



## **Glyphosate-Resistant Weeds Control with Double-Shooting in Soybean Pre-Planting**

### **Controle de Plantas Daninhas Resistentes ao Glifosato, por Pulverização Sequencial, em Pré-Plantio da Soja**

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#### **Abstract**

The weed resistance and tolerance to herbicides is an important soybean yield gap. The use of double-shooting, herbicide combinations, and action mechanisms rotation can be effective for an early control of these species. The aim of this study was to analyze the double-shooting performance of flumioxazin and glufosinate on the glyphosate-tolerant and glyphosate-resistant weeds. The experiment was conducted on Brazilian Yellow Argisolo distrocoesic, in a commercial soybean crop located in Mata Roma, Maranhão, Brazil, with a history of successive glyphosate use over a decade. The study was designed in randomized blocks, with six treatments, two additional controls, and five replicates. The treatments consisted of control 1 (no herbicide), control 2 (isolated glyphosate application - 1,500 g a.e. ha<sup>-1</sup>), and sequential spraying of glufosinate (500 and 600 g a.e. ha<sup>-1</sup>), flumioxazin (50 and 75 g a.i. ha<sup>-1</sup>), and glufosinate + flumioxazin (500/600 + 50/75 g a.i. ha<sup>-1</sup>). We evaluated weed control percentage, as well as the phytotoxicity, density, and soybean yield. At the end of this study, double-shooting control is recommended, which should be carried out with a first

application of glyphosate (1,500 g a.i. ha<sup>-1</sup>), followed by glufosinate (500 or 600 g a.i. ha<sup>-1</sup>) + flumioxazin (50 or 75 g a.i. ha<sup>-1</sup>). The implementation of this management until the 16 days early soybean planting reduces yield losses and increases glyphosate-resistant and glyphosate-tolerant weeds controls.

**Keywords:** *Glycine max* (L.) Merrill. Flumioxazin. Glufosinate. Early Management. Tolerant Weed.

### Resumo

A resistência ou tolerância de plantas daninhas a herbicidas é uma importante lacuna de produtividade da soja. A adoção de pulverizações sequenciais, misturas de herbicidas e rotação de mecanismos de ação podem ser efetivas no controle antecipado destas espécies. Objetivou-se analisar o desempenho de pulverizações sequenciais de flumioxazina e glufosinato sobre o controle de plantas daninhas tolerantes ou resistentes ao glifosato. O experimento foi conduzido em Argissolo Amarelo distrocoeso, numa lavoura comercial de soja, situada em Mata Roma, Maranhão, Brasil, com histórico de uso sucessivo de glifosato há mais de uma década. O estudo foi delineado em blocos casualizados, com 8 tratamentos e 5 repetições. Os tratamentos consistiram na testemunha 1 (sem herbicida), testemunha 2 (uso isolado de glifosato - 1.500 g e.a. ha<sup>-1</sup>) e pulverização sequencial de glufosinato (500/600 g i.a. ha<sup>-1</sup>), flumioxazina (50/75 g i.a. ha<sup>-1</sup>) e glufosinato + flumioxazina (500/600 + 50/75 g i.a. ha<sup>-1</sup>). Avaliou-se a porcentagem de controle de plantas daninhas, bem como, a fitotoxicidade, densidade e produtividade da soja. Ao término do estudo, recomenda-se o controle sequencial de plantas daninhas, o qual deve ser realizado com uma primeira aplicação de glifosato (1.500 g i.a. ha<sup>-1</sup>), seguido pelo uso de glufosinato (500/600 g i.a. ha<sup>-1</sup>) + flumioxazina (50/75 g i.a. ha<sup>-1</sup>). A implementação deste manejo, até os 16 dias antes do plantio da soja, reduz perdas de produtividade e melhora o controle plantas daninhas resistentes ou tolerantes ao uso de glifosato.

**Palavras-chave:** *Glycine max* (L.) Merrill. Flumioxazina. Glufosinato. Manejo Antecipado. Planta Daninha Tolerante.

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## 1 Introduction

Soybean [*Glycine max* (L.) Merrill] is one of the main agricultural crops worldwide due to its high economic potential, protein value, diverse uses in human and animal nutrition, play a role as a raw material for industrial and bioenergy purposes, and excellent adaptability to different regions (Seixas *et al.*, 2020). As a commodity, it generates significant gains in trade balance and directly contributes to strengthening the global economy (Montoya *et al.*, 2019).

