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Monitoring herbicide resistance at four weed species in Paraguay

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ABSTRACT: With the increasing reports of decreased effectiveness of herbicides in controlling *Digitaria insularis*, *Bidens subalternans*, *Chloris elata* and *Amaranthus hybridus*, as well as officially reported cases of resistance, it is believed that there may be biotypes of these species with herbicide resistance in Paraguay. This study aimed to monitor *D. insularis*, *B. subalternans*, *C. elata*, and *A. hybridus* suspected of having herbicide resistance. Seeds were collected during the 2016-2017, 2017-2018, and 2018-2019 growing seasons. In a greenhouse, herbicides clethodim, haloxyfop, and glyphosate were applied to *D. insularis* and *C. elata*, as well as glufosinate to *D. insularis*. *Bidens subalternans* and *A. hybridus* were treated with glyphosate, chlorimuron, imazethapyr, and lactofen. Glyphosate resistance was observed in *D. insularis*, multiple resistance (glyphosate, chlorimuron, and imazethapyr) was found in *B. subalternans*, and cross-resistance (chlorimuron and imazethapyr) was observed in *A. hybridus*. There was no indication of resistance to clethodim, haloxyfop, and glufosinate in *D. insularis* and lactofen in *B. subalternans* and *A. hybridus*. *Chloris elata* showed no signs of resistance to glyphosate, clethodim, and haloxyfop.
Keywords: *Digitaria insularis*; *Bidens subalternans*; *Chloris elata*; *Amaranthus hybridus*; herbicide effectiveness.

Monitoramento da resistência de herbicidas em quatro espécies de plantas daninhas no Paraguai

RESUMO: Com os crescentes relatos de diminuição da eficácia dos herbicidas no controle de *Digitaria insularis*, *Bidens subalternans*, *Chloris elata* e *Amaranthus hybridus*, bem como, devido aos casos de resistência notificados oficialmente, acredita-se que possam existir biótipos destas espécies com resistência a herbicidas no Paraguai. Objetivou-se monitorar *D. insularis*, *B. subalternans*, *C. elata* e *A. hybridus* com suspeita de resistência a herbicidas. As sementes foram coletadas nas safras 2016-2017, 2017-2018 e 2018-2019. Em casa-de-vegetação, foram aplicados os herbicidas cletodim, haloxyfop e glyphosate para *D. insularis* e *C. elata*, também glufosinate para *D. insularis*. *Bidens subalternans* e *A. hybridus* foram aplicados com glyphosate, clorimurom, imazethapyr e lactofen. Foi encontrado indício de resistência ao glyphosate para *D. insularis*, resistência múltipla (glyphosate, chlorimuron e imazethapyr) para *B. subalternans* e resistência cruzada (chlorimuron e imazethapyr) para *A. hybridus*. Não houve indicação de resistência ao cletodim, haloxyfop e glufosinate para *D. insularis*, bem como ao lactofen para *B. subalternans* e *A. hybridus*. Não houve indicação de resistência ao glyphosate, clethodim e haloxyfop para *C. elata*.
Palavras-chave: *Digitaria insularis*; *Bidens subalternans*; *Chloris elata*; *Amaranthus hybridus*; eficácia de herbicidas.

1. INTRODUCTION

Paraguay has an area of 406,752 km², with approximately 31 million ha of arable land, of which 3.7 million ha are cultivated with soybeans in the 2022-2023 growing season (Ministerio de Agricultura y Ganadería [MAG], 2022). In Paraguay, the first case of herbicide-resistant weed was reported in 1995 for wild poinsettia (*Euphorbia heterophylla* L.) resistant to imazethapyr, an herbicide with the mode of action of acetolactate synthase inhibitors (ALS).

Currently, there are cases with resistance to, the aforementioned *E. heterophylla* (HEAP, 2023), sourgrass (*Digitaria insularis* [L.] Mez ex Ekman) resistant to glyphosate (inhibitors of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase [EPSPs]), clethodim and haloxyfop (inhibitors of the enzyme acetyl-CoA carboxylase [ACCase]) (Krzyzaniak et al., 2023); and greater beggarticks (*Bidens subalternans* DC.) resistant to glyphosate (Heap, 2023), and Sumatran fleabane (*Conyza sumatrensis* [Retz.] E.Walker), with multiple resistance to the herbicides glyphosate, chlorimuron (ALS inhibitors) and paraquat (photosystem I inhibitors [PSI]) (ALBRECHT et al., 2020).

