

EFFECT OF DIFFERENT HERBICIDES ON *BIDENS PILOSA* AND
EUPHORBIA HETEROPHYLLA BIOTYPES RESISTANT TO ALS
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Select herbicides with different mechanism of action is a satisfactory option for resistant weed control. Then, the present work aimed to study the efficiency of different herbicides and their mixtures on *Bidens pilosa* (blackjack) and *Euphorbia heterophylla* (wild poinsettia) biotypes, resistant to ALS herbicides in two development stages. The trials we arranged in a completely randomized design with four replications. The treatments tested were (g a.i/a.e ha⁻¹): imazethapyr at 70 and 140 (WG formulation) + 1.0% Assist; imazethapyr at 57.6 and 72 (SL formulation) + 1.0% Assist; imazapic + imazethapyr at 56 and 70 + 1.0% Assist; glyphosate + imazethapyr (596); saflufenacil + glyphosate at 35 + 720 + 0.5% Dash in tank mix, glyphosate at 720 and, a control without herbicide application. Control efficiency was evaluated, as well as dry matter accumulation at the end of the studies. Plants of both species were more susceptible to herbicides at the early stage of development (2 to 4 leaves). The treatments with saflufenacil + glyphosate, (imazethapyr + glyphosate) and glyphosate promoted the best controls, regardless of the species studied and the application stage. The mixture with saflufenacil provided the highest control speed, and the mixture (imazethapyr + glyphosate) was less efficient among three excellent treatments when applied to plants in the 4-6 leaf stage. The treatments (imazethapyr, in both formulations) and (imazethapyr + imazapic) were ineffective in controlling the studied biotypes, regardless of dose and developmental stage studied.

Keywords: Acetolactate Synthase. Blackjack. Resistance. Wild Poinsettia.**1. Introduction**

Currently, weed resistance to herbicides is a major concern of modern agriculture. Forty-three cases have already been reported in Brazil, with an average of two confirmed cases of resistance to herbicides per year. These data place Brazil in the fifth position in the ranking of countries with the highest number of resistance reports (Francischini et al. 2019; Heap 2019).

ALS-inhibiting herbicides was introduced to the world market in 1982 and are currently the leader in resistance cases worldwide, with a record of 159 resistant weed species, which represent 63% of cases (Chapinotto et al. 2017; Bonow et al. 2018; Heap 2019). According to Takano et al. (2016), the first report of resistance to ALS inhibitors in *Bidens pilosa* L. was verified in soybean fields in the state of Mato Grosso do Sul in 1993.

Christoffoleti e Nicolai (2016) considered as the main consequences of weed resistance to herbicides the unfeasibility of using these products, loss of planting areas, reduced productivity and product quality, need for herbicide reapplication, and changes in the production system.

In a report by Ulguim et al. (2019), for better decision-making in management strategies, one must know the factors that influence the pressure when selecting resistant weed biotypes. Among them, the adoption of more convenient and economical agricultural practices by producers may lead to the emergence of herbicide-resistant biotypes, forcing a change in the adopted practices, which requires close monitoring of populations for early and rapid resistance detection, thus avoiding economic losses (Burgos et al. 2013).

Some strategies can be efficient in the control of persistent weeds, such as the use of herbicides with different mechanisms of action, as well as their association, inhibiting the selection of new resistant biotypes (Powles and Yu 2010; Oliveira Neto et al. 2010).

Thus, selecting herbicides with a diverse mechanism of action becomes a satisfactory option for resistant weed control together with a management strategy against resistant plant development since a broader range of mechanism of action of herbicides is required (Trezzi et al. 2009).

Recent results of resistant species biotypes have been proven by several researchers, such as Chiapinotto et al. (2017), who found high levels of resistance of *Cyperus iria* L. to ALS-inhibiting herbicides. Similarly, Ulguim et al. (2019) observed that almost half of the samples taken in the Rio Grande do Sul from plants of the genus *Cyperus* were resistant to ALS inhibitors probably due to the high pressure of selection of resistant biotypes by the consecutive use of Clearfield® technology, application of doses higher than that registered, and low crop rotation adoption. Also, Bonow et al. (2018) observed that accessions of *Echinochloa crus-galli* var. *mitis* (Pursh) Peterm. in paddy fields also showed resistance to ALS-inhibiting herbicides, indicating the adoption of herbicides with alternative mechanisms of action, such as EPSPS inhibitors (glyphosate).