In the 2024/25 growing season, soybean stood out as one of the most produced agricultural crops in the world, with a total of 421 million tons. In this context, Brazil was responsible for 40% of the global production (Embrapa, 2025). Among the main gaps in soybean yield, weed interference causes pod and grain losses, directly affecting the profitability of commercial crops (Martins; Andreani Junior, 2023; Pereira; Kerber; Fiorini, 2019).

In this scenario, chemical control has become an essential strategy for large-scale weed management in soybean farming (Galon *et al.*, 2023). However, the indiscriminate use of herbicides has led to serious issues, such as the selection of herbicide-resistant weed species, environmental impacts, and risks to human health (Agostini *et al.*, 2020).

Currently, more than fifty herbicide-resistant weed species have been identified in Brazil, among which twelve are resistant to glyphosate (Heap, 2020). This is a concerning situation, as glyphosate accounts for 60% of the global market of non-selective post-emergence herbicides. Since its introduction in Brazil, weed management practices have changed dramatically due to the exclusive and non-rotated use of this technology. This has led to the selection of glyphosate-resistant weeds, especially in crops with Roundup Ready (RR) transgenic cultivars (Codognoto *et al.*, 2023).

The annual cost of managing herbicide-resistant weeds has been estimated at approximately three billion dollars (Adegas *et al.*, 2022). Therefore, it is essential to adopt control practices based on the planned use of herbicides and natural resources, in line with the United Nations Sustainable Development Goals (SDGs), particularly Goals 2 and 12, which advocate for sustainable food production (Almeida *et al.*, 2024).

Sequential spraying (double-shooting) and herbicide combinations may offer benefits for food production and resistance prevention (Albrecht *et al.*, 2020). Although an old technique, tank mix prescriptions were only regulated in Brazil in 2018 (Brasil, 2018). This new regulation reinforces the need for studies on the effects of herbicide combinations on target species and agricultural crops (Gazziero, 2015).

There is a scarcity of scientific studies on sequential applications and combinations of flumioxazin and glufosinate for managing glyphosate-tolerant or -resistant weeds in pre-sowing soybean stages. Flumioxazin is a chlorophyll synthesis-inhibiting herbicide that acts on the enzyme protoporphyrinogen oxidase (PROTOX), is conditionally selective, non-systemic, and primarily absorbed by the roots. Glufosinate is a nitrogen assimilation-inhibiting herbicide that acts on the enzyme glutamine synthetase (GS), is non-selective and non-systemic, and has limited translocation within the plant (Albrecht *et al.*, 2023).

The hypothesis of this study is based on the premise that sequential spraying of flumioxazin and glufosinate formulations is effective in controlling glyphosate-tolerant or -resistant weed species in pre-sowing soybean cultivation. Therefore, the objective was to analyze the flumioxazin and glufosinate performance in the sequential control of glyphosate-tolerant or -resistant weeds in the pre-sowing phase of soybean.

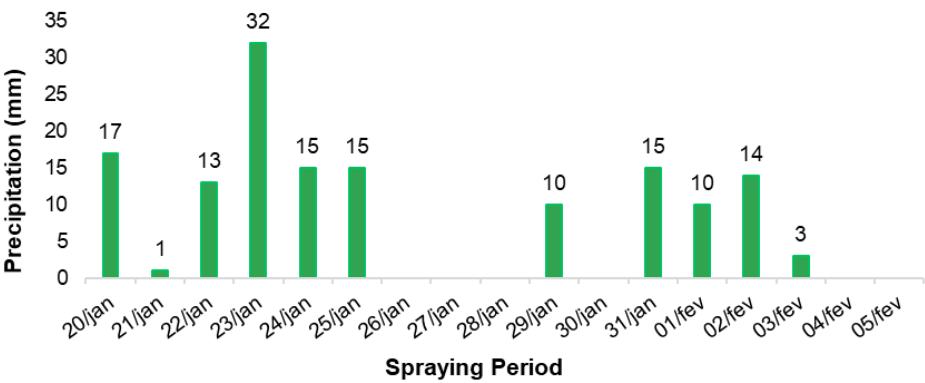
## **2 Material and Methods**

### **2.1 Study location**

The experiment was conducted in a commercial soybean field located in the municipality of Mata Roma (3° 14' 50" South, 43° 11' 13" West), Maranhão, Brazil, between January and May 2023. The climate in the region is classified as hot and humid tropical (Aw), with meteorological data during the study period indicating a total accumulated rainfall of 488.4 mm and an average temperature of

27 °C. Rainfall data recorded in the experimental area during the treatment application period are presented in Figure 1.

