



# Selectivity of Herbicides Applied Alone or in Tank Mixtures at Different Times of the Day in the Soybean Cultivar DM57i52 IPRO Over Two Growing Seasons

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## Abstract

The application of herbicides, alone or in mixtures, is essential in soybean cultivation, as interactions among chemicals can affect both weed control and crop selectivity. Application timing also matters in these interactions, as sunlight, temperature, and relative humidity vary throughout the day. This study evaluated the selectivity of glyphosate salts, applied alone or mixed with chlorimuron-ethyl, at two times of day, on soybean physiological traits and grain yield components. Experiments were conducted in a randomized block design, in a  $7 \times 2 + 1$  factorial arrangement with four replicates. Factor A consisted of herbicides (chlorimuron-ethyl, glyphosate isopropylamine, ammonium, potassium salts, and their mixtures), and Factor B was application time (6:30 am and 12:30 pm), with a weeded control. Phytotoxicity was assessed 7, 14, 21, 28, 35, and 42 days after treatment (DAT). Physiological traits were measured 28 DAT, and grain yield components at harvest. Chlorimuron-ethyl alone caused the highest visual phytotoxicity, peaking at 32.94%, 7 DAT and declining to 7.67%, 42 DAT. This was reflected in gas exchange, with a 17.1% reduction in photosynthetic rate compared to the control (16.47 vs. 19.86  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). Mixtures of glyphosate salts with chlorimuron-ethyl showed intermediate phytotoxicity (26.25–27.04%, 7 DAT), higher than glyphosate salts alone (maximum 6.56%, 7 DAT) but lower than chlorimuron-ethyl applied individually. These results highlight the influence of herbicide type, mixture, and application timing on soybean selectivity and physiological responses, providing insights for optimizing post-emergence herbicide strategies.

**Keywords** Glycine max · Soybean phytotoxicity · Herbicide mixtures · Weather conditions · Product interactions

## Introduction

Chemical weed control is the most widely used method, primarily due to its practicality, effectiveness, and lower cost compared to other control methods (Travlos et al. 2017). The use of pesticides (fungicides, herbicides and insecticides) in cultivated areas results in high operational costs; therefore, combined applications or tank mixes of differ-

ent products are used for application in most crops. This reduces time loss, fuel costs and plant crushing (Gazziero 2015; Gandini et al. 2020). Approximately 97% of pesticide users, all throughout Brazil, in some occasions end up by mixing six or more products in the sprayer tank to control pests in crops (Gazziero 2015).

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For weed control in soybeans, many producers combine glyphosate with other herbicides to broaden the control spectrum or even to control weeds that are resistant or tolerant to this product. Among the possible mixtures of glyphosate with other herbicides, one example is the chlorimuron-ethyl, an inhibitor of the acetolactate synthase (ALS) enzyme. This increases the control spectrum of problematic weed species that infest soybean fields (Silva et al. 2018).

In addition to herbicides, weather conditions can influence the effectiveness and selectivity of products applied at different times of the day in crops. The ideal conditions for applying pesticides are temperatures below 30 °C, relative humidity above 55% and wind speeds between 3 and 10 km h<sup>-1</sup> (Radons et al. 2022). These conditions are easily found in the early morning and late afternoon, when relative humidity is high and temperatures are lower, making these the most suitable periods for applying pesticides, including herbicides (Rodrigues et al. 2019).

Herbicides containing glyphosate are formulated as potassium, ammonium, and isopropylamine salts. Depending on the formulation, they may be more or less phytotoxic to crops, particularly when tank-mixed with other products. In addition, differences in active ingredient concentration can influence the extent of plant injury. Among these formulations, potassium salt shows greater absorption and translocation in plants compared to other glyphosate salts (Travlos et al. 2017; Zeeshan et al. 2024). These differences justify the evaluation of the selectivity of distinct glyphosate salts when applied to soybean crops.

Selectivity is characterized as a possible response that a given crop presents after the application of an herbicide, with or without the occurrence of damage. It varies according to the species, cultivar, herbicide, dose, mixtures and formulations applied, phenological stage of the plant, climate and soil conditions, among others (Merotto et al. 2015; Palma-Bautista et al. 2020; Gandini et al. 2020). Another possible characterization regarding selectivity is the plant's ability to metabolize the applied herbicide molecule (Nandula et al. 2019; Zhang and Yang 2021).

Herbicides, when applied to crops, can directly or indirectly affect plant growth and development. Some of these effects are changes in physiological and metabolic processes, causing deregulation of defense mechanisms, cellular oxidation, intoxication, changes in nutrient absorption, in addition to causing negative effects on crop yield components (Merotto et al. 2015; Bari et al. 2020).

Regarding the injuries caused by glyphosate salts to soybeans, some studies report that there is a high selectivity of these products to the crop, as no differences were found in the applications of isopropylamine, ammonium and potassium salts on some cultivars (Gonçalves et al. 2017; Andrade et al. 2020). However, when mixed, there are situa-

tions in which glyphosate salts can increase the phytotoxic effects on soybeans, as observed by Maciel et al. (2009). Studies involving the selectivity of glyphosate salts applied alone or in mixture with other herbicides at different times of the day are becoming increasingly important, especially for soybean crops, as many cultivars are launched annually, and also due to the continuous commercialization of new formulations of glyphosate-based herbicides (Palma Bautista et al. 2020; Martins-Gomes et al. 2022).

The results of this study will inform best management practices for weed control in soybean crops using herbicides applied alone or in tank mixtures, aiming to minimize potential phytotoxic effects and, consequently, to maximize grain yield and quality. The hypothesis of this study is that the use of glyphosate salts (isopropylamine, ammonium and potassium salt) associated with chlorimuron-ethyl when applied in the hottest hours of the day are more phytotoxic to soybeans and, consequently, affect plant physiology and grain yield. Therefore, the objective was to evaluate the selectivity of glyphosate salt formulations applied alone or associated with chlorimuron-ethyl, at different times of the day on physiological characteristics and grain yield components of soybeans.

## Materials and Methods

### Description, Location and Treatments Used in the Experiment

Two experiments were conducted under field conditions, in the experimental area of the Federal University of the Southern Frontier (UFFS), Erechim Campus/RS, 27° 43' 31" S, 52° 17' 40" W, 650 m ASL, in the crop seasons of 2019/20 and 2020/21. Two experiments were installed in different years to ensure repetition of the generated data and, thus, greater accuracy of the results.

The soil in the experimental area is classified as Acric Ferralsol (Streck et al. 2018). Soil pH correction and fertilization were carried out according to physical and chemical analyses and following technical recommendations for soybeans (CQFS-RS/SC). The chemical and physical characteristics of the soil are presented in Table 1.