The weed *D. insularis* is a perennial species of the family Poaceae, with such characteristics as an herbaceous plant, with the presence of clumps, erect, rhizomatous, striated stems and a height between 50 and 100 cm (VELDMAN; PUTZ, 2011). Gemelli et al. (2013) emphasized that with the introduction of the no-till system, the species grew in importance due to its aggressive characteristics, such as the formation of clumps, rhizomes, and the spread of propagules. Another important weed in grain crops in South America is *B. subalternans*. It belongs to the family Asteraceae; it has an erect shape and little branching (LAMEGO et al., 2011). It is a competitive species due to high seed production and dormancy mechanisms (RAIMONDO; SPADARO, 2017; SOUZA et al., 2018).

The family of Poaceae's tall windmill grass (*Chloris elata* Desv.) is native to America and reproduces by seeds and/or rhizomes (CARVALHO et al., 2005). Also noteworthy is the high production of seeds of the species, where a single plant can produce more than 96,000 seeds (BRIGHENTI et al., 2007). Another important weed is smooth pigweed (*Amaranthus hybridus* L.), which is native to America (SAUER, 1967) and sub-cosmopolitan (COSTEA et al., 2004). It presents reproduction by seeds, where a single plant can produce about 60,000 seeds (WEAVER, 1984; COSTEA et al., 2004).

Altogether, 46 cases of herbicide-resistant biotypes worldwide are found in these species, among Paraguay's most important weeds. Among the factors that lead to the selection of herbicide-resistant weed biotypes, the use of the same or different herbicides, however, with the same mechanism of action, in which the strong selection pressure leads to the selection of resistant biotypes, stands out. One of the main strategies for preventing the selection of resistant weed biotypes, as well as managing plants that already have cases of resistance, is the diversification of management practices, with emphasis on rotation and herbicide combinations integrated with non-chemical measures (COLEMAN et al., 2019; DEFFONTAINES et al., 2020; FRISVOLD et al., 2020).

Thus, the monitoring of populations of resistant weeds stands out, which makes it possible to identify the evolution and dispersion of cases of resistance, which, consequently, provides important information for decision-making for weed control (Owen & Powles, 2016; Albrecht et al., 2020). With the increasing reports of reduced effectiveness of some herbicides in the control of *D. insularis*, *B. subalternans*, *C. elata* and *A. hybridus*, as well as, due to officially reported cases of resistance, it is believed that there may be biotypes of these species with resistance to herbicides or with difficulties in chemical control. Therefore, the objective of this study was to monitor the biotypes of *D. insularis*, *B. subalternans*, *C. elata* and *A. hybridus* with suspected resistance in the departments of Canindeyú, Alto Paraná, Guairá, Caaguazú, Itapúa and San Pedro in Paraguay.

2. MATERIAL E METHODS

2.1. Seed collection

Seeds were collected at sites where plants of *D. insularis*, *B. subalternans*, *C. elata* and *A. hybridus* survived after herbicide application in pre-sowing burndown in the 2016-2017, 2017-2018 and 2018-2019 growing seasons. Seeds were collected in agricultural areas located in Canindeyú, Alto Paraná and Guairá, Paraguay (Supplementary file 1). Collection sites were chosen according to reports of control failures indicating potential cases of resistance. Seeds were collected according to the methodology proposed by Burgos et al. (2013). Seeds were collected from several plants for each site, combined in a single sample per site (with at least 1,000 physiologically mature seeds per sample).

2.2. Greenhouse study

In a greenhouse in the municipality of Katueté, Canindeyú, Paraguay (24°09'28.7" S, 54°52'10.4" W), at least 100 seeds were sown per pot for each collection site. After germination, seedlings were transplanted to 800 mL pots filled with substrate, one seedling per pot. Herbicides recommended for weed control were applied, in addition to the control (no application) (Table 1), for each collection site. A completely randomized design was used with eight replications for each herbicide applied at each collection site. Herbicides were applied at the 1-3 tillers stage of development for grasses and 6-8 leaves for broadleaves using a CO_2 pressurized backpack sprayer, with a constant pressure of 2 Kgf cm^{-2}, with a bar with four flat fan nozzles, 110.02 series (TeeJet Technologies, Wheaton, IL) positioned at 0.50 m from the target and at a speed of 1 m s^{-1}, providing a total spray volume of 200 L ha^{-1}.