**Figure 1** - Rainfall data during the treatment application period and weed control assessment in the experimental area



Source: research data.

The soil in the experimental area was classified as *Dystrophic Ultisol* (Santos et al., 2018), and the chemical analysis yielded the following results at depths of 0–10 cm and 10–20 cm (Table 1).

**Table 1** - Chemical analysis of soil at depths of 0–10 cm and 10–20 cm in the experimental area

| Depht      | pH*               | M.O. | P**                 | K                                | Ca  | Mg  | Al  | H + Al | SB  | CTC    | V    | m   | Ca   | Mg   | K   |
|------------|-------------------|------|---------------------|----------------------------------|-----|-----|-----|--------|-----|--------|------|-----|------|------|-----|
|            | CaCl <sub>2</sub> | %    | Mg dm <sup>-3</sup> | .....cmol dm <sup>-3</sup> ..... |     |     |     |        |     | .....% |      |     |      |      |     |
| 0 – 10 cm  | 5.2               | 1.3  | 1.9                 | 0.06                             | 1.4 | 0.5 | 0.0 | 1.6    | 2.0 | 3.6    | 54.0 | 0.0 | 38.9 | 13.9 | 1.7 |
| 10 – 20 cm | 4.9               | 0.9  | 1.8                 | 0.03                             | 1.2 | 0.4 | 0.0 | 1.9    | 1.6 | 3.5    | 47.0 | 0.0 | 34.3 | 11.4 | 0.9 |

\*pH estimated using the CaCl<sub>2</sub> extraction method; \*\*P estimated using the Mehlich<sup>-1</sup> extraction method.

Source: research data.

The experimental area was selected due to its history of conventional soil management, successive chemical control with glyphosate for over 10 years, and the occurrence of a high infestation of weeds suspected to be resistant to glyphosate, as reported by the local farmer.

## 2.2 Experimental design

The experiment was conducted in a randomized block design, with six treatments, two additional controls, and five replications. The treatments consisted of control 1 (no herbicide), control 2 (1500 g a.e. ha<sup>-1</sup> of glyphosate), and sequential sprayings of glufosinate (500 g a.i. ha<sup>-1</sup>; Finale®), glufosinate (600 g a.i. ha<sup>-1</sup>), flumioxazin (50 g a.i. ha<sup>-1</sup>; Sumyzin®), flumioxazin (75 g a.i. ha<sup>-1</sup>), glufosinate + flumioxazin (500 + 50 g a.i. ha<sup>-1</sup>), and glufosinate + flumioxazin (600 + 75 g a.i. ha<sup>-1</sup>). The experimental plots measured 3 m × 6 m. In all treatments, except control 1, mineral oil was added to the spray solution at a concentration of 0.25%.

A detailed description of the experimental design is presented in Table 2.

**Table 2** - Description of the experimental design, with sequential sprayings and herbicide combinations

| Treatment | Application 1 | Dose(g a.e ha <sup>-1</sup> ) | Application 2 | Dose(g a.i. ha <sup>-1</sup> ) |
|-----------|---------------|-------------------------------|---------------|--------------------------------|
| Control 1 | Not applied   | --                            | Not applied   | --                             |
| Control 2 | Gly           | 1.500                         | Not applied   | --                             |
| Trt 1     | Gly           | 1.500                         | Glu           | 500                            |
| Trt 2     | Gly           | 1.500                         | Glu           | 600                            |
| Trt 3     | Gly           | 1.500                         | Flu           | 50                             |
| Trt 4     | Gly           | 1.500                         | Flu           | 75                             |
| Trt 5     | Gly           | 1.500                         | Glu + Flu     | 500 + 50                       |
| Trt 6     | Gly           | 1.500                         | Glu + Flu     | 600 + 75                       |

\*a.e.: acid equivalent; a.i.: active ingredient; Ctrl: control; Trt: treatment; Gly: glyphosate; Glu: glufosinate; Flu: flumioxazin

Source: research data.

