Efficacy of imazapic/imazapyr and other herbicides in mixtures for the control of *Digitaria insularis* prior to soybean sowing

Efectividad de imazapic/imazapyr y otros herbicidas en mezclas para el control de *Digitaria insularis* en pre-siembra de soya

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ABSTRACT

Herbicide mixtures, use of multiple sites of action, and other weed management practices are necessary to avoid cases of biotype resistance. The aim of this study was to evaluate the efficiency of imazapic/imazapyr and other herbicides in mixtures to control *Digitaria insularis* at burndown before soybean sowing. This field research was conducted in Umuarama, State of Parana (PR), Brazil, in the 2018/19 soybean season. The experiment was conducted in a randomized block experimental design with four replicates and 11 treatments composed of the application of glyphosate, clethodim, haloxyfop, imazapic/imazapyr, glufosinate, 2,4-dichlorophenoxyacetic acid (2,4-D), dicamba, triclopyr, and saflufenacil, in mixtures. Weed control was evaluated as well as soybean injury and yield. An analysis of variance and F-test were performed, and the treatment means were compared by the Scott-Knott test. All treatments showed great control over the weed and low crop injury rate while maintaining soybean yield. The application of imazapic/imazapyr in mixtures with other herbicides was effective in controlling glyphosate-resistant *D. insularis* in burndown before soybean sowing and with sequential application of haloxyfop + glyphosate at V3 stage of soybean. This chemical management was also selective for soybean.

Key words: *Glycine max*, acetolactate synthase (ALS) inhibitors, weed, crop injury, chemical weed control.

RESUMEN

Las mezclas entre herbicidas, el uso de múltiples sitios de acción y otras prácticas de manejo de malezas son necesarias para evitar otros casos de resistencia de biotipos. El objetivo de este estudio fue evaluar la eficiencia de imazapic/imazapyr y otros herbicidas en mezclas para controlar *Digitaria insularis* en la desecación antes de la siembra de soya. Esta investigación de campo se realizó en Umuarama, Estado de Paraná (PR), Brasil, en la cosecha de soya de 2018/19. El experimento se realizó en un diseño experimental de bloques al azar, con cuatro repeticiones y 11 tratamientos, compuestos por la aplicación de glifosato, cletodim, haloxifop, imazapic/imazapir, glufosinato, ácido 2,4-diclorofenoxiacético (2,4-D), dicamba, triclopir y saflufenacil, en mezclas. Se evaluó el control de malezas, así como el daño y el rendimiento de la soya. Se realizaron un análisis de varianza y test F, y se compararon las medias de los tratamientos con el test de Scott-Knott. Todos los tratamientos mostraron un gran control sobre la maleza y una baja tasa de daño a los cultivos mientras se mantenía el rendimiento de la soya. La aplicación de imazapic/imazapir en mezclas con otros herbicidas fue eficaz en el control de *D. insularis* resistente al glifosato, en desecación antes de la siembra de soya y con aplicación secuencial de haloxifop + glifosato en la etapa V3 de la soya. Este manejo químico también fue selectivo para la soya.

Palabras clave: *Glycine max*, inhibidores de acetolactato sintasa (ALS), hierba, daño al cultivo, control químico de malezas.

Introduction

The weed *Digitaria insularis,* known as sourgrass, is native to tropical and subtropical areas of America. It can be found in pasture lands, coffee plantations, orchards, crop fields, roadsides, and in abandoned fields. It is referred to as rhizomatous and produces seeds throughout the summer, growing in clumps. These traits allow them to aggressively compete against cultivated crops (Moreira and Bragança, 2011; Lorenzi, 2014). There are four known *D. insularis* herbicide-resistant biotypes, all found in South America, from which three are glyphosate-resistant and one is resistant to haloxyfop and fenoxaprop. Weed resistance is a problem across the globe, and Brazil is ranked fifth with 50 registered resistance cases to EPSPs, ALS, ACCase, PSII, PSI, PPO, and auxins (Heap, 2020).