Thus, this research aimed to study the efficiency of some herbicides on *B. pilosa* and *E. heterophylla* biotypes resistant to ALS-inhibiting herbicides.

2. Material and Methods

The study was conducted under greenhouse conditions, with 2.5-L plastic pots filled with arable soil classified as a medium textured Dark Red Latosol. It consisted of four experiments, two per species (*B. pilosa* and *E. heterophylla*) resistant to ALS-inhibiting herbicides. For each species, herbicides were sprayed at two plant development stages (2–4 and 4–6 leaf pairs).

Treatments were arranged in a completely randomized design with four replications. Each experimental plot consisted of a pot with one plant per species. Treatments are shown in Table 1.

Table 1. Doses and herbicides tested in the control of *Bidens pilosa* and *Euphorbia heterophylla* plants.

| | Treatments | Dose (g a.i./a.e ha ⁻¹) |
|-----|--|-------------------------------------|
| 1. | Control | - |
| 2. | imazethapyr ¹ + Assist | 70.0 + 1.0% v/v |
| 3. | imazethapyr ¹ + Assist | 140.0 + 1.0% v/v |
| 4. | imazethapyr ² + Assist | 57.6 + 1.0% v/v |
| 5. | imazethapyr ² + Assist | 72.0 + 1.0% v/v |
| 6. | (imazapic + imazethapyr) ³ + Assist | 14.0 + 42.0 + 1.0% v/v |
| 7. | (imazapic + imazethapyr) ³ + Assist | 17.5 + 52.5 + 1.0% v/v |
| 8. | (glyphosate + imazethapyr) ⁴ | 509.9 + 86.0 |
| 9. | saflufenacil ⁵ + glyphosate ⁶ + Dash | 35.0 + 720.0 + 0.5% v/v |
| 10. | Glyphosate | 720.0 |

¹Pivot DG; ²Pivot 100 SL; ³Only; ⁴Alteza; ⁵Heat; ⁶Roundup Original; Assist and Dash = mineral oil/adjuvant.

The application was carried out using an air-pressurized stationary sprayer with a constant pressure of 200 kPa equipped with a boom with four Teejet XR 110.02VS flat fan spray tips, with a spacing of 0.5 m between them. The sprayer boom was displaced at a speed of 1 m s⁻¹, which resulted in a spray solution consumption of 200 L ha⁻¹.

Control efficiency of hairy beggarticks and wild poinsettia plants was evaluated at 1, 3, 7, 14, and 21 days after application (DAA) based on phytointoxication symptoms and using a scoring scale from 0 to 100%, where 0% represents the total absence of injury and 100% means plant death (SBCPD 1995). At the end of

the studies, plants of both species remaining in the pots were collected to determine their dry matter.

The results were subjected to analysis of variance by the F-test and means compared by the Tukey test at 5% probability level. The analysis of variance and comparison of means was performed by the program SISVAR.

3. Results and Discussion

Table 2 shows that the mixture of herbicides saflufenacil + glyphosate + Dash (Treatment 9) was the only treatment that provided injuries to *B. pilosa* plants at the stage of 2 to 4 leaf pairs at 1 DAA, reaching 71.5% control, which is considered good control. All herbicides and doses provided some intoxication effect on hairy beggarticks plants at 3 DAA, and the mixture contained in Treatment 9 reached an excellent control (97.5%) and all other treatments showed unsatisfactory controls.

Some treatments reduced and others increased the control at 7 DAA, such as imazethapyr + Assist at the two tested doses (Treatments 2 and 3), which provided low control increases, as well as Treatments 8 (glyphosate + imazethapyr) and 10 (glyphosate) applied alone, which had controls of 47.5 and 65%, respectively. Moreover, Treatment 9 (saflufenacil + glyphosate + Dash) provided the death (100%) of hairy beggarticks plants resistant to ALS inhibitors (Table 2).