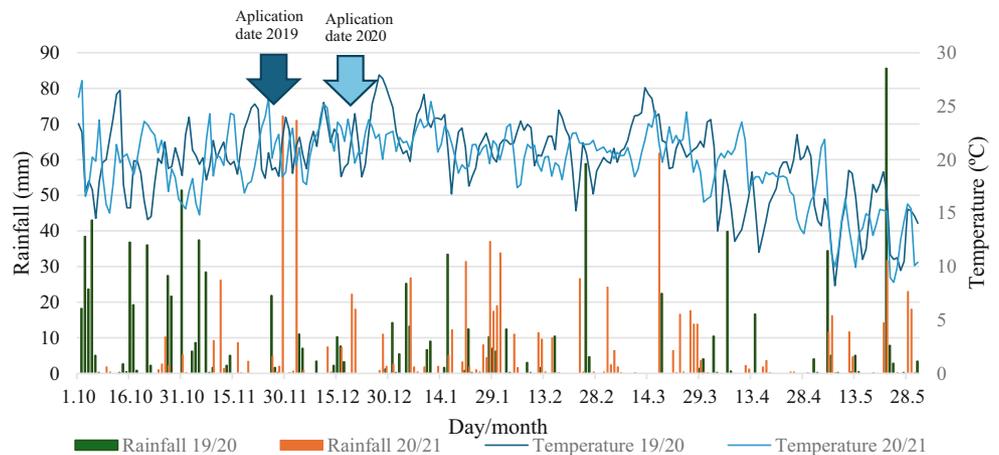
The region's climate is classified as Cfa (Köppen-Geiger), characterized as a humid subtropical climate with hot summers. It receives uniformly distributed precipitation, ranging from 1100 to 2000 mm annually, and the average temperature in the warmest month is below 22 °C. Additionally, the area experiences severe and frequent frosts, with an average occurrence of 10 to 25 days per year (Peel et al. 2007). Meteorological conditions, such as relative air humidity (%), precipitation (mm) and temperature (°C),

**Table 1** Chemical analysis of the Acric Ferralsol soil used in the experiment.

Sampled layer	pH Water	P mg dm <sup>-3</sup>	K <sup>+</sup>	H + Al Cmol <sub>c</sub> dm <sup>-3</sup>	Al <sup>3+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>
0–10 cm	4.8	6.3	106	8.7	0.9	5.1	3.3
SB	CTC (t)	CTC (T)	v	OM	Clay	Sand	Silt
			%				
8.67	10.7	18.5	53	8.4	3.3	72	22.9

SB Sum of bases; v base saturation; OM organic matter; CTC (t) cation exchange capacity effective; CTC ( $T_{pH=7.0}$ ) cation exchange capacity

**Fig. 1** Precipitation (mm) and temperature (°C) during the period of conducting the experiments from October to May 2019 to 2021



recorded during the period of experiments can be observed in Fig. 1.

The two experiments were conducted using a randomized block design in a factorial arrangement ( $7 \times 2 + 1$ ), with four replicates, during two crop seasons (2019/20 and 2020/21). Factor A consisted of the herbicide treatments (chlorimuron-ethyl, glyphosate isopropylamine salt, glyphosate ammonium salt, glyphosate potassium salt, and the mixtures of each glyphosate salt with chlorimuron-ethyl), while Factor B comprised the application times (6:30 am and 12:30 pm), in addition to a manually weeded control, as shown in Table 2. The control treatment without herbicide application was kept free of weed infestation by manual hoeing once a week throughout the entire crop cycle.

Each experimental unit was  $3 \times 5$  m (width and length), totaling an area of  $15 \text{ m}^2$ , with six seeding lines spaced 0.5 m apart. The useful area ( $6 \text{ m}^2$ ) corresponded to the four central lines, discarding the lateral borders (one line on each side of the plots) and the frontal borders (1 m at the beginning and at the end of the plots).

The experiments were sown using a seeder/fertilizer machine on October 24, 2019, and November 13, 2020, for the first and second crop years, respectively. The soybean cultivar DM57i52 IPRO (DONMARIO 57i52 IPRO), maturity group 5.7, medium size, glyphosate-resistant, was sown, in both crop seasons, at an average density of 28 seeds  $\text{m}^{-2}$ , which resulted in an approximate density of 280,000 seeds

$\text{ha}^{-1}$ . For initial fertilization,  $375 \text{ kg ha}^{-1}$  of the formula 05-20-20 ( $\text{N-P}_2\text{O}_5\text{-K}_2\text{O}$ ) were applied.

The herbicide treatments were applied when soybean plants were at the V3–V4 growth stage, on November 22, 2019, and December 22, 2020 (Table 3). Applications were performed using a  $\text{CO}_2$ -pressurized precision backpack sprayer (Névoa, São Paulo, Brazil) equipped with four TTeJet (XR TeeJet®, XR110/02-VP) flat-fan spray tips, operating at a constant pressure of 210 kPa and a travel speed of  $3.6 \text{ km h}^{-1}$ . This configuration provided an application volume of  $150 \text{ L ha}^{-1}$ , with a volumetric median diameter (VMD) of approximately  $160 \mu\text{m}$ . The spray was directed downward, using a single jet per nozzle. The water used for herbicide application had a pH between 6.0 and 6.5 and total hardness greater than  $3 \text{ mg CaCO}_3 \text{ L}^{-1}$ . Meteorological conditions at the time of application are presented in Table 3. The value of 100% luminosity recorded at both application times (6:30 am and 12:30 pm) refers to a qualitative assessment of sky conditions. On the days of herbicide application, the sky was completely clear, with no cloud cover, and therefore luminosity under this condition was classified as 100%, indicating full, unobstructed daylight conditions.

## Variables Evaluated

The phytotoxicity assessments of herbicides on soybean plants were carried out at 7, 14, 21, 28, 35 and 42 days

**Table 2** Treatments, active ingredient (a.i.) or acid equivalent (a.e.) rates, commercial products, doses (kg or L ha<sup>-1</sup>), and application times of herbicides used in experiments with the soybean cultivar DM57i52 IPRO. UFFS, Erechim, RS, Brazil, 2019/20 and 2020/21.