Table 1. Herbicides were applied to weeds for each collection point.
Tabela 1. Herbicidas aplicados nas plantas daninhas para cada ponto de coleta

Weed	Herbicide	Group - MoA	Rate (g ha^{-1})
D. insularis	Clethodim	A - ACCase inhibitors	96
	Haloxyfop	A - ACCase inhibitors	60
	Glyphosate	G - EPSPs inhibitors	720
	Glufosinate	H - GS inhibitors	500
	Without application	-	-
C. elata	Clethodim	A - ACCase inhibitors	96
	Haloxyfop	A - ACCase inhibitors	60
	Glyphosate	G - EPSPs inhibitors	720
	Without application	-	-
B. subalternans *A. hybridus*	Glyphosate	G - EPSPs inhibitors	720
	Chlorimuron	B - ALS inhibitors	20
	Imazethapyr	B - ALS inhibitors	100
	Lactofen	E - PPO inhibitors	180
	Without application	-	-

MoA = mode of action. Rates in g of acid equivalent (ae) ha^{-1} for glyphosate, imazethapyr and haloxyfop. For others, in g of active ingredient (ai) ha^{-1}. Average dose recommended for control at the stage of 2-4 tillers (grasses) and 6-8 leaves (broadleaves).

The weed control was evaluated 28 days after application (DAA). Visual scores were assigned to each experimental unit, where 0 represents no damage, and 100% indicates total plant death (VELINI et al., 1995). Results were presented descriptively, with maps elaborating the distribution of biotypes for which resistance was observed, according to the control effectiveness.

3. RESULTS

3.1. *Digitaria insularis*

The distributions of the collection sites for applying each herbicide, according to the control effectiveness of *D. insularis* biotypes, are shown in Figure 1. There was 100%

effectiveness in the control for applying glufosinate in all the collection sites, while for clethodim, a ≥86% control was found for all collection sites. For the application of haloxyfop in six sites, effectiveness was <86%, but still with control of at least 80%; however, it is already drawing attention to the risk of future resistance to ACCase inhibitors, which needs further study. Notably, at all collection sites, the application of glyphosate (720 g ae ha-1) was ineffective in controlling *D. insularis*, at most 40%, which indicates a resistance to that herbicide (Supplementary file 2).

3.2. *Chloris elata*

High effectiveness (≥86%) of herbicides was observed for almost all *C. elata* biotypes (Figure 2). Control (≥86%) was verified for all glyphosate or clethodim application collection sites. For the application of haloxyfop in only one collection site, the control was <86%; this was observed at site 02, with 78% control (Supplementary file 2), which demands further studies and suggests selection pressure and gradual loss of effectiveness of these products.

3.3. *Bidens subalternans*

Figure 3 shows the distribution of collection sites for applying each herbicide according to the effectiveness of controlling *B. subalternans* biotypes. There was 99-100% control of *B. subalternans* for applying lactofen in the 32 collection sites, for chlorimuron and imazethapyr, the values were quite varied.

Control ≥86% was registered for glyphosate application at 14 sites and chlorimuron or imazethapyr at 11 sites. In the range of 51 - 85% control, 7 sites were classified for glyphosate, 11 sites for chlorimuron and 13 sites for imazethapyr. While control ≤50% was observed in 11 sites for glyphosate application, in 10 sites for chlorimuron and in 8 sites for imazethapyr. It should be noted that at site 12, the control was below 50% for applying the three herbicides, while at eight sites, less than 50% was observed in two of these. *B. subalternans* populations resistant to glyphosate are suggested, as well as some cases of multiple and cross-resistance that are under further investigation (Supplementary file 2).

3.4. *Amaranthus hybridus*

The distributions of the 12 *A. hybridus* collection sites, with the respective control ranges for the application of herbicides, are illustrated in Figure 4. A high level of control was found for applying lactofen (99-100%) in all the collection sites. For glyphosate application in seven sites, a control of 70 - 85% was observed in the other sites; the control was ≥86%. Control ≤50% was found at six collection sites for chlorimuron application, and control ≥86% was observed in only three sites.

For the application of imazethapyr, a control of ≤50% was registered in five sites, while a control of ≥86% was detected in only three sites. It should be noted that at five sites, ≤50% control was observed for the application of chlorimuron and imazethapyr, which may indicate cases of cross-resistance to these acetolactate synthase (ALS) inhibitor herbicides and call attention to future cases of multiple resistance to ALS and glyphosate (Supplementary file 2).