Received for publication: 21 October, 2019. Accepted for publication: 9 November, 2020. Doi: 10.15446/agron.colomb.v38n3.83046

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In addition to resistance cases, as a perennial grass *D. insularis* is hard to control, especially during flowering development (Zobiole *et al*., 2016; Canedo *et al*., 2019). A study by Gazziero *et al*. (2019) shows that a cohabitation of six *D. insularis* plants m^{-2} among soybean crops reduces its yield by 40%. Thus, appropriate managing practices including different control methods are shown to be important to have an efficient outcome.

The association of different herbicides, rotation of sites of actions, preventive methods, among other practices are essential to manage and prevent new weed biotypes resistant to herbicides (Green, 2018; Heap and Duke, 2018; Neve *et al*., 2018; Frisvold *et al*., 2020). However, in the literature, there are reports of the antagonist effect of 2,4-D on the action of ACCase inhibitor graminicides, due to the translocation reduction and increase in the metabolism of herbicides from the ariloxifenoxipropionics group (Trezzi *et al*., 2007). Thus, there is a need for studies on the different mechanisms of control of *D. insularis*.

Pre-emergent herbicides used before sowing are emphasized as an important tool to weed management, especially in crop fields (Byker *et al*., 2013; Belfry *et al*., 2015). In this sense, ALS inhibitors are highlighted for weed control as pre-emergence herbicides used in soybean fields (Braz *et al*., 2017; Underwood *et al*., 2017). Imidazolinone herbicides are part of the group of the acetolactate synthase (ALS) (also called acetohydroxyacid synthase [AHAS]) inhibiting herbicides which hamper the synthesis of the branched-chain amino acids valine, leucine, and isoleucine. After their absorption, the herbicides are translocated to meristems and apexes that are actively growing areas, inhibiting the growth of susceptible plants. Chlorosis happens to sensitive plants followed by death in 7 to 14 d after treatment (Oliveira Júnior, 2011; Shaner and O'Connor, 2017).

These herbicides are applied at pre- and post-emergence, controlling a range of monocotyledons and dicotyledons in cereal and soybean crops and nonagricultural areas (Oliveira Júnior, 2011; Rodrigues and Almeida, 2018). Piasecki and Rizzardi (2016) report that imazapic/imazapyr (premix formulation) is efficient for controlling volunteer corn at soybean pre-emergence. Likewise, studies by Melo *et al*. (2017) show the efficiency of imazapic/imazapyr on the control of *D. insularis*.

It is believed that the imazapic/imazapyr is effective in controlling *D. insularis* when applied at pre-sowing burndown. Thus, this study aimed to evaluate the efficiency of imazapic/imazapyr (premix formulation) and other herbicides in mixtures to control *D. insularis* at burndown before soybean sowing.

Materials and methods

Conditions and experimental design

This field research was conducted in Umuarama, State of Parana (PR), Brazil (23°50'25.23" S, 53°13'45.70" W) during the 2018/19 crop season. The soil was classified as sandy (11% clay, 6.5% silt, and 82.5% sand) with the following chemical properties on the 0-20 cm layer: pH ($CaCl_2$) of 4.4, 1.33% organic matter, and 5.78 cmol$_c$ dm^{-3} cation exchange capacity. Under the Köppen classification, the climate is classified as Cfa, and Figure 1 shows the weather conditions during the time of the research.

