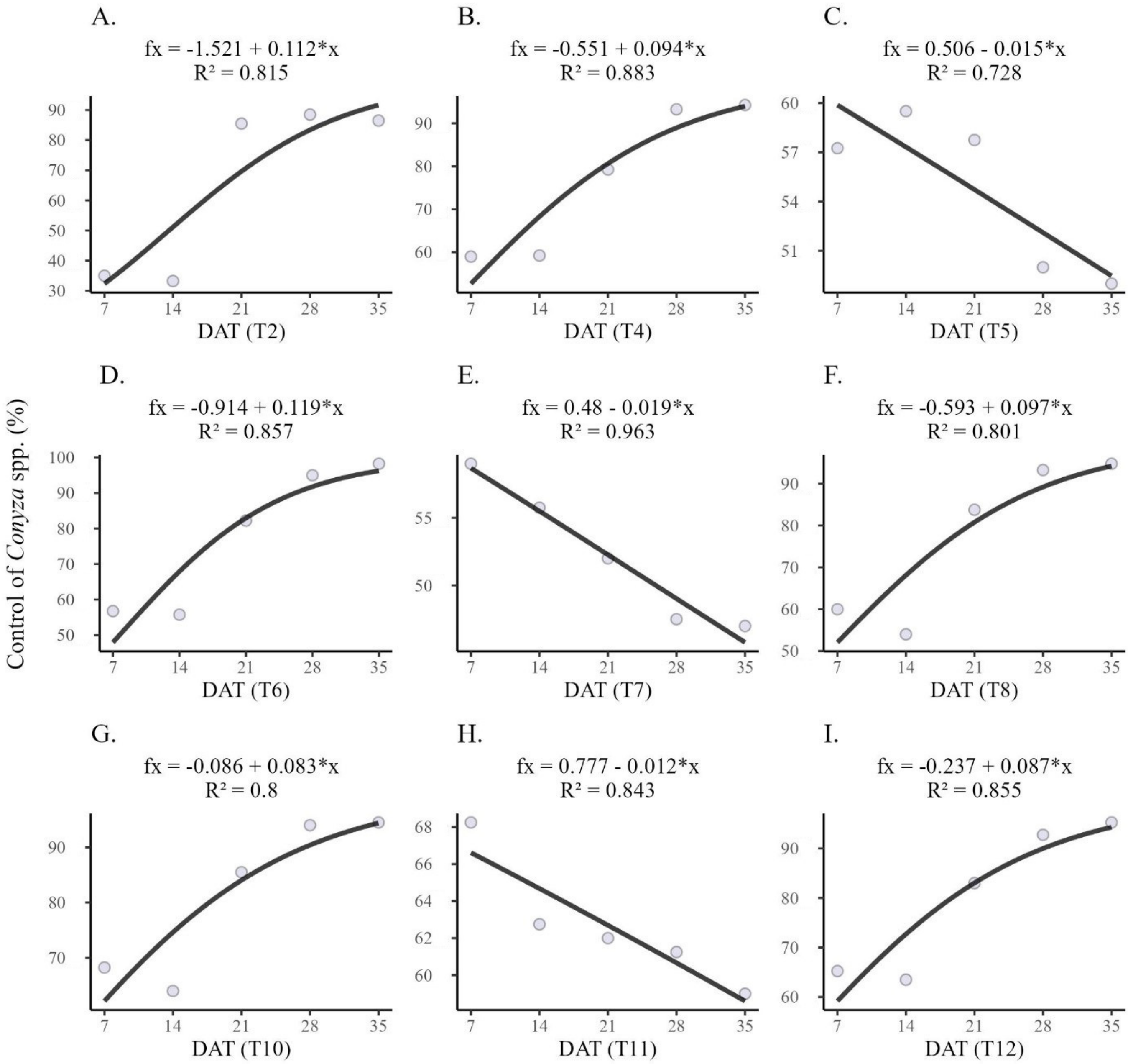
All treatments that received the application of glufosinate-ammonium showed a gradual evolution of the control percentages, and all treatments from 21 DAT already showed a control percentage higher than 80% (Figure 6). The treatments adjusted in the regression analysis with the following R^2 values: T2 ($R^2 = 0.815$; Figure 6A), T4 ($R^2 = 0.883$; Figure 6B), T5 ($R^2 = 0.728$; Figure 6C), T6 ($R^2 = 0.857$; Figure 6D), T7 ($R^2 = 0.963$; Figure 6E), T8 ($R^2 = 0.801$; Figure 6F), T10 ($R^2 = 0.80$; Figure 6G), T11 ($R^2 = 0.843$; Figure 6H), and T12 ($R^2 = 0.855$; Figure 6I). In this Figure, the adjustments for treatments T1, T3, and T9 were not presented, as they had R^2 values less than 0.6, respectively equal to 0.36, 0.501, and 0.460.