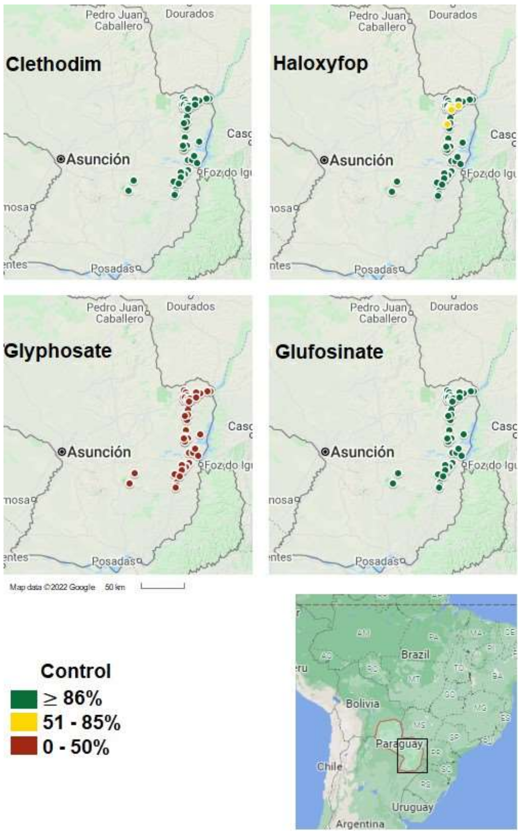

Figure 1. Distribution of *D. insularis* collection points and respective control levels at 28 days after herbicide application. Map data ©2022 Google
Figura 1. Distribuição dos pontos de coleta de *D. insularis* e respectivos níveis de controle aos 28 dias após aplicação do herbicida.

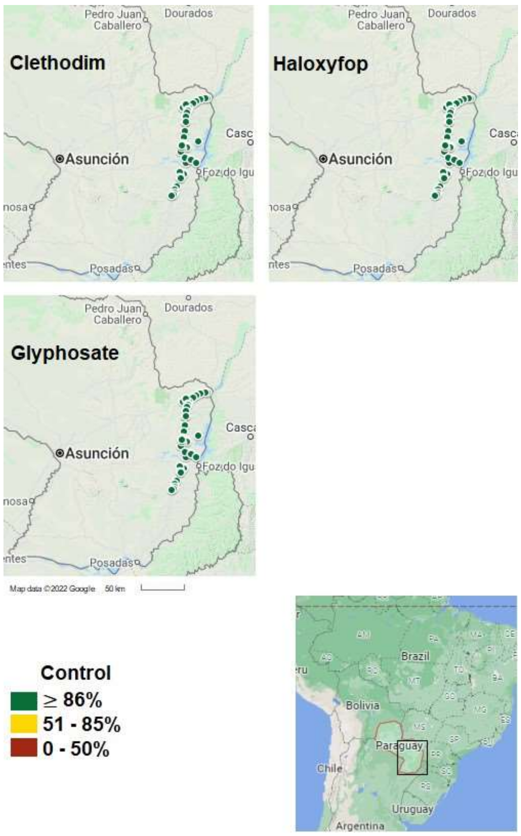

Figure 2. Distribution of *C. elata* collection points and respective control levels at 28 days after herbicide application. Map data ©2022 Google
Figura 2. Distribuição dos pontos de coleta de *C. elata* e respectivos níveis de controle aos 28 dias após aplicação do herbicida.

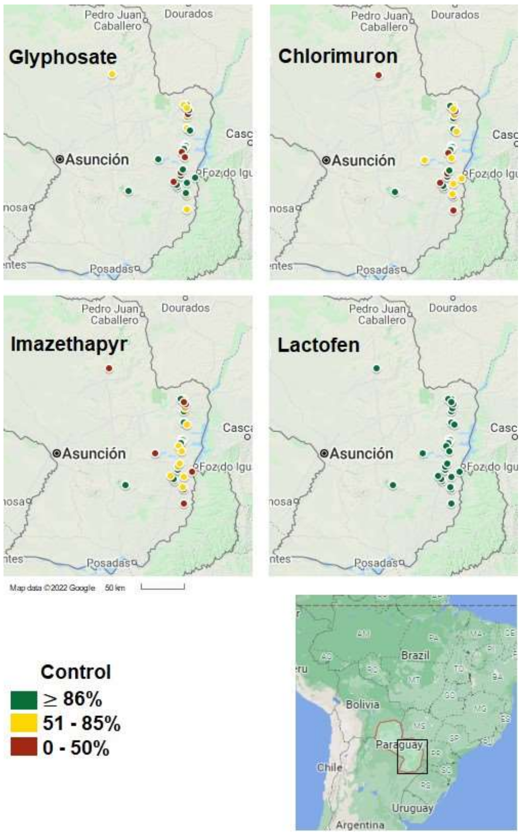

Figure 3. Distribution of *B. subalternans* collection points and respective control levels at 28 days after herbicide application. Map data ©2022 Google
Figura 3. Distribuição dos pontos de coleta de *B. subalternans* e respectivos níveis de controle aos 28 dias após aplicação do herbicida.

