AJCS 18(1):29-36 (2024) doi: 10.21475/ajcs.24.18.01.p4019

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Abstract

Interference from herbicide-resistant weeds is one of the biggest impediments in soybean crops. The aim was to evaluate the spatial dynamics and weed control under glyphosate and glufosinate combinations before soybean seeding. The experiment was conducted in a striped arrangement with thirteen treatments and seven replicates. The treatments consisted in no-treatment, glyphosate + fomesafen, glyphosate + saflufenacil, glyphosate + diclosulam, glyphosate + imazetapir, glyphosate + flumioxazine, glyphosate + carfentrazone-ethyl, glyphosate + imazetapyr + flumioxazine, glyphosate + glufosinate, glyphosate + saflufenacil, glufosinate + flumioxazine, glyphosate + glufosinate, glyphosate + saflufenacil, glufosinate + flumioxazine. The weed spatial dynamic was analyzed for geostatistics methods, while weed control by conventional and UAV (Unmanned Aerial Vehicle) methods. We identified twenty weed species, representing eighteen different genera and fourteen botanical families. It's worth noting that eudicotyledonous species were the dominant group, primarily characterized by an annual life cycle and sexual reproduction. The utilization of precision agricultural methods proved highly effective in conducting weed surveys before soybean seeding. Using glyphosate alone is not recommended in fields with a history of reactive management with this herbicide. Alternatively, combining glyphosate and glufosinate with Protox-inhibitors showed outstanding control efficacy against glyphosate-resistant or glyphosate-tolerant weeds, *Cenchrus echinatus, Spermacoce verticillata and Turnera subulata*.

Keywords: Brazil, drone, *Glycine max*, herbicide action mechanisms, weed survey.

Introduction

The soybean crop, scientifically known as *Glycine max* L. Merrill, holds a prominent position in Brazil's agricultural sector. The latest soybean harvest in Brazil reached an impressive 136 million tons, indicating an 8.9% increase compared to the previous one. Notably, in the north-eastern region of Brazil, the state of Maranhão emerges as the second-largest producer, with an estimated output of 3,285,600 tons cultivated across a vast area of 1,005,700 hectares (CONAB, 2021).

Weed interference can significantly hinder the economic performance of soybean cultivation. Chemical control stands out as a crucial method for effectively managing large-scale crops due to its efficiency, convenience, and costeffectiveness (Fadin et al., 2018). The success of herbicides depends on various factors, including spray conditions, historical weed infestations, soil properties, climate conditions, and environmental considerations. The implementation of early management strategies can result in healthier crop growth and a more efficient harvest, ultimately reducing production expenses and the necessity for subsequent herbicide applications, such as post-seeding glyphosate treatment (Bottcher et al., 2022).

The utilization of glyphosate accounts for 60% of the global market for non-selective herbicides. However, this widespread use has led to a rise in the occurrence of herbicide-resistant weeds, particularly those resistant to EPSP_s enzyme inhibitors. In the past decade, twelve glyphosate-resistant biotypes have been discovered, a notable figure among the sixteen recorded in global literature (Adegas et al., 2022). This highlights the importance of conducting research aimed at enabling the rotation of action mechanisms. This approach aims to move away from a solely reactive strategy for glyphosate management and instead proactively address the potential emergence of herbicide-resistant weeds in soybean fields (Kalsing et al., 2020).

Conducting weed surveys within commercial crops serves as a valuable tool for assessing the influence of agricultural practices on the dynamics of weed communities. Precision agriculture techniques have been leveraged for such monitoring endeavours, including the utilization of

ISSN:1835-2707

geostatistical maps, which offer the potential to enhance control strategies (Grego et al., 2020). Researchs employing proximal sensors has demonstrated promising outcomes in the early detection of weed densities. However, their applicability may be limited across expansive areas, thereby paving the way for the integration of alternative remote sensing platforms like Unmanned Aerial Vehicles (UAVs) (Khaliq et al., 2019).

The use of UAVs opens up new possibilities for weed management, offering the capability to accurately estimate weed prevalence rates with remarkable precision, rapid response times, and reduced operational costs. UAVs serve as a valuable source of data to improve weed monitoring, weed mapping, and weed control systems, which stands in contrast to conventional methods of phytosociological surveying (Shah et al., 2021; Yu et al., 2019).

Therefore, the primary aim of this study was to evaluate the efficiency of herbicides applied before soybean cultivation. The study specifically focused on assessing double and triple combinations involving glyphosate and glufosinate as a strategy to combat glyphosate-tolerant and glyphosate-resistant weeds.