Treatments	Rates (g ha <sup>-1</sup> of i.a or e.a)	Commercial name	Dose (kg or L ha <sup>-1</sup> )	Application times hours
Weeded control	...	–	...	6:30 am and 12:30 pm
Chlorimuron-ethyl	20*	Classic	0.08	
Glyphosate isopropylamine salt	1440**	Nufosate	3.00	
Glyphosate ammonium salt	1440**	Roundup WG	1.82	
Glyphosate potassium salt	1440**	Zapp QI 620	2.32	
Glyphosate isopropylamine salt + chlorimuron	1440 + 20	Nufosate + Classic	3.00 + 0.08	
Glyphosate ammonium salt + chlorimuron	1440 + 20	Roundup WG + Classic	1.82 + 0.08	
Glyphosate potassium salt + chlorimuron	1440 + 20	Zapp QI 620 + Classic	2.32 + 0.08	

\* and \*\* Amount of active ingredient and acid equivalent, respectively, applied per hectare of the herbicides

**Table 3** Meteorological conditions during the application of herbicides at different times in soybean crops in the 2019/20 and 2020/21.

Crop year	<b>06:30 am</b>						
	Application date	Luminosity (%)	Temperature °C		Relative humidity (%)	Soil conditions	Wind speed (km h <sup>-1</sup> )
			Air	Soil			
2019/20	22/11/2019	100	29.2	23.9	59.0	Moist	3.5
2020/21	22/12/2020	100	28.0	27.4	76.0	Moist	1.4
Crop year	<b>12:30 pm</b>						
	Application date	Luminosity (%)	Temperature (°C)		Relative humidity (%)	Soil conditions	Wind speed (km h <sup>-1</sup> )
			Air	Soil			
2019/20	22/11/2019	100	33.2	25.3	33.9	Moist	4.1
2020/21	22/12/2020	100	33.0	28.4	32.0	Moist	6.0

after application of treatments (DAT), assigning percentage scores, being zero (0%) the absence of injuries and one hundred (100%) the death of the plants (Velini et al. 1995).

After 28 DAT, gas exchange evaluations in plants were performed using an infrared gas analyzer (IRGA), model LCpro-SD (Analytical Development Co. BioScientific Ltd, Hoddesdon, UK). The variables evaluated were: internal CO<sub>2</sub> concentration (C<sub>i</sub>–μmol mol<sup>-1</sup>), transpiration coefficient (E–mol m<sup>-2</sup> s<sup>-1</sup>), gas conductance (G<sub>s</sub>–mol m<sup>-2</sup> s<sup>-1</sup>), and photosynthetic activity (A–μmol m<sup>-2</sup> s<sup>-1</sup>). Water use efficiency (WUE–mol CO<sub>2</sub> mol H<sub>2</sub>O<sup>-1</sup>) and carboxylation efficiency (EC–mol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) were obtained as follows:

$$WUE = \frac{A}{E}$$

$$EC = \frac{A}{C_i}$$

where WUE=water use efficiency (mol CO<sub>2</sub> mol H<sub>2</sub>O<sup>-1</sup>); A=photosynthetic activity (μmol m<sup>-2</sup> s<sup>-1</sup>); E=transpiration coefficient (mol m<sup>-2</sup> s<sup>-1</sup>); and C<sub>i</sub>=internal CO<sub>2</sub> concentration (μmol mol<sup>-1</sup>).

Each experimental block was evaluated under natural lighting for one day, between 7 and 11 am, under open sky, so that the environmental conditions were maintained homogeneous during the analyses. The chlorophyll index was measured with a portable chlorophyll meter model SPAD 502—Plus, Pequim, China, with measurements being made at five points on each plant, randomly on the lower, middle and upper leaves of the canopy.

Shortly before harvest, ten soybean plants were randomly collected in the useful area of each plot to evaluate the number of grains per pod, number of pods and number of grains per plant. After manual harvesting and threshing of the soybeans in an area of 6 m<sup>2</sup>, the weight of a thousand grains (g) and grain yield (kg ha<sup>-1</sup>) were determined. The weight of a thousand grains was measured by counting eight samples of 100 grains each, which were successively weighed on an analytical balance (Shimadzu, Brazil). For the analyses, grain moisture was standardized to a content of 13% and the yield data were extrapolated to kg ha<sup>-1</sup>.

## Statistical Analysis

The data were analyzed jointly for the two crop seasons in order to reduce the effect of year-to-year variation on the experimental results. Prior to analysis, the data were subjected to tests of normality and homogeneity of variances. When necessary, data were transformed to meet the assumptions of the analysis of variance, although the original means are presented for clarity.

The experiment followed a randomized block design in a factorial arrangement ( $7 \times 2 + 1$ ), in which herbicide treatments (Factor A), application times (Factor B), and their interaction ( $A \times B$ ) were considered fixed effects, and blocks were considered random effects. Analysis of variance (ANOVA) was performed using the F-test at a 5% significance level ( $p \leq 0.05$ ). When significant differences were detected, treatment means were compared using the Scott-Knott test at  $p \leq 0.05$ . All statistical analyses were performed using Sisvar software, version 5.6 (Ferreira 2011).

## Results and Discussion

Significant interactions between the tested factors (herbicides  $\times$  application times) were observed for phytotoxicity at 28 days after treatment application (DAT), transpiration rate, stomatal conductance, photosynthetic rate, carboxylation efficiency, chlorophyll index, number of pods and grains per plant, thousand-grain weight, and grain yield. For phytotoxicity assessed at 7, 14, 21, 35 and 42 DAT, as well as for the number of grains per pod, only a significant main effect of herbicides was detected. The remaining variables evaluated, namely internal  $\text{CO}_2$  concentration ( $C_i$ ) and water use efficiency (WUE), were not significantly affected by the factors, either individually or through their interaction; therefore, their results are not presented.

The lack of response in  $C_i$  and WUE, despite changes in photosynthesis and transpiration, may be explained by compensatory physiological mechanisms that maintained the balance between  $\text{CO}_2$  diffusion into the leaf and its assimilation by the Calvin cycle (Coêlho et al. 2024). This indicates that, although herbicides and application times altered gas exchange rates, they did not significantly disrupt the internal  $\text{CO}_2$  concentration or the proportional relationship between carbon assimilation and water loss (Bari et al. 2020), which justifies the absence of statistically detectable differences for these variables.

### Phytotoxicity of Herbicides and Application Times in Soybeans

The results indicate that the isolated application of chlorimuron-ethyl was more phytotoxic from 7 to 42 DAT com-

pared to the other treatments (Tables 4 and 5). However, over time, the damage caused by chlorimuron-ethyl decreased, reaching 42 DAT with an index of 7.67%.

Generally, phytotoxicity levels of 10 to 20% visible leaf damage are tolerable, as these injuries typically do not negatively affect soybean grain yield (Carvalho et al. 2022). Phytotoxicity rates in soybeans may vary depending on herbicide type, dose and formulation applied, plant development stage, environmental and soil conditions, tank mixes with other pesticides or foliar fertilizers, application time and other factors (Maciel et al. 2009; Merotto et al. 2015; McConwn et al. 2018; Arsenijevic et al. 2025).