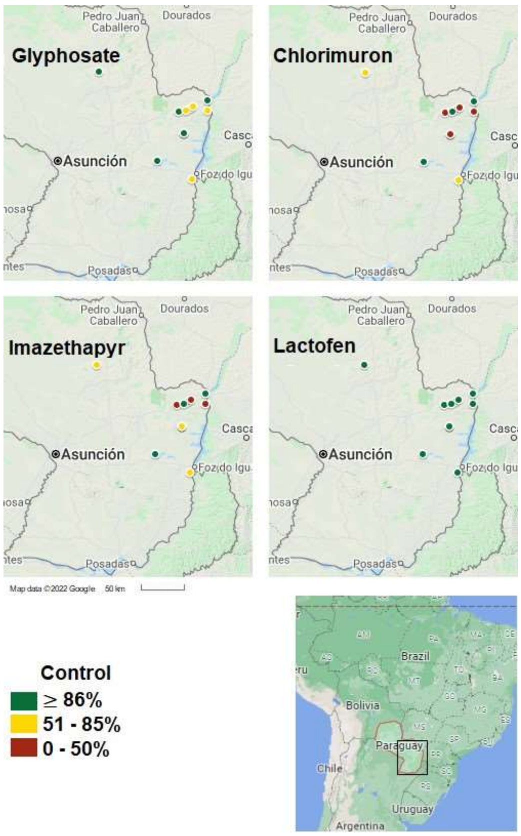

Figure 4. Distribution of *A. hybridus* collection points and respective control levels at 28 days after herbicide application. Map data ©2022 Google
Figura 4. Distribuição dos pontos de coleta de *A. hybridus* e respectivos níveis de controle aos 28 dias após aplicação do herbicida.

4. DISCUSSION

An indication of resistance to glyphosate was observed for *D. insularis* and *B. subalternans*. In Paraguay, *D. insularis* was reported as resistant to glyphosate in 2005, Argentina in 2014, and Brazil in 2008. In contrast, the herbicides haloxyfop, clethodim and especially glufosinate effectively controlled D. insularis at most sites, reinforcing the need to use different herbicides to ensure acceptable control and prevent the selection of new resistant biotypes. Other studies have previously highlighted the effectiveness of these herbicides in controlling *D. insularis* (CASSOL et al., 2019; BIANCHI et al., 2020; BAUER et al., 2021); however, this is softened in the form of a tank mix that can include the products to which there is confirmed resistance.

It is important to note that, despite the effectiveness of haloxyfop, a case of resistance of *D. insularis* to the herbicide above and fenoxaprop has been reported in Brazil. More recently, a biotype resistant to haloxyfop and pinoxaden has been reported (TAKANO et al., 2020) as a biotype with multiple resistance to glyphosate and ACCase inhibitors (haloxyfop and fenoxaprop). As in Paraguay, a biotype resistant to glyphosate, clethodim, and haloxyfop has been reported (KRZYZANIAK et al., 2023). Once again, the importance of diversifying management practices is reinforced to prevent the selection of resistant biotypes.

As for *D. insularis*, despite the low effectiveness of glyphosate, other investigated herbicides were effective in controlling *B. subalternans*, as also observed in other studies for the application of lactofen (SILVA et al., 2018; MENDES et al., 2019). It is suggested *B. subalternans* populations are resistant to glyphosate and even some cases of multiple resistance to glyphosate and ALS inhibitors. In Brazil, there is a case of multiple resistance to imazethapyr and glyphosate (MENDES et al., 2019). *B. subalternans* resistant to imazethapyr and chlorimuron have also been reported (GELMINI et al., 2002), among other reports of resistance to ALS inhibitors in *Bidens* spp., in Brazil (LAMEGO et al., 2009; TAKANO et al., 2016). Therefore, caution is recommended when using ALS-inhibiting herbicides to manage glyphosate-resistant *B. subalternans*.

The results of the present study indicate possible cases of cross-resistance to ALS-inhibiting herbicides chlorimuron (sulfonylurea group) and imazethapyr (imidazolinone group) for *A. hybridus*. Resistance to ALS-inhibiting herbicides in *A. hybridus* has already been reported for countries neighboring Paraguay. In Brazil, there are cases of *A. hybridus* resistant to glyphosate (RESENDE et al., 2022) and multiple resistance to glyphosate and chlorimuron (HEAP, 2023). In Argentina, multiple resistance to glyphosate and ALS inhibitors in Argentina can be highlighted (GARCÍA et al., 2020). In this sense, although glyphosate has effectively controlled most populations, caution is recommended, as there are already populations with lower control. Lactofen, in turn, was very effective, as also emphasized by Zanatta et al. (2008).