Results and Discussion

Floristic and phytosociological weed survey

In the before soybean seeding an amount of 20 weed species were identified, spanning across 18 genera and 14 botanical families. A majority of these species consisted of eudicotyledonous class (60%), exhibited an annual life cycle (60%) and sexual reproduction (95%) (Table 1). The Poaceae family emerged as a prominent contender with four species, followed for Cypereaceae (3), and Amaranthaceae (2). Inagaki et al. (2020) highlighted that the Poaceae family has extensive production of seeds and propagules, which contributes to its remarkable ability to disperse and colonize diverse environments, even under challenging conditions. As noted by Mesquita et al. (2016), Poaceae species can also form dense clusters with hard post-emergence control.

Concerning the arrangement in space, a distinct pattern was noticed for monocotyledonous species, where their occurrence was more concentrated, characterized by darkershaded circles on the map. The density exceeded 32 plants per square meter, showing a strong degree of clustering (87%). In contrast, eudicotyledonous species exhibited a more even distribution, with an average of 38 plants per square meter throughout the area. Moreover, there were small clusters ranging from 49 to 88 plants per square meter, indicating a high degree of clustering (94%) (Fig. 1). As per the findings of Gundy et al. (2017) and Rocha et al. (2015), comprehending this spatial distribution can enhance management strategies and help reduce the environmental impact of herbicides.

M. verticillata exhibited the highest Index Value Importance (73.76), followed for *T. subulata* (43.29) and *E. ciliaris* (32.79) (Fig. 2). The families and species identified in this study were also reported for Furtado et al. (2022) and Silva et al. (2021) in soybean fields of Maranhão (Brazil), as well as Caetano et al. (2018) in Bahia (Brazil), Alburqueque et al. (2017) in Roraima (Brazil), and in other countries including the United States (Webster and Nichols, 2012), Colombia (Ramírez et al., 2015), and China (He et al., 2019).

Some species such as *S. verticillata, E. indica, Cyperus sp., Amaranthus sp.,* and *C. echinatus* are listed as glyphosate-tolerant and glyphosate-resistant weeds in Brazilian states

such as Paraná (Takano et al., 2017), Rio Grande do Sul (Vargas et al., 2013), Bahia (Kalsing et al., 2020), and other countries with Malaysia and Colombia (Villalba, 2009).

Glyphosate-tolerant and glyphosate-resistant weeds control The control using UAV images demonstrated that all herbicides exhibited effectiveness compared to notreatement (Fig. 3). These results are in accordance with Kawamura et al. (2021) to validate the accuracy of UAV aerial mapping for weed survey in burn broadleaves operations. Glyphosate demonstrated its highest effectiveness when used in conjunction with other compounds, particularly Protox-inhibitors like flumioxazine, saflufenacil, and carfentrazone (Fig 4). Protox-inhibitor herbicides target the enzyme protoporphyrinogen oxidase (Protox), which is involved in chlorophyll synthesis (Gallon et al. 2019).

Additionally, Dalazen et al. (2015) observed synergistic effects between these mechanisms of action against glyphosate-tolerant weeds. Combining glyphosate with saflufenacil yielded improved control, aligning with the findings of Silva et al. (2014), Van Wely et al. (2015) and Agostineto et al. (2016). They reported enhanced control when glyphosate was paired with flumioxazine, fomesafen, and carfentrazone, respectively.

The isolated use of glyphosate is not recommended (Fig. 4). This could be attributed to the practice of glyphosate reactive management over a decade on farms. According to Albrecht et al. (2020), glyphosate remains a crucial herbicide for weed management, but it can be replaced or combined with other herbicides to prevent weed resistance.

Carneiro et al. (2020) noted that changes in processes of absorption and translocation can change the dose reaching, thus rendering it insufficient control. This was observed for *T. subulata*, which had unsatisfactory control with glyphosate alone and glyphosate combinations with fomesafen, diclosulam, and saflufenacil.

Ribeiro et al. (2015) pointed out that differential translocation could explain glyphosate tolerance in some botanical genera, but no instances of tolerance or glyphosate-resistance were identified in the *Turnera sp*, particularly the specie *T. subulata*. Conversely, Furtado et al. (2022), Silva et al. (2022), and Silva et al. (2021) indicated that this species was easily controlled.