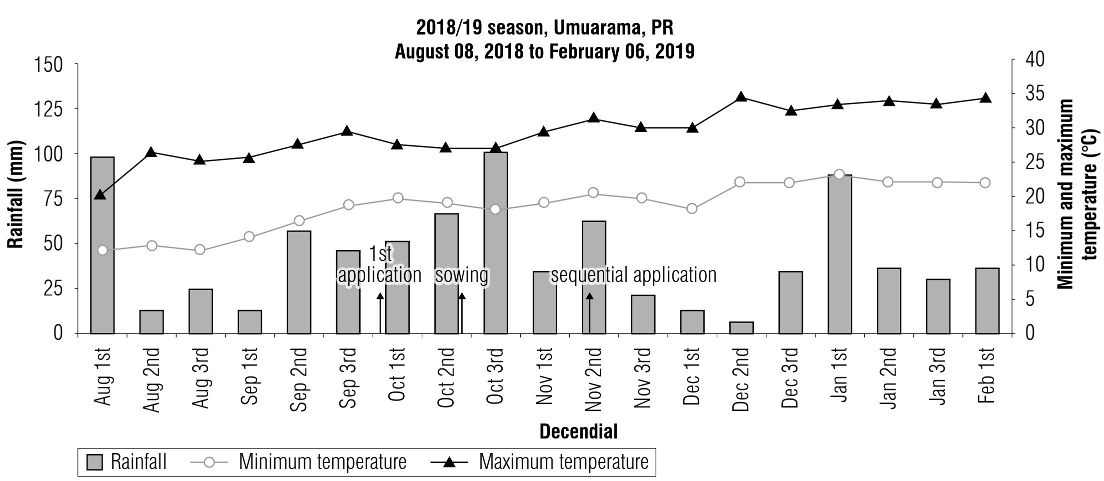

FIGURE 1. Representation of rainfall and minimum and maximum temperature for the study site. 2018/19 season, Umuarama, PR, Brazil.

The trial site was infested by *D. insularis,* identified as indicative of glyphosate resistance. Before application, the plants were at the flowering stage with 3.5 clumps m^{-2}. The area was previously cultivated with maize. A no-till sowing system was used for soybean, using 0.45 cm row spacing. The cultivar used was Monsoy® 6410 IPRO (Monsanto Co. do Brasil, São Paulo, SP, Brazil). The experiment was conducted in a randomized block experimental design, with four replications and 5x3 m plots. For the useful area, the four central lines were used, discarding the first and last meter of the plot. The treatments are described in Table 1. The commercial products used in the trial are presented in Supplementary material 1.

The first application was performed on October 5th, 2018. The soybean was sown on October 18th, 2018, and the sequential application was carried out on November 16th, 2018 when soybean plants were at the V3 growth stage (Fehr *et al*., 1971). One hundred mm of rainfall was reported in the area between the first application and seeding.

Both applications were performed using a CO_2 backpack sprayer at 3.6 km/h with AIXR 110.015 spray nozzles pressured at 2.5 kgf cm^{-2} with a volume of 150 L ha^{-1}. The weather conditions were a temperature of 23.1ºC, 62.3% relative humidity, and wind speed of 6.8 km/h. The sequential application had the following conditions: temperature of 23.1ºC, 60.1% relative humidity, and wind speed of 2.2 km/h.

Evaluations

Weed control was visually evaluated at 7, 14, 21, and 35 d after application (DAA) and on the 14th and 28th d after the sequential application. Soybean crop injury was evaluated at 7, 14, 21, 28, and 35 d after sowing (DAS). Percentage grades from 0% (absence of symptoms) to 100% (death of the plant) were given, based on the apparent symptoms related to the plant growth stage (Velini *et al*., 1995).

By the time of harvest, at the R8 stage (full maturation) of soybean plants (Fehr *et al*., 1971), the yield was calculated using only the plot's two central lines of 4 m long each. The grain moisture was corrected to 13% and the results converted to kg ha^{-1}.

Data analysis

An analysis of variance and the F-test ($P\leq0.05$) were performed as described by Pimentel-Gomes and Garcia (2002). The means of treatments were grouped together using the Scott-Knott (1974) test ($P\leq0.05$). For this purpose, the software Sisvar 5.6 was used (Ferreira, 2011).

Results

There was little or no control observed in the first application at 7 DAA (all below 35%); however, at 14 DAA all treatments associated with auxinic herbicides and saflufenacil showed inferior control compared to the others. At 21 DAA, the best results were observed for treatments with the application of ACCase inhibitors (haloxyfop and clethodim). Control superior to 80% was reported at 28 and 35 DAA by all herbicide combinations. It is important to highlight that at 35 DAA only the treatments involving glufosinate and 2,4-D showed lower performance (Tab. 2).

After the sequential application of glyphosate + haloxyfop at 14 DAA, weed control of at least 83.3% was observed. Besides that, the treatments involving auxinic herbicides and

TABLE 1. Treatments consisting of herbicide mixtures for the control of *D. insularis.* 2018/19 season, Umuarama, PR, Brazil.