It was observed that the use of glyphosate salts (isopropylamine, ammonium and potassium) applied alone to soybeans presented the lowest symptoms of phytotoxicity in all evaluated periods, from 7 to 42 DAT (Tables 4 and 5). In a study in which different formulations of glyphosate (isopropylamine and potassium salt) were applied, Santos et al. (2007) found few symptoms of phytotoxicity in soybeans. This is due to their resistance to this herbicide and their ability to metabolize it, similarly to what was observed in this study.

When glyphosate salts were applied in combination with chlorimuron-ethyl, phytotoxicity was consistently lower than that observed with chlorimuron-ethyl applied alone in all evaluations. This suggests a potential antagonistic interaction between the herbicides, in which glyphosate may reduce the uptake or activity of chlorimuron-ethyl, thereby mitigating its phytotoxic effects. Additionally, by 42 days after treatment (DAT), the visible symptoms of damage had decreased to less than 8% (Tables 4 and 5). Over time, the soybean plants were able to metabolize the herbicides, whether applied alone or in mixtures, with minimal symptoms. This recovery is attributed to the natural growth processes of the plants and their ability to activate detoxification mechanisms. According to Frene et al. (2018), plants have defense mechanisms that allow the metabolization of herbicides, which, over time, results in less injury to crops and corroborates the results observed in this study.

Although tank mixes can, in certain situations, cause phytotoxicity in crops, farmers choose this alternative to control pests—such as diseases, insects and weeds—thanks to the savings in resources, less trampling and agility of operations (Petter et al. 2013; Gazziero 2015; Gandini et al. 2020; Arsenijevic et al. 2025). However, when mixing herbicides, it is essential to know their potential effects on crops and to evaluate their effectiveness in controlling weeds. These mixtures can generate negative synergistic effects or act additively, causing high phytotoxicity, which can reduce grain yield or compromise adequate control of infesting pests (Gazziero 2015; Gandini et al. 2020; Symington et al. 2024).

**Table 4** Mean phytotoxicity (%) in the soybean cultivar DM57i52 IPRO as a function of the application of different herbicides at two times, in the 2019/20 and 2020/21 harvests.

Treatments	Phytotoxicity (%)				
	7 DAT <sup>1</sup>	14 DAT	21 DAT	35 DAT	42 DAT
Weeded control	0.00 d*	0.00 f	0.00 d	0.00 c	0.00 c
Chlorimuron-ethyl	32.94 a	29.40 a	24.79 a	14.42 a	7.67 a
Glyphosate isopropylamine salt	4.88 c	4.56 e	6.04 c	3.79 b	0.88 c
Glyphosate ammonium salt	5.54 c	5.79 e	8.63 c	3.17 b	2.25 b
Glyphosate potassium salt	6.56 c	5.63 e	4.81 c	3.94 b	2.31 b
Glyphosate isopropylamine salt + chlorimuron	26.25 b	13.77 d	13.94 b	3.50 b	2.89 b
Glyphosate ammonium salt + chlorimuron	27.04 b	23.50 b	17.19 b	6.56 b	4.06 b
Glyphosate potassium salt + chlorimuron	24.21 b	18.94 c	10.92 c	4.94 b	2.50 b
CV(%)	43.49	45.41	63.28	67.93	88.02
Overall Means	15.93	12.69	10.79	5.04	2.82
F <sub>herbicides</sub>	53.71	50.57	23.60	25.49	13.82
F <sub>times</sub>	0.53	0.43	9.75	0.19	0.04
F <sub>herbicides × times</sub>	0.62	0.55	1.83	1.94	1.03
P <sub>herbicides</sub>	0.00001	0.00001	0.00001	0.00001	0.00001
P <sub>times</sub>	0.47	0.52	0.06	0.67	0.85
P <sub>herbicides × times</sub>	0.74	0.80	0.09	0.07	0.42

F analysis of variance. P probability

<sup>1</sup> Days after applying treatments

\* Means followed by the same letter in the column do not differ from each other, according to the Scott-Knott test ( $p \leq 0.05$ )

The results indicate that the isolated application of chlorimuron-ethyl increased phytotoxicity in soybeans by 78.94% and 47.68% when compared, respectively, to glyphosate salts and the tank mix of herbicides, considering the average of the evaluations carried out at 7, 14, 21, 28, 35 and 42 DAT (Tables 4 and 5). The mixture of glyphosate salts with chlorimuron-ethyl showed an increase of 59.57% in phytotoxicity in relation to the isolated use of the salts, also considering the average of all evaluations. Recent studies corroborate these findings. Cesco et al. (2018) reported that mixtures of glyphosate with chlorimuron-ethyl and other herbicides increased phytotoxicity in RR2 soybeans and, in some cases, reduced productivity in experiments conducted in Paraná. Nonemacher et al. (2017), observed phytotoxicity of 62–67% when glyphosate was mixed with sulfometuron + chlorimuron-ethyl at 14 and 21 DAT. On the other hand, Da Silva et al. (2023) reported that the use of high rates of chlorimuron-ethyl in mixture with glyphosate caused phytotoxicity of around 30%; however, these injuries did not result in negative effects on soybean grain yield. It is worth noting that the different responses observed among studies regarding herbicide applications in soybean, with greater or lesser levels of phytotoxicity and consequent effects on grain yield, may be related to the cultivar used and the location where the study was conducted, as differences in climate, soil conditions, and management practices can substantially influence crop recovery following herbicide application.

It was observed that the isolated application or tank mix of chlorimuron-ethyl in soybean plants increases phytotoxicity, especially between 7 and 35 DAT, reaching acceptable levels after this period, without significantly compromising grain yield (Tables 4 and 5). Due to the young stage of the plants, the metabolization of the herbicide occurs more slowly, requiring time for the symptoms of phytotoxicity, especially those caused by chlorimuron-ethyl, to be eliminated. However, it was found that the phytotoxicity of chlorimuron-ethyl, whether applied alone or in mixtures with glyphosate salts, decreased, since the crop did not show losses in grain yield, except in the treatment carried out at 12:30 pm with glyphosate ammonium salt + chlorimuron-ethyl. Vidrine et al. (2002) and Albrecht et al. (2018) reported phytotoxicity levels between 4 and 30% with the application of chlorimuron-ethyl, isolated or associated with glyphosate, and found that the crop recovered from the damage over time, without compromising grain yield, corroborating the results observed in this research.