The first spraying was performed 16 days before soybean sowing (DBS), while the second occurred at 8 DBS. The sprayings were carried out on sunny days starting at 8:30 a.m., using a CO<sub>2</sub>-pressurized backpack sprayer equipped with a central boom with six nozzles, fitted with single flat-fan tips, operating under a pressure of 207 kPa and a spray volume of 100 L ha<sup>-1</sup>. Meteorological conditions recorded at the time of application included an average wind speed of 3.1 km/h, an average relative humidity of 65%, and an average temperature of 32 °C, all measured with a thermohygroanemometer (Akrom Kr825).

## 2.3 Variables analyzed

Weed surveys were conducted to describe the floristic composition of weeds present in the experimental area, as well as to estimate the percentage of control at 0, 8, and 16 days before soybean sowing. For this, the Quadrat Inventory Method was used, which consisted of positioning a 1 m<sup>2</sup> open square frame in two regular and central positions within each replication. After identifying the weed species using specialized literature, control was estimated by species according to the following equation:

Equation 1. Percentage of weed control by species:

$$\text{Control (\%)} = \frac{[(\text{Density in control 1} - \text{Density in treatment})]}{(\text{Density in control 1})} \times 100$$

The results were classified into the following control categories: none or scanty (0 to 40%), regular (41 to 60%), sufficient (61 to 70%), good (71 to 80%), very good (81 to 90%), and excellent (91 to 100%) (SBCPD, 1995).

Soybean stand evaluation was performed 25 days after sowing by visually diagnosing

phytotoxicity symptoms based on a symptom scale from 0 (no injury) to 100% (plant death) (EWRC, 1964), and by estimating plant density expressed as the count of plants per linear meter. The useful evaluation area consisted of three central rows, each with a length of 2 linear meters, arranged in each experimental unit.

At 135 days after soybean sowing, productivity ( $\text{kg ha}^{-1}$ ) was estimated according to the following equation:

Equation 2. Productivity :

$$\text{Productivity} = \frac{(\text{Plants per hectare}) \times (\text{Pods per plant}) \times (\text{Seeds per pods}) \times (\text{Thousand seed weight})}{10.000}$$

## 2.4 Statistical analysis

Data were subjected to analysis of variance (Anova) at  $p < 0.05$ , and when the null hypothesis was rejected, means were compared using Tukey's HSD test.

## 3 Results and Discussion

### 3.1 Weeds survey

The conventional weed survey identified 12 species, distributed across 10 botanical families, predominantly belonging to the class Eudicotyledons (67%). A total of 7,753 individuals were counted, resulting in an average density of 163 plants per  $\text{m}^2$  (Table 3).

**Table 3** - List of weed species identified in the floristic survey and classification by botanical class, scientific name, botanical family, number of individuals, and history of herbicide resistance and/or tolerance in Brazil (HRAC-BR, International Herbicide Resistant Weed Database)

| Class | Species                             | Family         | Individuals | Resistance                           | Tolerance |
|-------|-------------------------------------|----------------|-------------|--------------------------------------|-----------|
| E     | <i>Alternanthera tenella</i> Colla  | Amaranthaceae  | 265         | --                                   | --        |
| E     | <i>Amaranthus viridis</i> L.        | Amaranthaceae  | 60          | ALS<br>PSII<br>EPSP's                | --        |
| E     | <i>Euphorbia hirta</i> L.           | Euphorbiaceae  | 4           | --                                   | EPSP's    |
| E     | <i>Ipomea pueracea</i> (L) Roth.    | Convolvulaceae | 1662        | --                                   | EPSP's    |
| E     | <i>Senna obtusifolia</i> L.         | Fabaceae       | 2           | --                                   | --        |
| E     | <i>Sida rhombifolia</i> L.          | Malvaceae      | 7           | --                                   | EPSP's    |
| E     | <i>Spermacoce verticillata</i> L.   | Rubiaceae      | 624         | --                                   | EPSP's    |
| E     | <i>Turnera subulata</i> Sm.         | Turneraceae    | 1343        | --                                   | --        |
| M     | <i>Cenchrus echinatus</i> L.        | Commelinaceae  | 55          | --                                   | EPSP's    |
| M     | <i>Commelina benghalensis</i> L.    | Cypereaceae    | 7           | --                                   | EPSP's    |
| M     | <i>Cyperis rotundus</i> L.          | Poaceae        | 2218        | --                                   | --        |
| M     | <i>Eleusine indica</i> (L.) Gaertn. | Poaceae        | 1106        | ACCCase,<br>EPSP's<br>ACCCase/EPSP's | --        |

Abbreviations – E: Eudicotyledon; M: Monocotyledon; ACCCase: Acetyl-CoA carboxylase-inhibiting herbicides; ALS: Acetolactate synthase-inhibiting herbicides; EPSPs: 5-enolpyruvylshikimate-3-phosphate synthase-inhibiting herbicides; PSII: Photosystem II-inhibiting herbicides.