For the two variables, soybean phytotoxicity and weed control (*Conyza* spp.), the treatment was significant at 0.05 probability by the F-test of the deviance analysis. The Shapiro-Wilk normality test presented p-values of 0.985 and 0.06 for phytotoxicity and *Conyza* spp. control, respectively, representing a p-value greater than 0.05, indicating that the normal distribution adequately models the residues produced by the GAMLSS regression. For the coefficient of variation, a value of 15.24% was observed for phytotoxicity and 18.16% for *Conyza* spp. control, both indicating low variability (Table 4).

Regarding the herbicide treatments, the treatment 2,4-D + glyphosate + glufosinate-ammonium sequential (T2) showed a grain yield of 4,244.44 kg ha^{-1} and superior performance compared to treatment 2,4-D + chlorimuron-ethyl + glyphosate (T3) with a grain yield of 3,570.37 kg ha^{-1}. When comparing the 2,4-D + chlorimuron-ethyl + glyphosate (T3) treatment with the 2,4-D + chlorimuron-ethyl + glyphosate + glufosinate-ammonium sequential (T4) treatment, the treatment with the sequential application showed higher yield because its percentage of control was higher, causing lower soybean competition with weeds. The lowest yield was observed for the control without weeding with 1,329.62 kg ha^{-1}, demonstrating the impact of the presence of weeds on crop yield (Figure 7A).

All treatments resulted in adequate moisture. The treatments 2,4-D + glyphosate + sequential glufosinate-ammonium (T2) and 2,4-D + imazethapyr + glyphosate (T7) did not differ from each other and exhibited a moisture content higher than 12%, within the range considered suitable for soybeans. However, these treatments differed from 2,4-D + bentazon + glyphosate (T9), with moisture lower than 9%. The other treatments did not differ and showed moisture levels between 10 and 12%.

In the present experiment, the phytotoxic effects were not very expressive in soybeans, being less than 15%. A



*For details of the treatments, see Table 2. he points are the mean values, and the smoothed lines (Significant at $p \leq 0.05$ by t test) represent the fit of the logistic model, 1/[1+exp(-fx)]

Figure 6. Generalized additive models for location, scale, and shape (GAMLSS) regression with beta distribution and adjusted logit linkage function for the control of *Conyza* spp. (%) in relation to the days after the first application of the treatments (DAT)

Table 4. Results of the adjustment of the generalized additive models for location, scale, and shape (GAMLSS) models to the variables related to yield