Herbicide application can cause temporary or permanent stress in plants, depending on the physicochemical characteristics of the product, on the crop (whether cultivar or hybrid, considering the development stage, nutrition and water stress) and/or on environmental conditions (Carvalho et al. 2009, 2022; Grzanka et al. 2022). Temporary stress allows the plant to recover quickly from damage, reestablishing its initial growth rate—with or without yield losses—although significant changes occur in the crop cycle (Carvalho et al. 2009; Arsenijevic et al. 2025). In contrast, permanent stress

**Table 5** Phytotoxicity (%) at 28 days after application (DAT) and effect of herbicides on the physiological variables, transpiration rate (E, mol m<sup>-2</sup> s<sup>-1</sup>), stomatal conductance (GS, mol m<sup>-2</sup> s<sup>-1</sup>), photosynthetic rate (A, μmol m<sup>-2</sup> s<sup>-1</sup>), carboxylation efficiency (EC, mol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) and chlorophyll index (SPAD), as a function of application times in the soybean cultivar DM57i52 IPRO, in the 2019/20 and 2020/21 crop years.

Treatments	Herbicide application times—hours					
	06:30 am	12:30 pm	06:30 am	12:30 pm	06:30 am	12:30 pm
	Phytotoxicity 28 DAT <sup>l</sup>		Transpiration		Conductance	
Weeded control	0.00 Ad*	0.00 Ad	3.58 Aa	3.58 Aa	0.43 Aa	0.43 Aa
Chlorimuron-ethyl	15.89 Ba	28.33 Aa	2.85 Ab	2.75 Ab	0.39 Ab	0.40 Aa
Glyphosate isopropylamine salt	5.67 Ac	5.75 Ac	3.54 Aa	3.15 Ba	0.43 Aa	0.38 Bb
Glyphosate ammonium salt	3.63 Ac	3.25 Ad	3.14 Ab	3.36 Aa	0.43 Aa	0.45 Aa
Glyphosate potassium salt	4.63 Ac	6.13 Ac	3.56 Aa	3.02 Bb	0.45 Aa	0.42 Aa
Glyphosate isopropylamine salt + chlorimuron	8.00 Ab	5.83 Ac	3.32 Ab	2.98 Ab	0.38 Ab	0.38 Ab
Glyphosate ammonium salt + chlorimuron	9.50 Bb	17.50 Ab	3.09 Ab	3.35 Aa	0.44 Aa	0.40 Aa
Glyphosate potassium salt + chlorimuron	10.75 Ab	6.33 Bc	3.09 Ab	2.70 Bb	0.45 Aa	0.35 Bb
CV(%)	51.62		12.22		11.54	
Overall Means	7.81		3.19		0.41	
F <sub>herbicides × times</sub>	2.96		2.37		2.52	
P <sub>herbicides × times</sub>	0.007		0.03		0.02	
Treatments	Herbicide application times—hours					
	06:30 am	12:30 pm	06:30 am	12:30 pm	06:30 am	12:30 pm
	Photosynthesis		Carboxilation		Chlorophyll index	
Weeded control	19.86 Aa	19.86 Aa	0.08 Aa	0.08 Aa	41.58 Ab	41.58 Ac
Chlorimuron-ethyl	16.47 Ab	17.25 Ab	0.06 Ab	0.06 Ab	42.30 Aa	42.45 Ab
Glyphosate isopropylamine salt	18.94 Aa	18.10 Ab	0.07 Aa	0.07 Aa	41.38 Bb	43.27 Aa
Glyphosate ammonium salt	19.16 Aa	18.84 Aa	0.07 Aa	0.07 Aa	41.20 Ab	40.60 Ad
Glyphosate potassium salt	20.29 Aa	17.50 Bb	0.08 Aa	0.06 Bb	42.33 Aa	40.50 Bd
Glyphosate disopropylamine salt + chlorimuron	17.52 Ab	17.98 Ab	0.06 Ab	0.07 Aa	41.15 Ab	41.55 Ac
Glyphosate ammonium salt + chlorimuron	19.32 Aa	18.83 Aa	0.07 Aa	0.07 Aa	41.35 Bb	42.45 Ab
Glyphosate potassium salt + chlorimuron	19.77 Aa	15.27 Bc	0.07 A a	0.06 Bb	40.73 Ab	39.83 Bd
CV(%)	9.11		12.85		1.83	
Overall Means	18.43		0.07		41.49	
F <sub>herbicides × times</sub>	4.57		2.75		9.56	
P <sub>herbicides × times</sub>	0.0002		0.01		0.00001	

F analysis of variance. P probability

\* Means followed by different capital letters in the row and by different lowercase letters in the column differ from each other, according to the T-test and Scott-Knott test, respectively ( $p \leq 0.05$ )

results in a lower growth rate, increasing the probability of losses (Carvalho et al. 2009; Arsenijevic et al. 2025).

The use of chlorimuron-ethyl and the mixtures of glyphosate ammonium salt + chlorimuron-ethyl at 12:30h, as well as glyphosate potassium salt + chlorimuron-ethyl at 6:30h, resulted in the highest phytotoxicity scores at 28 DAT, considering the different application times for each treatment (Table 5). The highest phytotoxicity scores observed in treatments applied at 12:30h are probably associated with intense heat stress at that time, which enhances the damage of herbicides to plants. On the other hand, the accentuated phytotoxicity in treatments applied at 6:30h may be related to better hydration of plant cuticles, favoring the absorption of herbicides. Environmental factors such as relative humidity, time of exposure to light and temperature

can directly influence the efficacy and effects of herbicides on plants (Grzanka et al. 2022; Silva et al. 2022; Coêlho et al. 2024). Similar results were also reported by Coêlho et al. (2024).

### Effect of Herbicides and Application Times On Soybean Physiological Variables

The transpiration rate (E), stomatal conductance (GS), photosynthetic rate (A) and carboxylation efficiency (CE) were lower when chlorimuron-ethyl was applied alone or in mixtures with glyphosate isopropylamine salt at 6:30h (Table 5). On the other hand, the isolated application of glyphosate ammonium salt resulted in higher values of E, GS, A and CE when performed at 12:30h. For the other

**Table 6** Internal CO<sub>2</sub> concentration (Ci) and water use efficiency (WUE) at 28 days after herbicide application (DAT) as affected by different herbicides and application times in the soybean cultivar DM57i52 IPRO, during the 2019/20 and 2020/21 growing seasons.