Treatments[1]	Rates[2]
Control (without herbicide application)	-
Glyphosate + clethodim[3]	1,080 + 192
Glyphosate + haloxyfop[4]	1,080 + 120
Glyphosate + imazapic/imazapyr[5]	1,080 + 78.75/26.25
Glufosinate + imazapic/imazapyr[5]	500 + 78.75/26.25
Glyphosate + clethodim[3] + imazapic/imazapyr	1,080 + 192 + 78.75/26.25
Glyphosate + haloxyfop[4] + imazapic/imazapyr	1,080 + 120 + 78.75/26.25
Glyphosate + 2,4-D + imazapic/imazapyr[5]	1,080 + 670 + 78.75/26.25
Glyphosate + dicamba + imazapic/imazapyr[5]	1,080 + 288 + 78.75/26.25
Glyphosate + triclopyr + imazapic/imazapyr[5]	1,080 + 480 + 78.75/26.25
Glyphosate + saflufenacil + imazapic/imazapyr[5]	1,080 + 35 + 78.75/26.25

[1]Sequential application in all herbicide treatments of haloxyfop (66 g acid equivalent (a.e.) ha^{-1}) + glyphosate (720 g a.e. ha^{-1}), at post-emergence (V3) of soybean.

[2]Rates at g a.e. ha^{-1} for glyphosate, haloxyfop, 2,4-D and triclopyr. Rates at g active ingredient (a.i.) ha^{-1} for other herbicides. Addition of adjuvants: [3]Lanzar® 0.5% v/v; [4]Joint® 0.5% v/v; [5]Dash® HC 0.5% v/v.

the ones involving glufosinate were considered inferior as grass regrowth was frequently observed (≥89%). However, at 28 DAA efficient *D. insularis* control was observed by all the tested combinations of herbicides, computing a rate control of at least 94.8%. The application of glufosinate + imazapic/imazapyr is highlighted for its control levels of 85% at 35 DAA and 96.3% after the sequential application at 28 DAA (Tab. 2), indicating the importance of adding sequential applications to weed management practices.

Lower crop injury by all evaluations was also observed. In general, symptoms were characterized by small chloroses and yellowing of leaves. The application of glyphosate with clethodim or haloxyfop did not cause symptoms (Tab. 3).

TABLE 2. Control (%) of *D. insularis* under application of herbicides mixtures. 2018/19 season, Umuarama, PR, Brazil.

Treatments[1]	After 1st application					After 2nd application	
	7	14	21	28	35	14	28
	DAA						
Control (without application)	0.0 c	0.0 c	0.0 d	0.0 c	0.0 c	0.0 c	0.0 b
Glyphosate + clethodim	28.3 b	43.0 a	88.8 a	94.3 a	92.0 a	93.8 a	94.8 a
Glyphosate + haloxyfop	26.5 b	43.3 a	85.3 a	90.0 a	91.3 a	91.3 a	97.0 a
Glyphosate + imazapic/imazapyr	24.8 b	40.0 a	76.8 b	88.5 b	92.0 a	93.0 a	96.0 a
Glufosinate + imazapic/imazapyr	20.8 b	38.8 a	64.3 c	83.5 b	85.0 b	86.3 b	96.3 a
Glyphosate + clethodim + imazapic/imazapyr	34.8 a	42.5 a	88.8 a	92.5 a	94.0 a	93.3 a	97.5 a
Glyphosate + haloxyfop + imazapic/imazapyr	28.0 b	41.5 a	82.3 a	92.8 a	94.0 a	93.0 a	97.0 a
Glyphosate + 2,4-D + imazapic/imazapyr	25.3 b	30.0 b	74.5 b	83.0 b	83.8 b	83.8 b	96.0 a
Glyphosate + dicamba + imazapic/imazapyr	27.0 b	32.3 b	76.3 b	87.0 b	89.8 a	89.0 b	96.3 a
Glyphosate + triclopyr + imazapic/imazapyr	31.8 a	34.8 b	73.3 b	86.3 b	89.3 a	86.5 b	96.3 a
Glyphosate + saflufenacil + imazapic/imazapyr	26.5 b	35.0 b	77.0 b	91.8 a	91.3 a	92.8 a	97.0 a
Mean	24.9	34.6	71.5	80.9	82.0	82.0	87.6
CV (%)	14.2	12.3	8.8	4.3	3.0	4.5	1.4
F	*	*	*	*	*	*	*

DAA: days after application; CV: coefficient of variation.