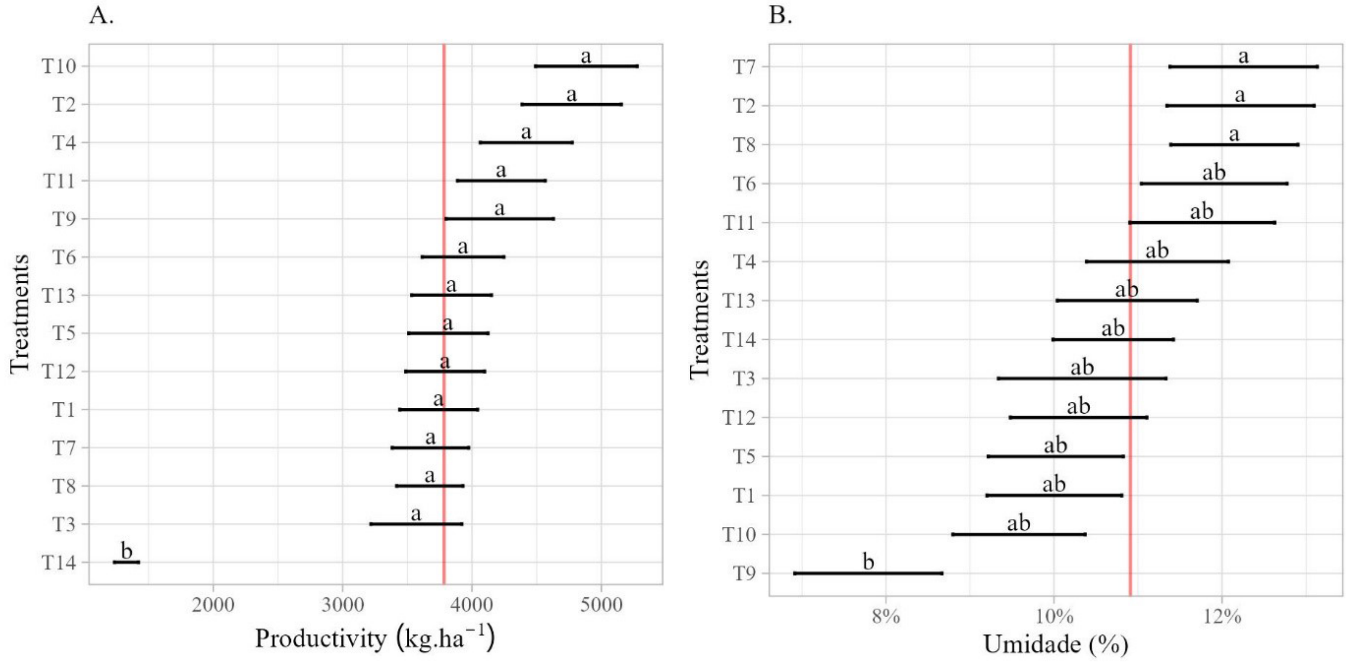
Variable	F test		
	Treatment (F Test)	Shapiro-Wilk test	Coefficient of Variation
Phytotoxicity	13,06**	0,985	15,24%
Conyza spp.	2,02**	0,06	18,16%

**Significant at 0.05 probability by the F test of the deviance analysis; SH - p value of the Shapiro-Wilk normality test; CV - Coefficient of variation

gradual reduction in the percentages of phytotoxicity was also observed, with values below 2% at 35 DAT. These results may be justified because the herbicides chlorimuron-ethyl, cloransulam-methyl, bentazon, imazethapyr, and imazamox are registered for soybean post-emergence and are therefore considered selective (Alencar et al., 2022; AGROFIT, 2024).

Silva et al. (2023) obtained similar results with cloransulam-methyl, chlorimuron-ethyl, bentazon, and imazethapyr in the post-emergence of soybean concerning phytotoxicity. The treatments cloransulam-methyl + glyphosate at three doses (30 + 1080 g a.i. ha^{-1}), (35 + 1080 g a.i. ha^{-1}), (40 + 1080 g a.i. ha^{-1}), and imazethapyr + glyphosate (100 + 1080 g a.i. ha^{-1}) exhibited phytotoxicity lower than 20%. In the case of bentazon + glyphosate (720 + 1080 g a.i. ha^{-1}) and three doses of chlorimuron-ethyl (15 + 1080 g a.i. ha^{-1}), (18 + 1080 g a.i. ha^{-1}), (20 + 1080 g a.i. ha^{-1}) $^{-1}$), the phytotoxicity indexes were close to or greater than 30%. The authors attributed these results to combined stress caused by exposure to herbicides, high temperatures, and water deficit (Albrecht et al., 2020).

However, it is important to note that the phytotoxic effects of herbicides may be associated with the climatic conditions to which the soybean crop was subjected at



*For details of the treatments, see Table 2. The red line indicates the mean observed in the experiment with 3783 kg ha^{-1} and 10.9% moisture. Treatments with the same letters do not differ from each other using the Tukey test ($p > 0.05$)

Figure 7. Multiple comparison results obtained by the Tukey test to compare yield (A) and moisture (B) between treatments

the time of application and during the execution of the experiment, including temperature and rainfall (Figure 1). Environmental stressors can amplify the phytotoxic effects on the crop. During the period from soybean sowing to vegetative development in the present experiment, the crop was not significantly affected by temperature, as the average maximum temperature did not exceed 35 °C.