Treatments	Herbicide application times—hours			
	06:30 am		12:30 pm	
	CO <sub>2</sub> concentration		Water use efficiency	
Weeded control	59.83 <sup>ns</sup>	259.83	6.50 <sup>ns</sup>	6.50
Chlorimuron-ethyl	266.17	270.25	6.14	6.74
Glyphosate isopropylamine salt	264.50	263.50	6.13	6.27
Glyphosate ammonium salt	259.50	273.17	6.49	6.66
Glyphosate potassium salt	258.00	271.25	6.65	6.59
Glyphosate isopropylamine salt + chlorimuron	265.75	260.50	6.22	6.71
Glyphosate ammonium salt + chlorimuron	261.41	269.25	6.85	6.81
Glyphosate potassium salt + chlorimuron	269.50	262.67	7.13	6.18
CV(%)	4.85		13.27	
Overall Means	264.69		6.53	
F <sub>herbicides</sub>	0.61		0.73	
F <sub>times</sub>	2.01		0.08	
F <sub>herbicides × times</sub>	1.50		1.17	
P <sub>herbicides</sub>	0.75		0.64	
P <sub>times</sub>	0.15		0.78	
P <sub>herbicides × times</sub>	0.18		0.33	

<sup>ns</sup> There was no significant effect ( $p \leq 0.05$ ) of herbicide application, application time, or the interaction between factors (herbicides × application times). *F* analysis of variance. *P* probability

treatments, there was a variation in the values of the physiological variables: in some cases, the application at 6:30 h had a positive effect, while in others, the use of herbicides at 12:30 h showed better results. This alternation in the effects of the applications may be associated with plant defense mechanisms. According to Silva et al. (2022), soybean plants subjected to adequate climatic conditions—without water stress and with an ideal amount of light—present higher CO<sub>2</sub> concentrations and higher photosynthetic rates. In contrast, plants under stress reduce their metabolism, directly impacting physiological variables.

When comparing the application times within each treatment, it was observed that the application of glyphosate potassium salt + chlorimuron-ethyl at 12:30 h resulted in lower values of E, GS, A and CE compared to the application at 6:30 h (Table 5). This effect may be associated with the decrease in stomatal opening and the reduction in photosynthetic activity under stress or low light conditions. Similar results were obtained by Manabe et al. (2014), in a trial to evaluate the application of herbicides in common bean plants and in weeds competing with this crop, and Coêlho et al. (2024), where midday applications of desiccant herbicides significantly suppressed photosynthetic pigments and physiological parameters, compared to early-day applications—highlighting how timing influences herbicide-induced stress responses on photosynthesis.

No statistical differences were observed between the application times (6:30 am and 12:30 pm) for the isolated use of chlorimuron-ethyl, glyphosate ammonium

salt and for the tank mixes of glyphosate isopropylamine salt + chlorimuron-ethyl and glyphosate ammonium salt + chlorimuron-ethyl, considering the GS and CE variables (Table 5). This result may be related to the fact that these variables depend directly on the opening and closing of the stomata, as previously discussed.

Regarding the chlorophyll index, chlorimuron-ethyl and glyphosate potassium salt showed the highest values when applied at 6:30 am, whereas at 12:30 pm only glyphosate isopropylamine salt differed statistically from the other treatments (Table 5). The remaining treatments did not differ from each other and generally presented lower chlorophyll index values. Notably, the weeded control exhibited a lower chlorophyll index than the best-performing herbicide treatments, indicating that some herbicide applications resulted in chlorophyll levels equal to or higher than the untreated, weed-free condition. When comparing application times within each treatment, it was observed that glyphosate isopropylamine salt and glyphosate ammonium salt + chlorimuron-ethyl applied at 6:30 am, as well as glyphosate potassium salt applied either alone or in mixture at 12:30 pm, resulted in the lowest chlorophyll index values for this variable (Table 5).

Some studies report an increase in soybean chlorophyll content 35 days after glyphosate application, which is associated with partial recovery of plants from the stress caused by the herbicide (Zobiolo et al. 2011; Da Cruz et al. 2019; Li et al. 2019). This effect may be related to the increase in the levels of chelated nutrients after glyphosate appli-

**Table 7** Number of grains per pod (NGP) in the soybean cultivar DM57i52 IPRO as a function of herbicide application in the 2019/20 and 2020/21 agricultural harvests.

Treatments	Number of grains per pod
Weeded control	2.62 b*
Chlorimuron-ethyl	2.65 b
Glyphosate isopropylamine salt	2.67 b
Glyphosate ammonium salt	2.78 a
Glyphosate potassium salt	2.64 b
Glyphosate disopropylamine salt + chlorimuron	2.58 b
Glyphosate ammonium salt + chlorimuron	2.56 b
Glyphosate potassium salt + chlorimuron	2.70 a
CV(%)	5.09
Overall Means	2.65
F <sub>herbicides</sub>	4.23
F <sub>times</sub>	3.42
F <sub>herbicides × times</sub>	0.84
P <sub>herbicides</sub>	0.0004
P <sub>times</sub>	0.07
P <sub>herbicides × times</sub>	0.55

F analysis of variance. P probability

\* Means followed by the same letter in the column do not differ from each other, according to the Scott-Knott test ( $p \leq 0.05$ )

cation, especially magnesium and manganese, allowing the gradual resumption of chlorophyll production in the plant (Da Cruz et al. 2019). Soon after glyphosate application, soybean plants showed lower chlorophyll content, possibly due to the reduction in the levels of these essential nutrients, which play a fundamental role in its synthesis and functionality (Zobiolo et al. 2011; Kanissery et al. 2019). According to Da Cruz et al. (2019), variations in chlorophyll levels are common and may be associated with several biotic and abiotic factors, such as water availability, temperature, solar radiation, salinity, herbicide application and competition with weeds, among others.

No interaction was observed between the factors tested, nor was there a significant effect of herbicides and application times, either when applied alone or in mixtures, for the variables Ci and WUE (Table 6).

At 28 days after application (DAT), no significant effects were observed for internal CO<sub>2</sub> concentration (Ci) or water use efficiency (WUE) as a function of herbicide treatments, application times, or their interaction (Table 6). Mean Ci values were relatively stable across treatments, ranging from 258.00 to 273.17 μmol mol<sup>-1</sup>, while WUE values varied minimally between 6.13 and 7.13 mol mol<sup>-1</sup>. These results indicate that, although some physiological parameters such as photosynthetic rate and transpiration were affected by the treatments, the internal CO<sub>2</sub> concentration and the ratio between carbon assimilation and water loss remained stable, suggesting the occurrence of compensatory

physiological mechanisms maintaining gas exchange balance in the plants.

Similar responses have been reported in soybean and other crops subjected to herbicide applications, where changes in certain physiological traits do not necessarily lead to significant alterations in Ci and WUE. Coêlho et al. (2024) observed that different application times of herbicides altered photosynthetic pigments and physiological indicators in cowpea without consistent impacts on all gas exchange variables. Likewise, Bari et al. (2020) reported that herbicide-induced stress in wheat affected some physiological parameters, while others remained unchanged due to plant regulatory mechanisms. In soybean, formulations of glyphosate and their mixtures may alter metabolism and photosynthetic performance, but these effects are often transient and may not significantly compromise internal CO<sub>2</sub> dynamics or water-use efficiency (Gonçalves et al. 2017; Li et al. 2019), which helps explain the lack of significant differences observed for Ci and WUE in the present study.