Source: research data.

The weed community presents botanical characteristics similar to cultivated species (Marques *et al.*, 2017; Santos; Rodrigues; Santos, 2017). Thus, the predominance of eudicotyledonous species is related to the history of soybean cultivation in the experimental area. The significant occurrence of this class highlights the importance of early management, since there are few selective herbicides available for post-emergence control in soybean (Silva *et al.*, 2021).

Among them, glyphosate is the most widely used due to its broad spectrum of control and selectivity in RR crops. However, this herbicide has been losing effectiveness, requiring higher doses, which in turn has increased the selection of resistant biotypes and the occurrence of phytotoxicity in soybean (Adegas *et al.*, 2022).

In this context, a significant number of weed species with a history of herbicide resistance or tolerance in Brazil were identified. Among the eudicotyledonous species, *Amaranthus viridis* (slender amaranth) has a history of single resistance to ALS-, EPSPs-, and PSII-inhibiting herbicides; *Ipomoea purpurea* (morning glory) and *Spermacoce verticillata* (buttonweed) are tolerant to EPSPs-inhibiting herbicides (Table 1). Regarding the monocotyledonous species, *Eleusine indica* (goosegrass) has records of both single and multiple resistance to ACCase- and EPSPs-inhibiting herbicides, while *Commelina benghalensis* (tropical spiderwort) and *Cyperus rotundus* (nutgrass) are tolerant to EPSPs-inhibiting herbicides (Table 1).

The identification of two herbicide-resistant and four herbicide-tolerant weed species to EPSPs inhibitors (glyphosate) suggests that the history of reactive glyphosate-based management for over ten years may be selecting hard-to-control species and potentially reducing the crop profitability. These species possess efficient dispersal and survival mechanisms, increasing their competitive advantage over soybean (Mendes; Silva, 2022).