The behavior of the different treatments tested at 14 DAA when compared to the control of *B. pilosa* plants was more explicit and consistent. The application of Treatments 8 (glyphosate + imazethapyr) and 10 (glyphosate) also resulted in the death of 100% of the plants, as reported for the mixture saflufenacil + glyphosate + Dash. Treatments 2 and 3 with imazethapyr + Assist in the two tested doses presented small increases and were still considered unsatisfactory. Also, Treatments 4 and 5 (imazethapyr + Assist) and 6 and 7 (imazethapyr + imazapic) + Assist were ineffective in controlling hairy beggarticks plants regardless of the dose, with the initial injuries disappearing completely. Monqueiro and Christoffoleti (2000) tested different herbicides on *B. pilosa* and *B. subalternans* DC. plants and observed that ALS-inhibiting herbicides were not efficient when applied alone or in mixture with each other. However, a satisfactory control was found in populations when a mixture of ALS-inhibiting herbicides was applied with alternative herbicides, making the control efficient in both susceptible and resistant populations, which corroborates the results obtained in the present study with Treatments 8, 9, and 10 (Table 2).

At the end of the study (21 DAA), only treatments with glyphosate (8, 9, and 10) provided control of hairy beggarticks plants, while treatments with imazethapyr (2 and 3) presented small injuries (Table 2). The lowest dry matter accumulations were also observed in these treatments. Treatments 4, 5, 6, and 7 still provided no injury to *B. pilosa* plants during this period, showing the biotype resistance to ALS-inhibiting herbicides, as well as to the two doses of imazethapyr + Assist (Treatments 2 and 3).

Bonow et al. (2018) observed that the application of ALS-inhibiting herbicides was not efficient in controlling resistant biotypes, recommending the adoption of products with alternative mechanisms of action such as ACCase inhibitors (cialofop-butyl, profloroxime and cletodime), glyphosate and propanil, corroborating the results found here.

Table 2. Effect of chemical treatments on the percentage of visual control and dry matter accumulation in *Bidens pilosa* plants at the stage of 2 to 4 leaf pairs during different evaluation periods after herbicide application.

| Treatments | Dose (g a.i./a.e ha ⁻¹) | Days After Application (DAA) | | | | | Dry Matter (g ⁻¹) |
|--------------------------------------|-------------------------------------|------------------------------|-------------------|-------------------|------------------|------------------|-------------------------------|
| | | 1 | 3 | 7 | 14 | 21 | |
| 1. control | - | 0.0 ^B | 0.0 ^E | 0.0 ^E | 0.0 ^C | 0.0 ^C | 4.0 ^A |
| 2. imazethapyr ¹ + Assist | 70.0 + 1.0% v/v | 0.0 ^B | 2.5 ^{CD} | 4.7 ^D | 8.0 ^B | 5.5 ^B | 1.6 ^{BC} |
| 3. imazethapyr ¹ + Assist | 140.0 + 1.0% v/v | 0.0 ^B | 2.5 ^{CD} | 6.2 ^D | 6.5 ^B | 5.0 ^B | 1.4 ^{BC} |
| 4. imazethapyr ² + Assist | 57.6 + 1.0% v/v | 0.0 ^B | 2.0 ^{DE} | 2.5 ^{DE} | 0.0 ^C | 0.0 ^C | 2.0 ^B |

| | | | | | | | |
|---|-------------------------|----------------------|----------------------|----------------------|----------------------|----------------------|---------------------|
| 5. imazethapyr ² + Assist | 72.0 + 1.0% v/v | 0.0 ^B | 3.2 ^{BCD} | 2.0 ^{DE} | 0.0 ^C | 0.0 ^C | 2.6 ^{AB} |
| 6. (imazapic + imazethapyr) ³ + Assist | 14.0 + 42.0 + 1.0% v/v | 0.0 ^B | 3.0 ^{BCD} | 2.7 ^{DE} | 0.0 ^C | 0.0 ^C | 2.5 ^{AB} |
| 7. (imazapic + imazethapyr) ³ + Assist | 17.5 + 52.5 + 1.0% v/v | 0.0 ^B | 3.2 ^{BCD} | 2.7 ^{DE} | 0.0 ^C | 0.0 ^C | 1.9 ^B |
| 8. (glyphosate + imazethapyr) ⁴ | 509.9 + 86.0 | 0.0 ^B | 4.5 ^{BC} | 47.5 ^C | 100 ^A | 100 ^A | 0.0 ^C |
| 9. saflufenacil ⁵ + glyphosate ⁶ + Dash | 35.0 + 720.0 + 0.5% v/v | 71.5 ^A | 97.5 ^A | 100 ^A | 100 ^A | 100 ^A | 0.0 ^C |
| 10. glyphosate | 720.0 | 0.0 ^B | 4.7 ^B | 65.0 ^B | 100 ^A | 100 ^A | 0.0 ^C |
| F treatments | | 608.65 ^{**} | 839.93 ^{**} | 359.76 ^{**} | 485.70 ^{**} | 729.89 ^{**} | 13.64 ^{**} |
| CV (%) | | 10.53 | 6.99 | 8.16 | 5.11 | 1.02 | 44.42 |
| LSD | | 1.82 | 2.08 | 4.60 | 3.88 | 0.76 | 1.71 |