For *T. subulata*, the best control was achieved with glufosinate + flumioxazine (100%) and glufosinate + saflufenacil (100%) (Fig. 5K, 5L), consistent with Takano et al. (2020). This underscores the significance of rotating mechanisms of action for effective management and prevention of resistant weeds. Conversely, according to Brunharo et al. (2014), glufosinate has gained worldwide recognition as a non-selective post-emergent herbicide, offering an alternative to glyphosate resistance. Its action mechanisms entail competitive inhibition of the enzyme glutamine synthetase, resulting in the accumulation of ammonium and eventual cell death (Albrecht et al., 2021).

Satisfactory control was observed for *M. verticillata, S. verticillata, S. dulcis, E. ciliaris, C. echinatus,* and *H. indicum* across all treatments analysed (Fig. 5). Excellent control was achieved for monocotyledonous weeds when categorizing treatments based on botanical classes, while good to excellent control for eudicotyledonous (Fig. 6). This emphasizes the importance of tailoring management strategies to target species within specific botanical classes, in alignment with Albrecht et al. (2021).

| Table 1 | L. Floristic weed | d classification by family | , scientific nome | nclature, EPPO o | ode, botanical | class (BC), lif | e cycle (LC) and | d reproductions f | forms |
|---------|-------------------|----------------------------|-------------------|------------------|----------------|-----------------|------------------|-------------------|-------|
| (RF). M | ata Roma, Mara | anhão, Brazil. | | | | | | | |

| Family | Species | EPPO | BC | LC | RF |
|------------------|----------------------------------|-------|----|-----|------|
| Amaranthaceae | Alternanthera tenella Colla | ALRTE | E | Р | As/S |
| Boraginacae | Amaranthus hybridus L. | AMACH | E | А | S |
| | | | | | |
| Commelinaceae | Helietropium indicum L. | HEOIN | E | А | S |
| | Commelina benghalensis L. | COMBE | М | Р | As/S |
| Cucurbitaceae | Cuncumis anguria L. | CUMAN | E | А | As/S |
| Cypereaceae | Cyperis iria L. | CYPIR | М | А | S |
| | Cyperis odoratus L. | CYPFE | М | A/P | As/S |
| | Cyperis rotundus L. | CYPRO | М | Р | As/S |
| Euphorbiaceae | Euphorbia hirta L. | EPHHI | E | А | S |
| Fabaceae | Mimosa pudica L. | MIMPU | E | Р | S |
| Lecythidaceae | Lecythis lurida (Miers) S.A.Mori | LCYLU | E | Р | S |
| Molluginaceae | Mollugo verticillata L. | MOLVE | E | А | S |
| Phyllanthanceae | Phyllanthus niruri L. | PYLNI | E | А | S |
| | | | | | |
| Poaceae | Cenchrus echinatus L. | CCHEC | М | А | S |
| | Eleusine indica (L.) Gaertn | ELEIN | М | A/P | S |
| | Eragrostis ciliaris (L.) R. Br | ERACI | М | A/P | S |
| | Paspalum plicatulum Michx. | PASPL | М | Р | As |
| | | | | | |
| Rubiaceae | Spermacoce verticillata L | BOIVE | E | Р | S |
| Scrophulariaceae | Scoparia dulcis L. | SCFDU | E | A | S |
| Turneraceae | Turnera subulata L. | TURSU | E | Р | S |

Note: E – Eudicotyledonous; M – Monocotyledonous; A – Annual; P – Permanent; As – Asexual; S – Sexual.



Figure 1. Weed spatial distribution by botanical class in before soybean seeding. The database was expressed in weed density, number plants for meter square (pl m⁻²). **1A:** Monocotyledonous weeds. **1B:** Eudicotyledonous weeds. Mata Roma, Maranhão, Brazil.

Table 2. Soils chemical characteristics in 0-10 cm and 10-20 cm depth. Mata Roma, Maranhão, Brazil.

| | | | Sorptive complex | | | | | | | | |
|-------|-----|--------|------------------|-----------------------|-----|-----|-----|------|-----|-----|--|
| Depth | рН | MO dag | Р | к | Са | Mg | Al | H+Al | SB | СТС | |
| (cm) | | kg⁻¹ | mg dm⁻³ | cmol dm ⁻³ | | | | | | | |
| 0-10 | 5.2 | 1.3 | 1.9 | 0.06 | 1.4 | 0.5 | 0.0 | 1.6 | 2.0 | 3.6 | |
| 10-20 | 4.9 | 0.9 | 1.8 | 0.03 | 1.2 | 0.4 | 0.0 | 1.9 | 1.6 | 3.5 | |

pH - CaCl₂ method; P - Mehlich-1 method.



Figure 2. Weed Importance Value Index in before soybean seeding. Mata Roma, Maranhão, Brazil.