All herbicides were effective in the control of *C. elata*, although some had effectiveness below 100%; the effectiveness, especially of glyphosate in combination with ACCase inhibitors, was verified by Albrecht et al. (2021). What stands out is the case of resistance to glyphosate reported in Brazil (BARROSO et al., 2014; BRUNHARO et al., 2016). In this situation, population monitoring is also important. Therefore, monitoring weed resistance cases is an essential practice to understand, identify and quantify the frequency of these plants in advance (SCHULTZ et al., 2015). Thus, resistance monitoring studies lead to increased research and new techniques for controlling resistant or tolerant plants (BECKIE et al., 2019; COMONT; NEVE, 2021).

Therefore, works in this category are essential for creating integrated weed management plans. Uniquely, this design becomes imperative in countries such as Paraguay due to insufficient research. Not only monitoring but continuing research is positive, aimed at proving resistance and shaping strategies to control target weeds.

5. CONCLUSIONS

An indication of resistance to glyphosate was found for *D. insularis* populations and possible multiple resistance (glyphosate, chlorimuron and imazethapyr) for *B. subalternans* as well as cross-resistance (chlorimuron and imazethapyr) for *A. hybridus* at several sampled sites.

There was no indication of resistance to clethodim, haloxyfop and glufosinate for *D. insularis* populations and lactofen for populations of *B. subalternans* and *A. hybridus*. She indicated the need for further studies, especially for the multiple resistance of *D. insularis*, which showed differences in control.

The tested populations of *C. elata* did not show resistance to glyphosate, clethodim or haloxyfop; however, there were control differences.

Additional similar studies should be carried out in Paraguay, as this was unprecedented work in this country. It must be continued, as resistance cases can evolve substantially due to the high selection pressure observed in this environment.

The rapid increase in the spread of cases of weed resistance to herbicides in Paraguay is highlighted. At the same time, there is a lack of resistance monitoring work and strategies for controlling and mitigating this problem in this country.

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APPENDICES

Supplementary file 1. List of weed biotypes and information on collection areas. Paraguay, 2016-2017, 2017-2018 and 2018-2019 seasons.
Arquivo complementar 1. Lista de biótipos de plantas daninhas e informações sobre áreas de coleta. Paraguai, safras 2016-2017, 2017-2018 e 2018-2019.