¹Pivot DG; ²Pivot 100 SL; ³Only; ⁴Alteza; ⁵Heat; ⁶Roundup Original; Assist and Dash = mineral oil/adjuvant. ^{**}Significant at 1% probability. Means followed by the same letter in the column do not differ statistically from each other by the Tukey test ($p > 0.05$).

Table 3 shows that some herbicides had already caused intoxication to the most developed *B. pilosa* plants (4 to 6 leaf pairs) at 1 DAA, especially the mixture saflufenacil + glyphosate + Dash (Treatment 9), with a 42% control, but lower than that found when plants had 2 to 4 leaf pairs (Table 2), which reached 71.5% control during this same period. Some treatments showed an evolution in the control at 3 DAA, but still unsatisfactory, standing out the Treatment 9, with 83.2% control.

At 7 DAA, chemical treatments behaved similarly to those applied in the first tested stage (2 to 4 leaf pairs), but with lower controls (Table 3). A continuous increased control of *Bidens pilosa* plants was also observed in some treatments at 14 DAA, such as Treatments 8 (imazethapyr + glyphosate), 9 (saflufenacil + glyphosate + Dash), and 10 (glyphosate), with 66.2, 100, and 94.5% control, respectively, besides showing that the mixture of imazethapyr + glyphosate had an inferior behavior when compared to the isolated application of glyphosate. The mixture of saflufenacil + glyphosate + Dash led to the death of hairy beggarticks plants only at 14 DAA, unlike at the stage of 2 to 4 leaf pairs, in which plant death occurred at 7 DAA (Table 2). It shows that adequate management of the mechanism of action of herbicides, coupled with the correct plant development stage for the application, provides more efficient control over weeds, especially for resistant biotypes. These results corroborate those observed by Braz et al. (2011), when they applied glyphosate to control resistant biotypes in a GR cotton crop, proving to be a viable alternative to ALS-inhibiting herbicides.

An increase in the control of *B. pilosa* plants in relation to the previous evaluation was observed at 21 DAA for treatments with imazethapyr + glyphosate and glyphosate, with 90 and 99.5% control, respectively, while the mixture saflufenacil + glyphosate + Dash reached 100% control. However, the mixture of imazethapyr + glyphosate provided a lower control at this more advanced stage of plant development (4 to 6 leaves). Table 3 also shows that dry matter accumulation in the plants was significantly lower in treatments that visually presented the best controls (Treatments 8, 9, and 10). The use of herbicides with different mechanism of action or combined with ALS-inhibiting herbicides has become an alternative for the control of resistant *B. pilosa* plants since herbicides such as imazethapyr stand out for their efficient control over other weed species, such as *Setaria* spp., *Chenopodium album* L., *Solanum* spp., *Amaranthus retroflexus* L., and *Digitaria sanguinalis* (L.) Scop (Alister and Kogan 2005).

All treatments with imazethapyr + Assist (Treatments 2 and 3), imazethapyr + Assist (Treatments 4 and 5), and imazethapyr + imazapic + Assist (Treatments 7 and 8) were not effective in controlling *B. pilosa* plants at the stage of 4 to 6 leaf pairs, showing the resistance of the biotype to the group of ALS-inhibiting herbicides (Table 3). Takano et al. (2016) worked with dose-response curves of the herbicide imazethapyr on resistant and susceptible *B. pilosa* biotypes and found that the resistant biotype was not controlled even at high doses (800 g ha⁻¹), which is $\approx 8x$ the recommended dose, with control lower than 50%.