The crop also did not suffer from water stress because, in October, the accumulated rainfall was 100 mm, favoring the rapid recovery of possible phytotoxic effects. In addition, the accumulated total rainfall during crop development was close to 850 mm, which is higher than the minimum water amount demanded by soybeans, which is 450 to 800 mm (Báez et al., 2020). This association of factors contributed to the gradual decrease in the percentages of soybean phytotoxicity.

The herbicides glyphosate, glufosinate-ammonium, and 2,4-D were selective in soybean, with no significant phytotoxic effects, even though there was no post-emergence record for this crop. This behavior is attributed to the soybean cultivar (B5595CE- ENLIST® technology), which is tolerant to the herbicides 2,4-D, glufosinate-ammonium, and glyphosate (Silva et al., 2021a). In this context, the ENLIST® package represents a gain in the management of weeds in the post-emergence of soybeans because it allows the positioning of previously non-selective herbicides without phytotoxic effects on the crop. Thus, it allows a change in technological advent, altering the positioning of glufosinate-ammonium, previously positioned only in soybean pre-sowing or pre-harvest desiccation.

Albrecht et al. (2020) demonstrated the use of glufosinate-ammonium in soybean pre-sowing to control *Conyza* spp. The author highlighted that the association of glufosinate-ammonium with 2,4-D or saflufenacil + imazethapyr and their sequential placement resulted in greater effectiveness in controlling *Conyza* spp. with sizes larger than 15 cm. Thus, in the event of a short window between pre-sowing desiccation and soybean sowing, the ENLIST® package allows the sequential application of glufosinate-ammonium already in the post-emergence of the crop, bringing a gain in management, allowing a greater efficiency production system in terms of logistics and time.

In the positioning of the 2.4-D for the *Conyza* spp. control, there was a reduction in the percentages of control over the evaluation time for the treatments applied individually. A possible explanation for this behavior is linked to the symptoms of rapid necrosis in the leaves of *Conyza* spp., indicating a possible presence of resistant biotypes in the experimental area. This assumption is based on the 2015 report on biotypes of *Conyza* spp. resistant to 2,4-D in Paraná, which had rapid dissemination biotypes with expressive frequency in Mato Grosso do Sul (HEAP, 2024). This resistance is characterized by the rapid necrosis of the leaf part of the plant, symptoms expressed quickly, approximately 2 hours after application, and regrowth occurring through the axillary buds after three weeks (Queiroz et al., 2020).

Souza et al. (2023) studied resistant and susceptible *Conyza sumatrensis* plants, showing different responses to the 2,4-D application. In the results obtained for the resistant plants, a reduction in the translocation levels of the herbicide was observed, 98.8% of the 2,4-D, which accumulated in the leaf. In the susceptible plant, 13% was translocated by the plant after 96 h of application. Absorption occurred faster in resistant biotypes. Plants resistant to the 2,4-D herbicide did not metabolize it, whereas susceptible plants metabolize it. One of the factors highlighted by the author and the resistance of *Conyza sumatrensis* plants is the necrosis in the leaves, with rapid cell death interfering with the translocation of 2,4 D, not culminating in translocation to the apical meristem of the plant, thus allowing the survival of resistant biotypes.

Another relevant point concerns the control of *Conyza* spp. Until 14 DAT, regardless of the herbicide used, no control percentages higher than 80% were observed, emphasizing the ineffectiveness of single applications, which do not represent a viable management option. Therefore, it is essential to resort to sequential applications to establish satisfactory control. This is because *Conyza* spp. in advanced phenological stages exhibits greater herbicide tolerance and, consequently, lower control efficacy (Schneider et al., 2021).