### Effect of Herbicides and Application Times On Soybean Grain Yield Components

The number of grains per pod was higher when glyphosate ammonium salt and the mixture of glyphosate potassium salt + chlorimuron-ethyl were applied, even surpassing the weeded control and the other treatments (Table 7). Since the soybean plants did not suffer significant effects of phytotoxicity and were able to metabolize these herbicides, the mixtures of chlorimuron-ethyl with glyphosate did not negatively impact grain yield. Similar results were observed by Maciel et al. (2009) when evaluating different herbicides, applied alone or in mixtures to soybean, corroborating the findings of this study.

The application of glyphosate isopropylamine salt, glyphosate ammonium salt, and glyphosate potassium salt + chlorimuron-ethyl at 6:30 am resulted in the highest number of grains per plant (Table 8), while the other treatments did not differ from each other. This result is probably related to the fact that herbicides applied at cooler times of the day are less phytotoxic to the crop, or even that plants can metabolize them more efficiently compared to applications made at periods of higher temperatures. Coêlho et al. (2024), evaluating different herbicides in cowpea, observed that the lowest toxic effects on the crop occurred when the products were applied at 6:30 am, compared to applications at 12:30 pm, a result that, in part, resembles this study. This effect is mainly due to the heat stress caused by the application of herbicides close to noon (Amaral et al. 2020; Abu-Nassar and Matzrafi 2025). According to Barrozo et al. (2020), high temperatures during the hottest times of the day can cause irreversible damage to plants,

**Table 8** Number of pods per plant (NPP), number of grains per plant (NGP), and weight of a thousand grains (WTG) of the soybean cultivar DM57i52 IPRO based on the application of herbicides at different times, in the 2019/20 and 2020/21 agricultural seasons.

Treatments	Herbicide application times—hours			
	06:30 am	12:30 pm	06:30 am	12:30 pm
	Number of pods per plant		Number of grains per plant	
Weeded control	52.75 Aa*	52.75 Aa	145.35 Aa	145.35 Aa
Chlorimuron-ethyl	46.76 Ab	48.05 Ab	128.91 Ab	134.35 Ab
Glyphosate isopropylamine salt	51.38 Aa	45.17 Ab	142.47 Aa	126.06 Bb
Glyphosate ammonium salt	57.33 Aa	50.87 Aa	165.94 Aa	147.32 Ba
Glyphosate potassium salt	46.17 Ab	50.60 Aa	128.33 Aa	140.47 Ab
Glyphosate isopropylamine salt + chlorimuron	49.18 Ab	51.00 Aa	133.37 Ab	138.15 Ab
Glyphosate ammonium salt + chlorimuron	53.95 Aa	57.73 Aa	146.58 Aa	158.90 Aa
Glyphosate potassium salt + chlorimuron	54.30 Aa	43.92 Bb	150.18 Aa	126.06 Bb
CV(%)	12.86		13.25	
Overall Means	50.74		141.16	
F <sub>herbicides × times</sub>	2.83		2.38	
P <sub>herbicides × times</sub>	0.009		0.03	
Treatments	Weight of a thousand grains (g)		Yield (kg ha <sup>-1</sup> )	
Weeded control	163.51 Ac	163.51 Ac	2540.01 A <sup>ns</sup>	2540.00 Aa
Chlorimuron-ethyl	169.54 Ab	173.09 Aa	2702.81 A	2705.00 Aa
Glyphosate isopropylamine salt	164.56 Ac	166.28 Ab	2643.82 A	2523.61 Aa
Glyphosate ammonium salt	176.89 Aa	169.00 Bb	2565.19 A	2197.04 Bb
Glyphosate potassium salt	168.45 Ab	159.69 Bc	2734.48 A	2789.62 Aa
Glyphosate isopropylamine salt + chlorimuron	161.90 Ac	164.20 Ac	2536.97 A	2825.82 Aa
Glyphosate ammonium salt + chlorimuron	170.15 Ab	166.41 Ab	2646.22 A	2315.52 Ab
Glyphosate potassium salt + chlorimuron	170.04 Ab	168.03 Ab	2318.33 A	2641.80 Aa
CV(%)	2.68		13.68	
Overall Means	167.20		2576.62	
F <sub>herbicides × times</sub>	4.27		2.06	
P <sub>herbicides × times</sub>	0.0003		0.05	

ns not significant ( $p \leq 0.05$ ). *F* analysis of variance. *P* probability

\* Means followed by different letters (uppercase in rows and lowercase in columns) differ from each other by the t-test and the Scott-Knott test, respectively, at a significance level ( $p < 0.05$ )

interfering with their metabolism and intensifying the toxic effects of herbicides.

Applications of chlorimuron-ethyl alone and in mixtures with glyphosate isopropylamine salt resulted in the lowest number of grains per plant when applied at 6:30 am and 12:30 pm, compared to the weeded control (Table 8). In addition, the application at 12:30 pm of glyphosate isopropylamine salt, glyphosate potassium salt and the tank mix of chlorimuron-ethyl + glyphosate potassium salt also decreased the number of grains per plant, both compared to the weeded control and the other treatments.

Applications of glyphosate ammonium salt and chlorimuron-ethyl resulted in the highest weight of one thousand grains when applied at 6:30 am and 12:30 pm, respectively, outperforming all other treatments, including the weeded control (Table 8). Similar results were observed by Reddy and Zablutowicz (2003), who found that application of glyphosate in the isopropylamine salt or ammonium salt formulations did not decrease soybean yield. Accord-

ing to Correia and Durigan (2007), variations in yield of RR soybean cultivars are generally associated with specific genetic characteristics of each cultivar and not with the use of different glyphosate formulations.

The weeded control and glyphosate isopropylamine salt, applied alone or mixed with chlorimuron-ethyl, showed the worst results for the weight of one thousand grains when applied at 6:30 am and 12:30 pm (Table 8). Similar results were observed by Albrecht et al. (2012), who, when using the mixtures of glyphosate + chlorimuron-ethyl, found a negative effect on the weight of a thousand grains of the soybean cultivar CD 214 RR. According to the authors, this mixture of herbicides interferes with the formation and filling of grains, resulting in reduced soybean yield.