Table 3. Effect of chemical treatments on the percentage of visual control and dry matter accumulation in *Bidens pilosa* plants at the stage of 4 to 6 leaf pairs during different evaluation periods after herbicide application.

| Treatments | Dose (g a.i./a.e ha ⁻¹) | Days After Application (DAA) | | | | | Dry Matter (g ⁻¹) |
|---|-------------------------------------|------------------------------|----------------------|----------------------|----------------------|----------------------|-------------------------------|
| | | 1 | 3 | 7 | 14 | 21 | |
| 1. control | - | 0.0 ^B | 0.0 ^C | 0.0 ^D | 0.0 ^C | 0.0 ^C | 6.96 ^A |
| 2. imazethapyr ¹ + Assist | 70.0 + 1.0% v/v | 0.0 ^B | 0.0 ^C | 0.0 ^D | 0.0 ^D | 0.0 ^C | 7.47 ^A |
| 3. imazethapyr ¹ + Assist | 140.0 + 1.0% v/v | 0.0 ^B | 0.0 ^C | 4.2 ^D | 0.0 ^D | 0.0 ^C | 7.96 ^A |
| 4. imazethapyr ² + Assist | 57.6 + 1.0% v/v | 0.0 ^B | 2.7 ^B | 5.2 ^D | 0.0 ^D | 0.0 ^C | 6.61 ^A |
| 5. imazethapyr ² + Assist | 72.0 + 1.0% v/v | 0.0 ^B | 0.0 ^{BC} | 4.2 ^D | 0.0 ^D | 0.0 ^C | 6.07 ^A |
| 6. (imazapic + imazethapyr) ³ + Assist | 14.0 + 42.0 + 1.0% v/v | 0.0 ^B | 0.0 ^C | 2.2 ^D | 7.5 ^C | 0.0 ^C | 5.40 ^{AB} |
| 7. (imazapic + imazethapyr) ³ + Assist | 17.5 + 52.5 + 1.0% v/v | 0.0 ^B | 9.7 ^C | 1.0 ^D | 3.0 ^C | 0.0 ^C | 5.85 ^A |
| 8. (glyphosate + imazethapyr) ⁴ | 509.9 + 86.0 | 0.0 ^B | 97.5 ^B | 14.7 ^C | 66.2 ^B | 90.0 ^B | 2.23 ^{BC} |
| 9. saflufenacil ⁵ + glyphosate ⁶ + Dash | 35.0 + 720.0 + 0.5% v/v | 85.0 ^B | 100 ^A | 98.5 ^A | 100 ^A | 100 ^A | 0.92 ^C |
| 10. glyphosate | 720.0 | 0.0 ^B | 97.5 ^B | 30.0 ^B | 94.5 ^A | 99.5 ^A | 0.92 ^C |
| F treatments | | 1734.00 ^{**} | 995.48 ^{**} | 639.67 ^{**} | 932.08 ^{**} | 319.25 ^{**} | 3.80 ^{**} |
| CV (%) | | 15.19 | 8.42 | 14.97 | 10.22 | 8.54 | 28.56 |
| LSD | | 3.11 | 6.84 | 5.81 | 6.69 | 6.13 | 3.47 |

¹Pivot DG; ²Pivot 100 SL; ³Only; ⁴Alteza; ⁵Heat; ⁶Roundup Original; Assist and Dash = mineral oil/adjutant. ^{**}Significant at 1% probability. Means followed by the same letter in the column do not differ statistically from each other by the Tukey test (p>0.05).

Table 4 shows that the mixture of saflufenacil + glyphosate + Dash was the only chemical treatment that caused damage to *E. heterophylla* plants (2 to 4 leaf pairs) at 1 DAA, reaching 85% control. The mixture of saflufenacil + glyphosate + Dash increased the control over wild poinsettia plants at 3 DAA, reaching 96.2%. On the other hand, Treatments 8 (imazethapyr + glyphosate) and 10 (glyphosate) presented controls of the order of 30%, and the others did not provide visual injuries during this period (Table 4).