[1]Sequential application in all herbicide treatments of haloxyfop (66 g acid equivalent (a.e.) ha^{-1}) + glyphosate (720 g a.e. ha^{-1}), at post-emergence (V3) of soybean.

*Means followed by the same letter in the column do not differ from each other by the Scott-Knott (1974) test ($P \leq 0.05$).

TABLE 3. Crop injury (%) and yield (kg ha^{-1}) of soybean plants under application of herbicide mixtures, for the control of *D. insularis*. 2018/19 season, Umuarama, PR, Brazil.

Treatments[1]	Crop injury				Yield
	14	21	28	35	
	DAS				
Control (without application)	0.0 a	0.0 a	0.0 a	0.0 a	825 b
Glyphosate + clethodim	0.0 a	0.0 a	0.0 a	0.0 a	2,432 a
Glyphosate + haloxyfop	0.0 a	0.0 a	0.0 a	0.0 a	2,444 a
Glyphosate + imazapic/imazapyr	3.8 b	5.0 b	2.8 b	2.5 b	2,506 a
Glufosinate + imazapic/imazapyr	3.5 b	4.5 b	2.0 b	2.0 b	2,450 a
Glyphosate + clethodim + imazapic/imazapyr	3.8 b	5.3 b	2.5 b	2.8 b	2,432 a
Glyphosate + haloxyfop + imazapic/imazapyr	3.5 b	4.3 b	2.5 b	2.0 b	2,379 a
Glyphosate + 2,4-D + imazapic/imazapyr	3.3 b	5.0 b	2.3 b	2.0 b	2,516 a
Glyphosate + dicamba + imazapic/imazapyr	4.0 b	5.8 b	3.5 c	3.3 b	2,292 a
Glyphosate + triclopyr + imazapic/imazapyr	4.5 b	6.3 b	4.0 c	2.8 b	2,433 a
Glyphosate + saflufenacil + imazapic/imazapyr	3.3 b	4.3 b	2.0 b	2.3 b	2,415 a
Mean	2.7	3.7	2.0	1.8	2,284
CV (%)	11.7	11.6	8.3	16.8	10.8
F	*	*	*	*	*

DAS: days after sowing; CV: coefficient of variation.

[1]Sequential application in all herbicide treatments of haloxyfop (66 g acid equivalent (a.e.) ha^{-1}) + glyphosate (720 g a.e. ha^{-1}), at post-emergence (V3) of soybean.

*Means followed by the same letter in the column do not differ from each other by the Scott-Knott (1974) test ($P \leq 0.05$).

The treatment glyphosate + triclopyr + imazapic/imazapyr showed the greatest values of injury, with 6.3% at 21 DAS, followed by the treatment glyphosate + dicamba + imazapic/imazapyr (5.8%). However, from 21 DAS the symptoms of all treatments started to decrease, and all percentages were considered acceptable and not harmful to the crop. Despite the symptoms, there was no change in yield (Tab. 3).

Discussion

Less than 30 d passed between the application of imazapic/imazapyr and the sowing of soybean, in accordance with the label recommendations (Rodrigues and Almeida, 2018). Additionally, about 100 mm of precipitation were observed in this period, also a necessary volume according to the label (Rodrigues and Almeida, 2018). This precipitation combined with the sandy texture of the soil may have favored the leaching of the product, which favored the selectivity for soybean.

Every herbicide mixture performed in this study showed efficient performance on *D. insularis* control with minimum injury while maintaining soybean yield. The use of imazapic/imazapyr and other herbicides in mixtures was potentially selective at burndown before soybean sowing. Melo *et al*. (2017) observed 100% of *D. insularis* control at 35 DAA using imazapic/imazapyr (52.5/17.5 g a.i. ha^{-1}) at postemergence. Francischini *et al*. (2012) also noted imazapic/imazapyr efficiency controlling *D. insularis* along with other weeds that is similar to that verified in the present study.