The performance of herbicides in the *Conyza* spp. control is directly related to its phenological stage, particularly to the height of the plants. Plants less than 10 cm in height are more susceptible and, therefore, provide better control, while those more than 20 cm in height do not respond effectively to herbicides (Silva et al., 2021b). In addition, the advanced phenological stage of *Conyza* spp. results in an increase in the number of trichomes, which hinders the interception of spray droplets by the leaf surface, reducing herbicide absorption (Gazola et al., 2022).

Silva et al. (2021b) conducted an experiment to control *Conyza* spp. in soybean pre-sowing, with applications of 2,4-D + glyphosate, followed or not, by sequential applications, alone or associated with pre-emergent. They observed that the treatment 2,4-D + glyphosate (975 + 1025 g a.i. ha^{-1}) with sequential glufosinate-ammonium (500 g a.i. ha^{-1}) showed satisfactory control of more than 80% as early as 14 DAT, which remained at this level in the following evaluations—demonstrating the value of ENLIST® technology in the positioning of glufosinate-ammonium in soybean post-emergence.

In this experiment, some herbicides that normally result in effective *Conyza* spp. control in soybean post-emergence, such as chlorimuron-ethyl and cloransulam-methyl, were ineffective. However, it is important to emphasize that the satisfactory control of these herbicides is directly related to their application in plants with heights less than 10 cm. In the present study, *Conyza* spp. had a height of more than 20 cm. Blainsk et al. (2015) obtained a control of *Conyza* spp. higher than 80% using applications in the post-emergence of soybean using cloransulam-methyl (25, 30, 35, 40 g a.i. ha^{-1}) and chlorimuron-ethyl (20 g a.i. ha^{-1}). This confirms the efficacy of these treatments when applied in the initial stages of this weed.

Conyza spp., in advanced phenological stages, shows greater herbicide tolerance, consequently resulting in lower control efficacy, requiring sequential application for satisfactory results (Schneider et al., 2021), as observed in the present study. However, it is important to note that late weed control is not ideal, as this practice hinders the deposition of soybean seeds in the soil at the time of planting. Additionally, the initial competition of weeds with the crop compromises the yield potential of the latter (Albrecht et al., 2019). This is because the critical period for preventing interference (CPPI) begins when the crop coexists with weeds, highlighting the importance of more effective and early control strategies.

Conyza spp. plants in advanced stages of development at the time of soybean sowing significantly increase the dispersal potential of their seeds while compromising the effectiveness of herbicides. This results in regrowth after application and, consequently, adverse economic consequences. Infestation by *Conyza* spp. represents a significant challenge in soybean production units, with rates ranging from 40.8 to 49% of the areas, reaching densities of up to 16,207,463 plants ha^{-1}. This infestation generates substantial competition with soybeans, leading to drastic reductions in yield (Lucio et al., 2019). As observed by Albrecht et al. (2019), the interference of *Conyza* spp. in soybean crops results in notable reductions in grain yield - even a moderate infestation, ranging from 0.16 to 0.62 plants m^{-2}, results in significant reductions of 12.54 to 13.72%.

In the context of the dissemination and proliferation of resistant biotypes of *Conyza* spp. and other weeds that are challenging to control, the introduction of the ENLIST® system marks a crucial moment of diversification and the incorporation of new mechanisms of action in soybean post-emergence, with a focus on selectivity. This plays a key role in reducing the incidence of herbicide-resistant biotypes and promoting the rotation of mechanisms of action.

Therefore, the sequential approach that uses glufosinate-ammonium stands out as an additional weed control tool, even in advanced phenological stages, as evidenced in this study. This allowed the effective control of *Conyza* spp. through sequential applications, even when the plants reached heights greater than 20 cm, emphasizing the importance of this study in the management of resistance and challenging weeds.

Conclusion

1. The phytotoxic effects of all treatments on soybeans were low, with an impact of less than 15%. However, the control of *Conyza* spp. after single application was unsatisfactory, with efficacy below 80% at 7 and 14 days after treatment (DAT).

2. In contrast, 21 DAT, the treatments that received sequential applications of glufosinate-ammonium achieved control greater than 80%.

3. Regardless of the herbicides combined with 2,4-D and glyphosate, sequential application significantly increased the control, reaching adequate levels.

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