Most chemical treatments provided some toxic effect to *E. heterophylla* plants at 7 DAA and showed evolution in their control, except for the application of Treatment 6 (imazethapyr + imazapic + Assist) at the lowest dose. The mixture of herbicides of Treatment 9 (saflufenacil + glyphosate + Dash) resulted in the death of wild poinsettia plants during this period (100%), while Treatments 8 (imazethapyr + glyphosate) and 10 (glyphosate) provided a similar and efficient control, with a value of 97.5% (Table 4). All other treatments and doses provided unsatisfactory controls, which proves the resistance of this biotype of *E. heterophylla* to ALS-inhibiting herbicides. Similarly, Chiapinotto et al. (2017) worked with dose-response to confirm resistance in biotypes of *C. iria* and found the existence of resistant biotypes to ALS-inhibiting herbicides, as the control level and dry matter reduction was lower and gradual when compared to those of the susceptible biotype. According to these researchers, the emergence of resistance necessarily occurs due to a change in the structure of the ALS enzyme and its site of action, caused by a mutation in the gene (Ntoanidou et al. 2016).

Most chemical treatments showed decreases in the control of *E. heterophylla* plants at 14 DAA, except for Treatments 8 (imazethapyr + glyphosate) and 10 (glyphosate), which provided a 100% control of wild poinsettia plants (Table 4), as in Treatment 9 (saflufenacil + glyphosate + Dash).

At the end of the study (21 DAA), treatments with persistent visual intoxication symptoms slowed these effects, which also allowed weed recovery, except for treatments with glyphosate, which had already reached 100% control. Dry matter accumulation in plants of *E. heterophylla* was null in treatments with glyphosate (8, 9, and 10), while the other treatments were similar to the control without herbicide application (Table 4). Monqueiro and Christoffoleti (2000) observed that *E. heterophylla* biotypes resistant to ALS-inhibiting herbicides were efficiently controlled by Protox-, GS-, and EPSEs-inhibiting herbicides, which could be used to minimize the selection effects and manage the resistant population of *E. heterophylla*, as observed. These results showed that the tested biotype was susceptible to the mixture of saflufenacil + glyphosate + Dash and herbicides imazethapyr + glyphosate and glyphosate applied alone at this early development stage, as they have another mechanism of action. All other treatments with imazethapyr + Assist and imazethapyr + imazapic + Assist were ineffective in controlling *E. heterophylla* biotype regardless of the dose and formulation.

Table 4. Effect of chemical treatments on the percentage of visual control and dry matter accumulation in *Euphorbia heterophylla*, plants at the stage of 2 to 4 leaf pairs during different evaluation periods after herbicide application.

| Treatments | Dose (g a.i./a.e ha ⁻¹) | Days After Application (DAA) | | | | | Dry Matter (g ⁻¹) |
|---|-------------------------------------|------------------------------|-------------------|----------------------|----------------------|----------------------|-------------------------------|
| | | 1 | 3 | 7 | 14 | 21 | |
| 1. control | - | 0.0 ^B | 0.0 ^C | 0.0 ^C | 0.0 ^D | 0.0 ^C | 3.73 ^A |
| 2. imazethapyr ¹ + Assist | 70.0 + 1.0% v/v | 0.0 ^B | 0.0 ^C | 7.5 ^B | 7.0 ^{BC} | 0.0 ^C | 4.12 ^A |
| 3. imazethapyr ¹ + Assist | 140.0 + 1.0% v/v | 0.0 ^B | 0.0 ^C | 11.2 ^B | 6.0 ^{BC} | 0.0 ^C | 4.41 ^A |
| 4. imazethapyr ² + Assist | 57.6 + 1.0% v/v | 0.0 ^B | 0.0 ^C | 8.5 ^B | 8.7 ^B | 1.2 ^{BC} | 3.86 ^A |
| 5. imazethapyr ² + Assist | 72.0 + 1.0% v/v | 0.0 ^B | 0.0 ^C | 5.0 ^{BC} | 3.0 ^{CD} | 2.2 ^{BC} | 4.03 ^A |
| 6. (imazapic + imazethapyr) ³ + Assist | 14.0 + 42.0 + 1.0% v/v | 0.0 ^B | 0.0 ^C | 0.0 ^C | 0.0 ^D | 0.0 ^C | 2.78 ^{AB} |
| 7. (imazapic + imazethapyr) ³ + Assist | 17.5 + 52.5 + 1.0% v/v | 0.0 ^B | 0.0 ^C | 9.7 ^B | 6.7 ^{BC} | 3.2 ^B | 2.68 ^{AB} |
| 8. (glyphosate + imazethapyr) ⁴ | 509.9 + 86.0 | 0.0 ^B | 30.0 ^B | 97.5 ^A | 100 ^A | 100 ^A | 0.0 ^B |
| 9. saflufenacil ⁵ + glyphosate ⁶ + Dash | 35.0 + 720.0 + 0.5% v/v | 85.0 ^A | 96.2 ^A | 100 ^A | 100 ^A | 100 ^A | 0.0 ^B |
| 10. glyphosate | 720.0 | 0.0 ^B | 30.0 ^B | 97.5 ^A | 100 ^A | 100 ^A | 0.0 ^B |
| F treatments | | 734.55 ^{**} | 153.90 | 995.48 ^{**} | 229.18 ^{**} | 503.39 ^{**} | 8.15 ^{**} |
| CV (%) | | 15.19 | 30.99 | 8.42 | 5.82 | 3.87 | 50.59 |
| LSD | | 3.11 | 11.96 | 6.84 | 4.65 | 2.87 | 3.13 |