P	Dep.	Latitude	Longitude	Crop	P	Dep.	Latitude	Longitude	Crop
					D. insularis				
01	Canindeyú	24°09'55"S	54°51'55"W	Maize	34	Alto Paraná	25°00'27"S	54°51'47"W	Soybean
02	Canindeyú	24°04'33"S	54°57'05"W	Maize	35	Alto Paraná	25°03'28"S	54°55'17"W	Soybean
03	Canindeyú	24°06'19"S	54°55'09"W	Turnip	36	Alto Paraná	25°03'45"S	54°55'36"W	Soybean
04	Canindeyú	24°08'39"S	54°38'33"W	Maize	37	Alto Paraná	25°16'30"S	54°50'15"W	Soybean
05	Canindeyú	24°15'31"S	54°48'36"W	Maize	38	Alto Paraná	25°16'43"S	54°44'47"W	Soybean
06	Canindeyú	24°12'30"S	54°52'00"W	Maize	39	Alto Paraná	25°20'48"S	54°38'51"W	Soybean
07	Canindeyú	24°16'23"S	54°55'46"W	Maize	40	Alto Paraná	25°29'13"S	54°50'53"W	Soybean
08	Canindeyú	24°11'51"S	54°58'12"W	Maize	41	Alto Paraná	25°32'21"S	54°54'03"W	Soybean
09	Canindeyú	24°15'40"S	54°56'20"W	Maize	42	Alto Paraná	25°34'11"S	54°54'59"W	Soybean
10	Canindeyú	24°10'33"S	54°53'20"W	Maize	43	Alto Paraná	25°37'56"S	54°58'16"W	Soybean
11	Canindeyú	24°26'48"S	54°51'45"W	Maize	44	Alto Paraná	25°51'16"S	55°07'06"W	Soybean
12	Canindeyú	24°33'15"S	54°51'39"W	Maize	45	Guairá	25°52'41"S	56°07'12"W	Soybean
13	Canindeyú	24°33'25"S	54°52'48"W	Maize	46	Alto Paraná	25°55'11"S	55°06'52"W	Soybean
14	Alto Paraná	24°39'25"S	54°52'48"W	Maize	47	Alto Paraná	25°57'31"S	55°08'22"W	Soybean
15	Canindeyú	24°23'15"S	54°51'33"W	Oat	48	Alto Paraná	25°00'14"S	54°56'50"W	Soybean
16	Canindeyú	24°28'27"S	54°51'57"W	Wheat	49	Alto Paraná	25°36'44"S	54°58'44"W	Soybean
17	Alto Paraná	25°45'04"S	55°04'39"W	Oat	50	Alto Paraná	25°36'23"S	54°59'47"W	Soybean
18	Alto Paraná	25°41'31"S	55°09'28"W	Oat	51	Alto Paraná	25°34'34"S	54°59'23"W	Soybean
19	Alto Paraná	24°55'56"S	54°36'20"W	Crotalaria	52	Alto Paraná	25°33'29"S	54°59'19"W	Soybean
20	Canindeyú	24°11'60"S	54°56'10"W	Chia	53	Alto Paraná	25°31'29"S	54°59'56"W	Soybean
21	Canindeyú	24°05'32"S	54°22'21"W	Soybean	54	Alto Paraná	25°37'14"S	54°59'57"W	Soybean
22	Canindeyú	24°05'42"S	54°26'40"W	Soybean	55	Guairá	25°41'21"S	56°00'44"W	Soybean
23	Canindeyú	24°07'17"S	54°36'13"W	Soybean	56	Alto Paraná	25°44'30"S	55°03'00"W	Soybean
24	Canindeyú	24°11'05"S	54°42'38"W	Soybean	57	Canindeyú	24º14'08"S	54º52'25"W	Maize
25	Canindeyú	24°15'39"S	54°51'15"W	Soybean	58	Canindeyú	24º14'54"S	54º49'17"W	Soybean
26	Canindeyú	24°15'41"S	54°52'51"W	Soybean	59	Alto Paraná	25º11'52"S	54º43"06"W	Maize
27	Canindeyú	24°23'29"S	54°50'45"W	Soybean	60	Canindeyú	24º13'47"S	54º49'14"W	Soybean
28	Canindeyú	24°27'16"S	54°51'53"W	Soybean	61	Canindeyú	24º33'30"S	54º55'46"W	Maize
29	Canindeyú	24°32'38"S	54°51'33"W	Soybean	62	Canindeyú	24º06'43"S	54º40'48"W	Soybean
30	Alto Paraná	24°50'53"S	54°54'58"W	Soybean	63	Canindeyú	24º16'48"S	54º40'44"W	Maize
31	Alto Paraná	24°57'33"S	54°56'13"W	Soybean	64	Canindeyú	24º16'48"S	54º50'44"W	Maize
32	Alto Paraná	24°59'06"S	54°54'22"W	Soybean	65	Canindeyú	24º12'55"S	54º41'51"W	Soybean
33	Alto Paraná	25°00'01"S	54°52'59"W	Soybean					
					C. elata				
01	Canindeyú	24°16'23"S	54°55'46"W	Soybean	26	Alto Paraná	25°00'27"S	54°51'47"W	Soybean
02	Canindeyú	24°17'07"S	54°50'04"W	Soybean	27	Alto Paraná	25°01'09"S	54°50'29"W	Soybean
03	Canindeyú	24°33'15"S	54°51'33"W	Soybean	28	Alto Paraná	25°02'50"S	54°51'13"W	Soybean
04	Canindeyú	24°33'13"S	54°52'59"W	Soybean	29	Alto Paraná	25°20'57"S	54°52'25"W	Soybean
05	Canindeyú	24°28'27"S	54°51'57"W	Soybean	30	Alto Paraná	25°03'45"S	54°55'36"W	Soybean
06	Alto Paraná	25°48'05"S	55°04'07"W	Soybean	31	Alto Paraná	25°07'39"S	54°57'56"W	Soybean
07	Alto Paraná	24°55'56"S	54°36'20"W	Soybean	32	Alto Paraná	25°14'32"S	54°53'33"W	Soybean
08	Canindeyú	24°05'45"S	54°24'21"W	Soybean	33	Alto Paraná	25°16'47"S	54°45'06"W	Soybean
09	Canindeyú	24°05'42"S	54°26'40"W	Soybean	34	Alto Paraná	25°16'43"S	54°44'47"W	Soybean
10	Canindeyú	24°06'39"S	54°33'10"W	Soybean	35	Alto Paraná	25°20'48"S	54°38'51"W	Soybean
11	Canindeyú	24°08'41"S	54°38'40"W	Soybean	36	Alto Paraná	25°32'21"S	54°54'03"W	Soybean
12	Canindeyú	24°11'05"S	54°42'38"W	Soybean	37	Alto Paraná	25°34'11"S	54°54'59"W	Soybean
13	Canindeyú	24°15'28"S	54°47'30"W	Soybean	38	Alto Paraná	25°37'42"S	54°58'13"W	Soybean
14	Canindeyú	24°13'18"S	54°52'09"W	Soybean	39	Alto Paraná	25°36'44"S	54°58'44"W	Soybean
15	Canindeyú	24°12'27"S	54°51'59"W	Soybean	40	Alto Paraná	25°35'18"S	54°59'34"W	Soybean
16	Canindeyú	24°18'14"S	54°49'22"W	Soybean	41	Alto Paraná	25°33'29"S	54°59'19"W	Soybean
17	Canindeyú	24°20'09"S	54°49'41"W	Soybean	42	Alto Paraná	25°30'54"S	55°00'06"W	Soybean
18	Canindeyú	24°22'23"S	54°50'26"W	Soybean	43	Alto Paraná	25°37'14"S	54°59'57"W	Soybean
19	Canindeyú	24°27'16"S	54°51'53"W	Soybean	44	Alto Paraná	25°38'47"S	54°59'06"W	Soybean
20	Canindeyú	24°32'38"S	54°51'33"W	Soybean	45	Alto Paraná	25°51'16"S	55°07'06"W	Soybean
21	Alto Paraná	24°43'54"S	54°53'05"W	Soybean	46	Alto Paraná	25°55'11"S	55°06'52"W	Soybean
22	Alto Paraná	24°50'53"S	54°54'58"W	Soybean	47	Alto Paraná	25°57'31"S	55°08'22"W	Soybean
23	Alto Paraná	24°57'33"S	54°56'13"W	Soybean	48	Alto Paraná	25°58'34"S	55°10'27"W	Soybean
24	Alto Paraná	24°59'36"S	54°54'09"W	Soybean	49	Alto Paraná	25°02'44"S	54°56'46"W	Soybean
25	Alto Paraná	25°00'01"S	54°52'59"W	Soybean	50	Alto Paraná	25°00'14"S	54°56'50"W	Soybean
					B. subalternans				
01	Canindeyú	24°12'30"S	54°52'00"W	Maize	17	Alto Paraná	25°07'39"S	54°57'56"W	Soybean
02	Canindeyú	24°17'07"S	54°50'04"W	Soybean	18	Alto Paraná	25°12'59"S	54°54'47"W	Soybean
03	Canindeyú	24°26'48"S	54°51'45"W	Soybean	19	Alto Paraná	25°36'30"S	54°59'48"W	Soybean