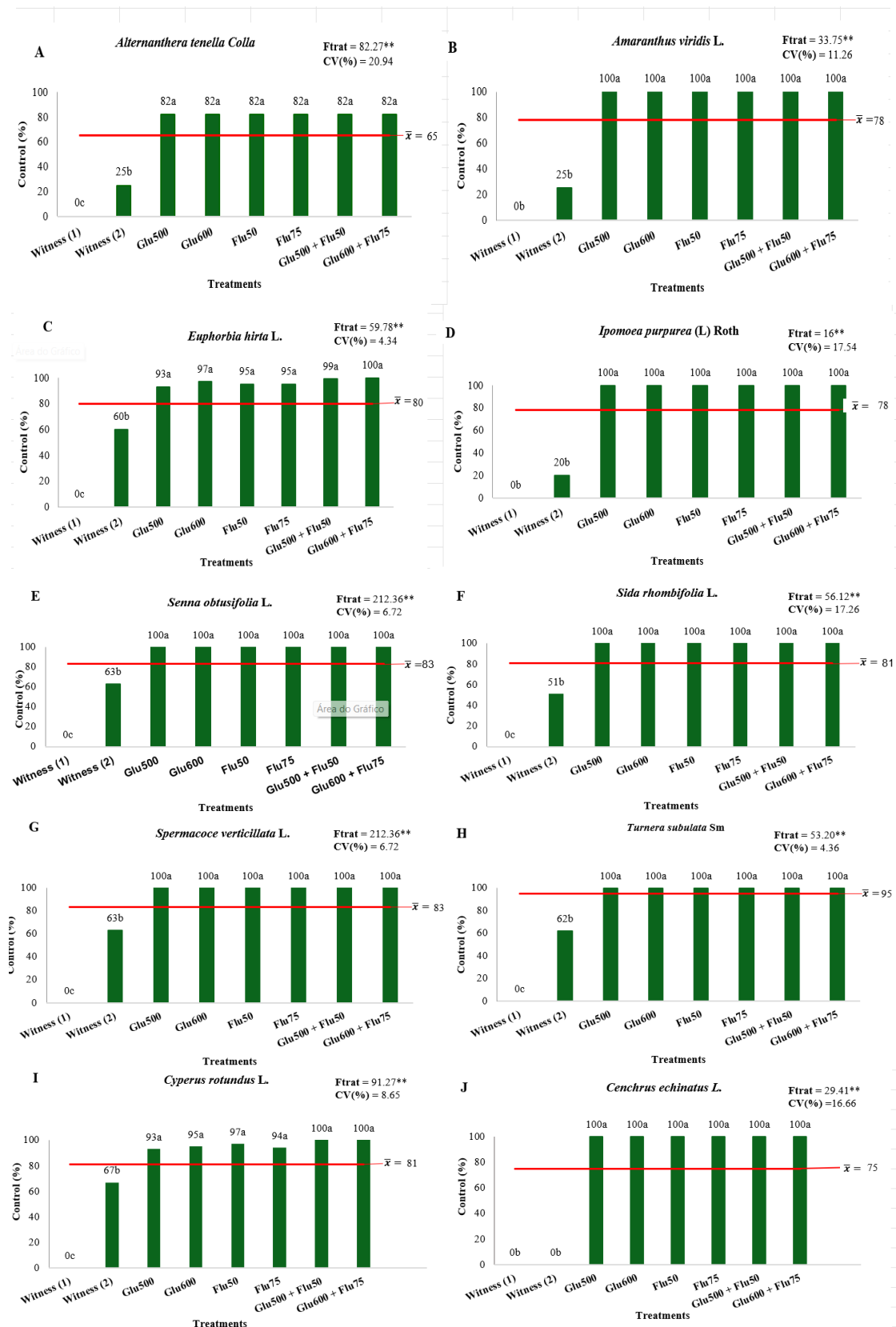
Therefore, these results highlight the importance of monitoring and controlling such species using effective strategies, considering their broad adaptation to the soil, climate, and management practices of the study region (Almeida *et al.*, 2024). Preventive management of hard-to-control weed species is crucial for crop sustainability and profitability (Silva *et al.*, 2024b).

### 3.2 Weed control

The results indicated that the sequential use of flumioxazin and glufosinate enhanced the control performance of herbicide-resistant weeds, achieving 100% control for most of the identified

species (Figure 2).

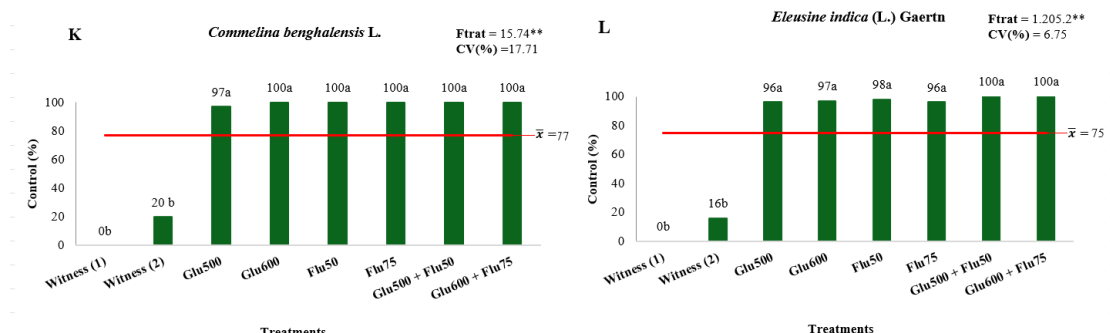
**Figure 2** - Control of weed species subjected to different herbicide treatments in pre-plant soybean management





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1A: *Alternanthera tenella* Colla. 1B: *Amaranthus viridis* L. 1C: *Euphorbia hirta* L. 1D: *Ipomea puerperacea* (L) Roth. 1E: *Senna obtusifolia* L. 1F: *Sida rhombifolia* L. 1G: *Spermacoce verticillata* L. 1H: *Turnera subulata* Sm. 1I: *Cenchrus echinatus* L. 1J: *Cyperis rotundus* L. 1K: *Commelina benghalensis* L. 1L: *Eleusine indica* (L.) Gaertn.