¹Pivot DG; ²Pivot 100 SL; ³Only; ⁴Alteza; ⁵Heat; ⁶Roundup Original; Assist and Dash = mineral oil/adjuvant. ^{**}Significant at 1% probability. Means followed by the same letter in the column do not differ statistically from each other by the Tukey test (p>0.05).

The effect of different chemical treatments on *E. heterophylla* plants at the stage of 4 to 6 leaf pairs showed that only the mixture saflufenacil + glyphosate + Dash (Treatment 9) caused plant injury to wild poinsettia at 1 DAA, but with a very poor control of 18.7% (Table 5), which is much lower than that found at the stage of 2 to 4 leaf pairs (85%) (Table 4). All herbicides provided some toxic effect to *E. heterophylla* plants at 3 DAA, except for the treatment with the herbicide imazethapyr + imazapic + Assist (Treatment 6) at the lowest dose. Plants treated with the saflufenacil + glyphosate + Dash showed significant control increases, reaching a value of 57.5% (Table 5).

Only the mixture saflufenacil + glyphosate + Dash provided an efficient control of wild poinsettia plants at 7 DAA, with a value of 96.7% (Table 5). However, the other chemical treatments were still ineffective to control wild poinsettia plants, unlike that observed at the first studied development stage,

when the mixture of imazethapyr + glyphosate and the isolated application of glyphosate provided efficient controls during this period, with a value of 97.7% control for both treatments (Table 4) and 46.2 and 67.5%, respectively (Table 5). An increase in control efficiency of some of the tested treatments was observed at 14 DAA, with only the treatments saflufenacil + glyphosate + Dash, imazethapyr + glyphosate, and glyphosate providing controls ranging from good to excellent (99, 82.5, and 97%, respectively) (Table 5).

Increases in the action of treatments with imazethapyr + glyphosate, saflufenacil + glyphosate + Dash, and glyphosate were observed again on *E. heterophylla* plants at 21 DAA, with values of controls of 88.7, 100, and 98%, respectively, while the mixture of imazethapyr + glyphosate remained inferior. The high toxic visual effects recorded in wild poinsettia plants in these three treatments reflected statistically on dry matter accumulation of plants, with lower accumulations when compared to other treatments that did not provide efficient control of plants (Table 5).

These results showed that the tested biotype was susceptible to Treatments 8, 9, and 10, as observed for the development stage of 2 to 4 leaf pairs (Table 4). However, a lower action of these treatments was evidenced at the stage of 4 to 6 leaf pairs, but the mixture saflufenacil + glyphosate + Dash showed a total control (Table 5), evidencing the importance of the rotation of herbicides of different mechanisms of action. In this sense, Vargas et al. (2013) studied plants of *E. heterophylla* resistant to ALS inhibitors and observed an efficient control with glyphosate application. This research highlighted the importance of using crop rotation to prevent the selection of resistant biotypes, mixtures of herbicides with different mechanisms of action as a strategy to avoid resistance to herbicides, and constant monitoring of the occurrence of plant escape.