Soybean grain yield did not differ between treatments applied at 6:30 am (Table 8). Typically, when herbicides are applied under conditions of high light, high temperatures, and low relative humidity, the phytotoxic effect on the crop is more intense (Peterson et al. 2016). However, in

this study, applications made at 6:30 am occurred under less adverse conditions, which may explain the lack of impact of treatments on soybean yield. Similar results were observed by Agostinetto et al. (2009), who did not identify differences in grain yield of soybean cultivars when applying glyphosate formulations (ammonium salt, isopropylamine salt, or potassium salt) at different growth stages of the crop. Similarly, Silva et al. (2018) reported that the isolated or combined application of glyphosate with other herbicides, including the tank mix of glyphosate + chlorimuron-ethyl in the cultivar NS 6700 RR2, did not significantly affect soybean yield. These results corroborate the findings of this study.

The isolated application of glyphosate ammonium salt and its tank mix with chlorimuron-ethyl resulted in the lowest soybean grain yields when applied at 12:30 pm (Table 8). On the other hand, the other treatments performed at this time showed higher and statistically equivalent yield, with no differences when compared to the weeded control. The phytotoxic effects of herbicides should not be evaluated exclusively based on visual symptoms, as there are known cases of products that reduce crop yield without causing visible damage (Simões et al. 2016). On the other hand, some herbicides can cause apparent injuries that disappear as the plants develop, without compromising crop yield (Silva et al. 2018; Da Cruz et al. 2019; Arshad et al. 2025). Variation in soybean yield in response to the isolated use of glyphosate or in mixture with other herbicides may be associated with several factors, such as the cultivar's tolerance to the application, the dose and the physicochemical characteristics of the molecules, in addition to climatic conditions, soil characteristics and application technology. These factors may influence the selectivity of the crop, making it more or less susceptible to the effects of herbicides (Merotto et al. 2015; Silva et al. 2018; Arshad et al. 2025).

The differences in phytotoxicity observed between glyphosate (in its various salt forms) and chlorimuron-ethyl can be better understood by considering their distinct physicochemical properties and how these interact with application conditions (time of day, temperature, humidity). Glyphosate, as a highly polar and hydrophilic molecule, has a very low octanol-water partition coefficient ( $\log K_{ow} < -3.2$ ), and its salts (isopropylamine, ammonium, potassium) are highly water-soluble, influencing absorption and translocation within the plant (Reddy and Zablotowicz 2003; Agostinetto et al. 2009; Travlos et al. 2017). Potassium salts, in particular, may penetrate the plasma membrane more efficiently than ammonium or isopropylamine salts, potentially facilitated by plant Na/K transport mechanisms. Chlorimuron-ethyl, a sulfonyleurea, differs in polarity, solubility ( $\sim 1200 \text{ mg L}^{-1}$  at pH 7), and pKa ( $\sim 3.8\text{--}4.2$ ), such that a substantial fraction is non-ionized at typical leaf-surface pH, enhancing lipophilic uptake

through the cuticle (Maciel et al. 2009; Albrecht et al. 2018).

Application timing further modulates uptake and efficacy: cooler, more humid conditions in the early morning favor cuticular penetration and reduce rapid evaporation, whereas higher midday temperatures and lower humidity can limit absorption (Mohr et al. 2007; Abu-Nassar and Matzrafi 2025). In tank mixtures with chlorimuron-ethyl, physicochemical compatibility, solubility, and ionic form may influence absorption dynamics and synergistic or antagonistic interactions, explaining the observed variation in phytotoxicity among herbicide types and application times. Collectively, these factors provide a mechanistic basis for the differences in selectivity and effectiveness observed in the present study.

Comparing the different application times, it was observed that the treatments that received glyphosate ammonium salt at 12:30 pm showed lower grain yield compared to the application carried out at 6:30 am (Table 8). On the other hand, the other treatments presented equivalent yields, regardless of the application time. The reduction in yield caused by the application of glyphosate ammonium salt at 12:30 pm is probably associated with more intense heat stress and lower humidity during this period, which compromise the absorption and efficacy of the herbicide, as previously discussed. These conditions limit the physiological recovery of the plant, reducing its productive potential. In contrast, the other treatments maintained stable yield between application times, suggesting better tolerance to environmental variations. DeWerff et al. (2015) also did not find effects on soybean grain yield related to the timing of glyphosate application from stages V1 to R1, reinforcing the results observed both by Mohr et al. (2007) and in this study. According to Andrade et al. (2020), the lower the toxic effect of the glyphosate formulation, the greater the synthesis of secondary metabolites, which favors growth and production of photoassimilates by the plant. The increase in the availability of photoassimilates allows for more intense transport of these compounds to the grains, resulting in heavier grains and, consequently, higher yield (Andrade et al. 2020; Manghwar et al. 2024).

Although, in some situations, herbicide application at 12:30 pm does not negatively affect certain variables in soybean or may not differ significantly from applications made at 6:30 am, the present results indicate that applications during the hottest periods of the day can intensify herbicide effects on soybean plants, especially when products are applied in tank mixtures. Therefore, it is recommended that farmers prioritize spraying at times when weather conditions are milder. In this sense, lower temperatures and higher relative humidity favor herbicide absorption and translocation, increasing weed control efficiency and, in most cases, ensuring greater selectivity to the crop.

In addition, applications carried out under less intense thermal stress reduce the risk of evaporation and volatilization, minimizing drift to neighboring areas. This is especially important in regions where crops sensitive to certain herbicides are present, helping to prevent crop injury, economic losses, environmental impacts, and potential legal disputes.

## Conclusions

Isolated applications of chlorimuron-ethyl caused the highest levels of phytotoxicity in the soybean cultivar DM57i52 IPRO57. In contrast, phytotoxicity was lower when glyphosate was used in the isopropylamine salt, ammonium salt and potassium salt formulations. However, tank mixes of chlorimuron-ethyl with these glyphosate salts increased phytotoxicity in this cultivar.

Among the tested combinations, the mixture of glyphosate ammonium salt + chlorimuron-ethyl applied at 12:30 pm was the most harmful to soybean, resulting in the highest phytotoxicity and the lowest grain yield. These effects are likely associated with increased plant stress under unfavorable environmental conditions at midday, which intensified herbicide absorption and damage.

The weeded control, as well as the isolated application of glyphosate ammonium salt or in association with chlorimuron-ethyl, presented better values for the physiological variables of soybean plants, including transpiration rate, stomatal conductance, photosynthetic rate and carboxylation efficiency. In general, the weeded control and the application of glyphosate ammonium salt showed the best results for the soybean grain yield components. In addition, the application of herbicides at 6:30 am was less harmful to the crop than applications at 12:30 pm.

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**Data availability statement** The data presented in this study are available from the corresponding author upon reasonable request (L.Galon).

**Conflict of interest** L. Galon, R.A. Kujawinski, G.E. Zamboni, I. Aspiazu, R.J. Tonin, J.C. Onesko, M.A.M. Bagnara, G. Concenço and G.F. Perin declare that they have no competing interests.

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