Source: research data.

The isolated use of glyphosate (1,500 g a.e. ha<sup>-1</sup>), without sequential spraying, showed null or low levels of control for *C. benghalensis* (20%), *C. echinatus* (0%), *E. indica* (16%), *A. tenella* (25%), *A. viridis* (25%), and *I. purpurea* (20%), with results statistically similar to control 1 (no herbicide). These findings suggest that the indiscriminate use of glyphosate for over 10 years in the experimental area may be selecting species tolerant or resistant to glyphosate, such as *C. benghalensis*, *C. echinatus*, *E. indica*, *A. tenella*, *A. viridis*, and *I. purpurea*.

These results are consistent with those reported by Markus *et al.* (2021), who described that selection pressure caused by continuous use of a single herbicide over a long period—without proper rotation of the mechanism of action—can result in severe changes in floristic composition, increased production costs, and decreased crop yields.

In this context, *E. indica*, for which the isolated application of glyphosate (1,500 g a.e. ha<sup>-1</sup>) was ineffective, is one of the weed species that requires greater attention, as it is currently one of the most difficult-to-control species in Brazil (Adegas *et al.*, 2017; Takano *et al.*, 2016), being present in approximately 75% of grain production areas (Almeida *et al.*, 2024; Takano *et al.*, 2017). Furthermore, Ofusu *et al.* (2023) highlight that this species occurs in over 60 countries and causes yield losses in at least 46 perennial and annual food crops.

Other weed species identified in this study, such as *C. rotundus* (67%), *E. hirta* (60%), *S. obtusifolia* (63%), *S. rhombifolia* (51%), *S. verticillata* (63%), and *T. subulata* (62%), also exhibited low control levels with isolated glyphosate application (1,500 g a.e. ha<sup>-1</sup>), supporting results reported by Silva *et al.* (2024a) and Amorim *et al.* (2023) in soybean crops. This emphasizes the poor performance of this herbicide against hard-to-control species, especially when sequential control strategies are not adopted.

Moreover, the low percentage of glyphosate control on species with prostrate growth habits—such as *A. tenella*, *C. echinatus*, *C. benghalensis*, and *E. indica*—may be related to protective advantages from spray droplet interception by taller weed species, a phenomenon known as the "umbrella effect" (Guimarães Neto *et al.*, 2023; Holkem *et al.*, 2022).

The effective use of application technology and sequential spraying is essential for accurate management of hard-to-control weeds in pre-sowing conditions (Kalsing *et al.*, 2018). Accordingly, the sequential control strategy adopted in this study resulted in "excellent" control levels for most of the listed species.

The use of glyphosate in the first application was important for broad-spectrum control of susceptible weeds, allowing greater contact exposure to flumioxazin and glufosinate, which are low-translocation, non-selective herbicides (Alonso *et al.*, 2013). The excellent control level achieved by glufosinate (500 and 600 g a.i. ha<sup>-1</sup>) may be attributed to its broad-spectrum activity (Carmo *et al.*, 2023) and high efficacy against juvenile weed plants (Teixeira *et al.*, 2023).

Glufosinate has been widely used worldwide as a post-emergence herbicide alternative to glyphosate, particularly following the increase in resistance cases to EPSPs inhibitors (Brustolin *et al.*, 2020). This herbicide acts as a competitive inhibitor of the enzyme glutamine synthetase, promoting ammonia accumulation and cell death in susceptible plants (Mundt *et al.*, 2021).

The use of flumioxazin (50 and 75 g a.i. ha<sup>-1</sup>) provided a control level statistically similar to that of glufosinate. Flumioxazin inhibits the enzyme protoporphyrinogen oxidase (PROTOX), which catalyzes the oxidation of protoporphyrinogen to protoporphyrin IX, a precursor in chlorophyll synthesis (Ponte *et al.*, 2024). Flumioxazin enables rapid weed control in both pre- and post-emergence applications (Brunetto *et al.*, 2023), showing good performance in combination with glufosinate (Takano *et al.*, 2019).

In this context, the pre-emergence residual effect of flumioxazin may have contributed to controlling the weed seed bank, in addition to its post-emergence effectiveness. Therefore, the combination of glufosinate and flumioxazin resulted in a synergistic interaction, achieving control levels close to or equal to 100% up to soybean planting.