All treatments with imazethapyr + Assist and imazethapyr + imazapic + Assist were ineffective in controlling *E. heterophylla* plants at the stage of 4 to 6 leaf pairs (Table 5), which corroborates the results obtained by Aarestrup et al. (2008), who verified that a resistant biotype of *E. heterophylla* was not controlled by another ALS-inhibiting herbicide (chlorimuron-ethyl).

Table 5. Effect of chemical treatments on the percentage of visual control and dry matter accumulation in *Euphorbia heterophylla*, plants at the stage of 4 to 6 leaf pairs during different evaluation periods after herbicide application.

| Treatments | Dose (g a.i./a.e ha ⁻¹) | Days After Application (DAA) | | | | | Dry Matter (g ⁻¹) |
|---|-------------------------------------|------------------------------|-------------------|---------------------|----------------------|----------------------|-------------------------------|
| | | 1 | 3 | 7 | 14 | 21 | |
| 1. control | - | 0.0 ^B | 0.0 ^B | 0.0 ^C | 0.0 ^C | 0.0 ^C | 6.00A |
| 2. imazethapyr ¹ + Assist | 70.0 + 1.0% v/v | 0.0 ^B | 0.7 ^B | 0.7 ^C | 0.0 ^C | 0.0 ^C | 5.52 ^A |
| 3. imazethapyr ¹ + Assist | 140.0 + 1.0% v/v | 0.0 ^B | 0.0 ^B | 0.0 ^C | 0.0 ^C | 0.0 ^C | 6.50 ^A |
| 4. imazethapyr ² + Assist | 57.6 + 1.0% v/v | 0.0 ^B | 0.7 ^B | 2.5 ^B | 1.2 ^C | 0.0 ^C | 6.00 ^A |
| 5. imazethapyr ² + Assist | 72.0 + 1.0% v/v | 0.0 ^B | 0.5 ^B | 6.2 ^C | 3.7 ^C | 1.7 ^C | 5.00 ^A |
| 6. (imazapic + imazethapyr) ³ + Assist | 14.0 + 42.0 + 1.0% v/v | 0.0 ^B | 0.0 ^B | 0.0 ^C | 0.0 ^C | 0.0 ^C | 6.75 ^A |
| 7. (imazapic + imazethapyr) ³ + Assist | 17.5 + 52.5 + 1.0% v/v | 0.0 ^B | 0.7 ^B | 1.0 ^C | 0.0 ^C | 0.0 ^C | 4.75 ^A |
| 8. (glyphosate + imazethapyr) ⁴ | 509.9 + 86.0 | 0.0 ^B | 0.7 ^B | 46.2 ^B | 82.5 ^B | 88.7 ^B | 2.50 ^B |
| 9. saflufenacil ⁵ + glyphosate ⁶ + Dash | 35.0 + 720.0 + 0.5% v/v | 18.7 ^A | 57.5 ^A | 96.7 ^A | 99.0 ^A | 100 ^A | 3.15 ^B |
| 10. glyphosate | 720.0 | 0.0 ^B | 1.7 ^B | 67.5 ^B | 97.0 ^A | 98.0 ^A | 3.00 ^B |
| F treatments | | 61.36 ^{**} | 134.46 | 63.62 ^{**} | 512.12 ^{**} | 538.00 ^{**} | 2.16 ^{**} |
| CV (%) | | 80.74 | 49.05 | 39.74 | 13.94 | 4.75 | 32.85 |
| LSD | | 3.65 | 7.48 | 21.31 | 9.53 | 3.31 | 3.28 |

¹Pivot DG; ²Pivot 100 SL; ³Only; ⁴Alteza; ⁵Heat; ⁶Roundup Original; Assist and Dash = mineral oil/adjuvant. **Significant at 1% probability. Means followed by the same letter in the column do not differ statistically from each other by the Tukey test (p>0.05).

4. Conclusions

Plants of both species were more susceptible to herbicides at the early development stage (2 to 4 leaves). Treatments with saflufenacil + glyphosate + Dash, imazethapyr + glyphosate, and glyphosate promoted the best controls regardless of the species and application stage, and the mixture with saflufenacil provided the highest control rate; the mixture imazethapyr + glyphosate was less efficient among the best treatments when applied to plants of both species at the stage of 4 to 6 leaves; and the treatments imazethapyr + Assist (both formulations) and imazethapyr + imazapic + Assist were ineffective in controlling the studied biotypes regardless of the dose and stage of development.

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