Besides successfully controlling *D. insularis* in different mixtures as shown in this study, imazapic/imazapyr is also efficient in the control of volunteer corn (Piasecki and Rizzardi, 2016) and is selective to soybean. Additionally, post-application of imazapic/imazapyr was also reported as efficient in controlling different grasses and broadleaved weeds, such as eudicotyledons *Bidens pilosa* and *Raphanus raphanistrus* (Santos *et al*., 2012) and monocotyledons *Hymenachne amplexicaulis* (Silva *et al*., 2012), *Oryza sativa*, *Echinochloa crus-galli* and *Cyperus esculentus* (Helgueira *et al*., 2018).

Regarding effectiveness, the glyphosate + imazapic/imazapyr mixture is highlighted when combined with auxinic herbicides, especially triclopyr and dicamba. This mixture is equivalent to that of glyphosate + imazapic/imazapyr with ACCase inhibitors (haloxyfop or clethodim). The application of these mixtures shows a broad control spectrum considering the effectiveness of auxins for the control of eudicotyledons combined with the control of *D. insularis* verified in this study.

High levels of infestation of weeds such as *D. insularis* and other grasses require ACCase inhibitor herbicides such as haloxyfop and clethodim. However, applying synthetic auxins such as 2,4-D and dicamba is not always an option because of the antagonism that can be created between these herbicides and ACCase inhibitors (Trezzi *et al*., 2007; Pereira *et al*., 2018; Gomes *et al*., 2020). The antagonism between these mixtures is possibly explained by the reduction of translocation of these herbicides to their action sites, compared to the cases when herbicides are pulverized alone (Scherder *et al*., 2005). Thus, eudicotyledon control is hampered and glyphosate or some other herbicide is used as an option for chemical management. There are few studies in the literature that provide information on antagonism, additive effect, or synergism of imazapic/imazapyr in a mixture with synthetic auxins, glufosinate, or saflufenacil, so the present work is unprecedented.

The mixture of saflufenacil, glyphosate, and imazapic/imazapyr was effective on the control of *D. insularis* (≥91.3%) at 28 and 35 DAA. In general, saflufenacil does not show high control of *Digitaria* spp. (Soltani *et al*., 2014). However, in this study, it was identified as a tool for *D. insularis* control when used in mixtures with glyphosate and imazapic/imazapyr since mixtures like this have a broad spectrum of action. Dalazen *et al*. (2015) observe synergism for saflufenacil + glyphosate, and Datta *et al*. (2013) observe it for imazapic + saflufenacil. It is noteworthy that these treatments had slower action, compared to treatments with ACCase inhibitors (haloxyfop and clethodim). Bianchi *et al*. (2020) observe a synergistic effect of clethodim + glyphosate for the control of *D. insularis* at 21 DAA. In the present study, treatments without ACCase inhibitors achieved greater levels of control after 28 DAA.

The results of this study showed that glufosinate + imazapic/imazapyr application as a burndown technique prior to sowing, and when applied along with glyphosate (only at soybean post-emergence) associated with haloxyfop they were efficient for controlling *D. insularis* at a 96.3%. Studies by Everman *et al*. (2007), Melo *et al*. (2012), Gemelli *et al*. (2013), and Silva *et al*. (2017) also emphasize the use of glufosinate in the control of *D. insularis* in different chemical weed management programs.

In this sense, it is important to emphasize the four biotype resistance cases of *D. insularis* in the world. Three of these biotypes are resistant to glyphosate and one to haloxyfop and fenoxaprop (Heap, 2020). Therefore, other mechanisms of action are useful to manage this grass; thus, imazapic/imazapyr (ALS inhibitors) and glufosinate (GS inhibitor) can be used to control *D. insularis* offering soybean selectivity as verified in this study.

Conclusion

The application of imazapic/imazapyr in mixtures with other herbicides was effective in controlling glyphosate-resistant *D. insularis* in burndown before soybean sowing and with sequential application of haloxyfop + glyphosate at the V3 stage of soybean. This chemical management was also selective for soybean.

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SUPPLEMENTARY MATERIAL 1. Commercial products used.

Commercial products	Herbicides
Roundup® Original	Glyphosate
Select® 240 EC	Clethodim
Verdict® R	Haloxyfop
Amplexus™	Imazapic/imazapyr
Finale®	Glufosinate
DMA® 806 BR	2,4-D
Atectra®	Dicamba
Triclon®	Triclopyr
Heat®	Saflufenacil