### 3.3 Soybean selectivity and yield

Phytotoxicity and soybean plant density were not statistically influenced ( $p < 0.05$ ) by the treatments evaluated, indicating excellent selectivity of the herbicides and doses tested in this study—

particularly flumioxazin at different rates—given the herbicide residual half-life in the soil (Table 4).

**Table 4** - Statistical analysis of soybean injury (phytotoxicity), plant density, and yield under different pre-sowing treatments in Mata Roma, Maranhão, Brazil

| Treatment                       | Phytotoxicity (%) | Density (pl m <sup>-1</sup> ) | Productivity (kg ha <sup>-1</sup> ) |
|---------------------------------|-------------------|-------------------------------|-------------------------------------|
| Control (1)                     | --                | 12                            | 1.860 c                             |
| Control (2)                     | 0                 | 13                            | 2.520 b                             |
| Glu500 <sup>seq</sup>           | 0                 | 13                            | 3.300 a                             |
| Glu600 <sup>seq</sup>           | 0                 | 12                            | 3.180 a                             |
| Flu50 <sup>seq</sup>            | 0                 | 13                            | 2.820 b                             |
| Flu75 <sup>seq</sup>            | 0                 | 14                            | 3.120 a                             |
| (Glu500 + Flu50) <sup>seq</sup> | 0                 | 12                            | 2.940 aB                            |
| (Glu600 + Flu75) <sup>seq</sup> | 0                 | 14                            | 3.300 a                             |
| F <sub>trat</sub>               | --                | --                            | 6.20**                              |
| C.V. (%)                        | --                | 5.53                          | 4.43                                |

\*significant at the 1% probability level ( $p < 0.01$ ). Means followed by the same letter in the column do not differ from each other according to Tukey's HSD test. Seq = sequential application following the use of glyphosate (1,500 g a.e. ha<sup>-1</sup>); Glu = Glufosinate; Flu = Flumioxazin.

Source: research data.

These results are consistent with Pontes *et al.* (2024), Silva *et al.* (2024b), and Silva *et al.* (2022), in performance studies involving different dosages and/or mixtures of flumioxazin applied in pre-emergence of soybean and in dystrophic cohesive Yellow Argisol.

The treatments composed of glufosinate and flumioxazin combinations, as well as both glufosinate doses (500 and 600 g a.i. ha<sup>-1</sup>) and the higher flumioxazin dose (75 g a.i. ha<sup>-1</sup>), achieved the highest yield performances. Therefore, the application of these treatments is recommended—particularly the mixtures of flumioxazin and glufosinate—due to their synergistic effects in the post-emergence control of glyphosate-susceptible, tolerant, or resistant weed species (Mundt *et al.*, 2021). Additionally, flumioxazin complementary pre-emergence control contributed to yield increases of 1,434 and 774 kg ha<sup>-1</sup>, respectively, in the present study.

Conversely, competition with weeds in the treatment using glyphosate alone (1,500 g a.e. ha<sup>-1</sup>) resulted in a 24% yield loss, confirming the risks associated with the indiscriminate use of glyphosate, particularly regarding the selection of tolerant or resistant weeds and their interference in soybean phenological development.

Rotation of mechanisms of action, proper planning of application timing, and knowledge of the predominant weed infestation are essential for managing herbicide-tolerant or -resistant species. Thus, the implementation of these sequential chemical control strategies should be integrated with other control methods and preventive practices, aiming to improve sustainability and productivity

indicators in large-scale agriculture.

#### 4 Conclusion

Twelve weed species were identified, distributed across 10 botanical families, predominantly belonging to the class Eudicotyledons (67%).

Among the species identified, *C. benghalensis* (20%), *C. echinatus* (0%), *E. indica* (16%), *A. tenella* (25%), *A. viridis* (25%), and *I. purpurea* (20%) showed tolerance or resistance to the isolated application of glyphosate (1,500 g a.e. ha<sup>-1</sup>), resulting in null or low control levels.

Sequential control is recommended, starting with an initial application of glyphosate (1,500 g a.e. ha<sup>-1</sup>), followed by glufosinate (500 or 600 g a.i. ha<sup>-1</sup>) + flumioxazin (50 or 75 g a.i. ha<sup>-1</sup>). This planned management, applied up to 16 days before sowing, effectively reduced yield losses and controlled weed species with a history of tolerance or resistance to glyphosate.

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