Figure 3. Weed control by UAV methods in thirteen herbicides treatments, before soybean seeding. **5A:** Herbicides treatments ortomosaic. **5B:** Green Leaf Index map. **5C:** Weed classification by k-means methods. Note: T1 – no-treatment, T2 – glyphosate, T3 – glyphosate + fomesafen, T4 – glyphosate + saflufenacil, T5 – glyphosate + diclosulam, T6 – glyphosate + imazetapir, T7 – glyphosate + flumioxazine, T8 – glyphosate + carfentrazone-ethyl, T9 – glyphosate + imazetapyr + flumioxazine, T10 – glyphosate + glufosinate, T11 – glyphosate + flumioxazine + carfentrazone, T12 – glufosinate + saflufenacil, T13 – glufosinate + flumioxazine. Mata Roma, Maranhão, Brazil.



Figure 4. Weed coverage estimated by UAV methods. Note – Car: carfentrazone-ethyl, Dic: diclosulam, Flu: flumioxazine, Fom: fomesafen; Gly: glyphosate, Glu: glufosinate, Ima: imazetapyr, No-treat: no-treatment, Saf: saflufenacil. Mata Roma, Maranhão, Brazil.



Figure 5. Weed control (%) before soybean seeding and under different treatments. Note: * n - not present specie weeds in the treatment. TURSE: *Turnera subulata*, MOLVE: *Mollugo verticillata*, ERACI: *Eragrostis ciliares*, HEOIN: *Helietropium indicum*, CCHEC: *Cenchrus echinatus*, BOIVE: *Spermacoce verticillata*, SCFDU: *Scoparia dulcis*. Mata Roma, Maranhão, Brazil.

Figure 6. Weed control in eudicotyledonous and monocotyledonous species under different treatments. Note: *n – not present weeds, Car: carfentrazone-ethyl, Dic: diclosulam, Flu: flumioxazine, Fom: fomesafen; Gly: glyphosate, Glu: glufosinate, Ima: imazetapyr, Saf: saflufenacil. Mata Roma, Maranhão, Brazil.

The use of pre-emergent molecules showed as a promising option in before soybean cultivation control (Fig. 6), consistent with Marchi et al. (2013). Favourable outcomes were observed in combinations of diclosulam and imazetapyr with glyphosate and glufosinate. In addition to satisfactory performance with casual pre-emergents, such as saflufenacil and flumioxazine.

Materials and Methods

Location

The research was conducted in a commercial soybean field located in Mata Roma, Maranhão, Brazil, geographic coordinates 3º 14' 50" South latitude and 43º 11' 13" West longitude. The selection of this field was a collaborative effort with the farmer, and it had a history of continuous glyphosate usage for over a decade.

The experimental field was characterized as having a typical yellow Argissolo distrocoeso soil type, as described by Dantas et al. (2014). The chemical properties of the soil are detailed in Table 2. The climate in this region is classified as tropical, characterized as hot and humid (Aw). The recorded meteorological data included accumulated rainfall of 145 mm, a maximum temperature of 25°C, and a minimum temperature of 34°C.

Experimental screening

The experimental screening followed a randomized block design, in a striped arrangement, comprising thirteen treatments and seven repetitions. The treatments included a no-treatment, glyphosate (1,620 g a.e. ha⁻¹), glyphosate + fomesafen (1,620 g a.e. ha⁻¹ + 250 g a.i. ha⁻¹), glyphosate + saflufenacil (1,620 g a.e. ha⁻¹ + 28.6 g a.i. ha⁻¹), glyphosate + diclosulam (1,620 g a.e. ha⁻¹ + 34 g a.i. ha⁻¹), glyphosate + flumioxazine (1,620 g a.e. ha⁻¹ + 50 g a.e. ha⁻¹), glyphosate + flumioxazine (1,620 g a.e. ha⁻¹ + 50 g a.i. ha⁻¹), glyphosate + carfentrazone-ethyl (1,620 g a.e. ha⁻¹ + 40 g a.i. ha⁻¹), glyphosate + imazetapyr + flumioxazine (1,620 g a.e. ha⁻¹ + 40 g a.i. ha⁻¹), glyphosate + imazetapyr + flumioxazine (1,620 g a.e. ha⁻¹ + 100 g a.e. ha⁻¹ + 50 g a.i. ha⁻¹), glyphosate + glufosinate

(1,620 g a.e. $ha^{-1} + 600$ g a.i. ha^{-1}), glyphosate + flumioxazine + carfentrazone (1,620 g a.e. $ha^{-1} + 50$ g a.i. $ha^{-1} + 20$ g a.i. ha^{-1}), glufosinate + saflufenacil (400 g a.i. $ha^{-1} + 28.6$ g a.i ha^{-1}), glufosinate + flumioxazine (400 g a.i. $ha^{-1} + 50$ g i.a ha^{-1}). Except in no-treatment, all treatments were added 0.25% mineral oil.

The treatments spraying occurred 15 days before soybean seeding with a knapsack sprayer equipped with a spray boom housing six nozzles and 3.0 meters in size. The nozzles were single fan tip type, working pressure of 207 kPa, and a rate of 150 L ha⁻¹. Spraying took place in the morning, with a recorded wind velocity of 3.14 km h⁻¹, relative air humidity at 65%, and an air temperature of 32.6 °C.

Weed conventional survey

The first weed survey took place three days before the burn operations, while the second survey occurred 10 days after the application of treatments (DAA). In the initial survey, we collected 100 georeferenced samples using a regular grid measuring 20.0 meters by 10.0 meters. This involved 10 linear pathways, each with 10 sampling points. The second survey at 10 DAA was conducted in treatment plots, and we used sampling squares measuring 1.0 meter by 1.0 meter. During the sampling process, we identified the weeds using specialized literature and quantified them to calculate phytosociological indices and control using the following equations:

| Eq. | 1: | Relative density | (RD) = | species density × 100 total species density | | | | |
|--|-----------|--------------------------------------|-------------------------|--|--|--|--|--|
| Eq. 2: Re | elative f | requency (RF) = | species fi total spe | requency × 100 cies frequency | | | | |
| Eq. 3: Re | elative a | bundace (RA) = | species al total spe | bundace × 100 cies abundace | | | | |
| Eq. 4: Importance Value Index (IVI) = RD + RF + RA | | | | | | | | |
| Eq. 5: Co | ontrol = | (notreatment density - notreatmen | treatment density | ^{density)} × 100 | | | | |

The control was categorized in none to poor (0 to 40%), fair (41 to 60%), sufficient (61 to 70%), good (71 to 80%), very good (81 to 90%), and excellent (91 to 100%). *Weed aerial survey*

In addition to traditional analysis methods, we assessed the weed control at 10 DAA using aerial imagery captured by a Phantom 4 Pro UAV. The UAV operated at an altitude of 11 meters, resulting in a Ground Sampling Distance (GSD) of 3.07 mm per pixel. It was equipped with an RGB visible spectrum camera. The UAV flights were planned and executed using the DroneDeploy software, while the production of orthomosaics was carried out using WebODM software.

To evaluate weed control, we calculated the Green Leaf Index (GLI) and generated a corresponding GLI map. This map was then subjected to classification using an unsupervised model known as k-means, which divided the data into two classes. The first class was designated as "weeds" (comprising green or blue pixels), while the second class was labelled "no-weed" (corresponding to zero pixel value). For each treatment, we defined a rectangular area measuring 2.5 meters by 18.0 meters. Classifications for control efficiency were assigned based on the percentage of weed coverage and categorized as follows: none or poor (91 to 100% soil coverage), fair (81 to 90%), sufficient (71 to 80%), good (61 to 70%), very good (41 to 60%), and excellent (21 to 40%).

Statistics methods

The weed phytosociological data collected through the traditional approach were subjected to descriptive statistical analysis and presented visually using bar graphs. Additionally, we delved into the weed spatial distribution with a specific emphasis on monocotyledonous and eudicotyledonous density. The weed spatial variability was computed for the Degree Spatial Dependence (DSD), it was categorized into three levels: weak (DSD < 25%), moderate (25% < DSD < 75%), and strong (DSD > 75%).

Conclusion

We identified twenty weed species, representing eighteen different genera and fourteen botanical families. It's worth noting that eudicotyledonous species were the dominant group, primarily characterized by an annual life cycle and sexual reproduction. The utilization of precision agricultural methods proved highly effective in conducting weed surveys before soybean seeding.

Using glyphosate alone is not recommended in fields with a history of reactive management. Alternatively, combining glyphosate with Protox-inhibitors showed outstanding control efficacy against glyphosate-resistant and glyphosate-tolerant weeds, such as *Cenchrus echinatus, Spermacoce verticillata and Turnera subulata*.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgments

The Coordination for the Advancement of Higher Education Personnel (CAPES) provided a scholarship for the first author. The Foundation for the Support of Scientific and Technological Research of Maranhão (FAPEMA), with financial support under code 750/22. The Soybean Producers Association of Maranhão, Meio-Norte branch, provided logistical assistance.

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