P	Dep.	Latitude	Longitude	Crop	P	Dep.	Latitude	Longitude	Crop
04	Alto Paraná	25°48'05"S	55°04'07"W	Oat	20	Alto Paraná	25°34'34"S	54°59'23"W	Soybean
05	Alto Paraná	25°45'04"S	55°04'39"W	Oat	21	Alto Paraná	25°31'29"S	54°59'56"W	Soybean
06	Canindeyú	24°18'17"S	54°49'25"W	Maize	22	Guairá	25°52'41"S	56°07'12"W	Soybean
07	Alto Paraná	25°41'31"S	55°09'28"W	Oat	23	Alto Paraná	25°27'16"S	54°57'16"W	Maize
08	Canindeyú	24°11'60"S	54°56'10"W	Chia	24	Caaguazú	25°16'05"S	55°28'30"W	Maize
09	Canindeyú	24°23'05"S	54°50'38"W	Soybean	25	Canindeyú	24°15'36"S	54°52'06"W	Soybean
10	Alto Paraná	24°39'13"S	54°52'35"W	Soybean	26	Alto Paraná	24°42'55"S	54°48'35"W	Maize
11	Alto Paraná	24°40'37"S	54°52'18"W	Soybean	27	Alto Paraná	25°42'55"S	54°51'26"W	Maize
12	Alto Paraná	24°59'06"S	54°54'22"W	Soybean	28	Alto Paraná	25°57'59"S	54°51'26"W	Maize
13	Alto Paraná	24°59'36"S	54°54'09"W	Soybean	29	Alto Paraná	25°37'01"S	54°41'56"W	Maize
14	Alto Paraná	25°00'01"S	54°52'59"W	Soybean	30	Alto Paraná	25°55'22"S	54°52'38"W	Soybean
15	Alto Paraná	25°02'58"S	54°54'12"W	Soybean	31	Itapúa	26°14'06"S	54°51'57"W	Maize
16	Alto Paraná	25°03'36"S	54°55'25"W	Soybean	32	San Pedro	23°36'08"S	56°28'45"W	Maize
				A. hybridus					
01	Alto Paraná	24°43'40"S	54°52'48"W	Maize	07	Canindeyú	24°14'49"S	54°45'46"W	Soybean
02	Caaguazú	25°15'49"S	55°27'46"W	Soybean	08	Alto Paraná	25°37'19"S	54°42'04"W	Maize
03	Alto Paraná	24°44'06"S	54°53'23"W	Soybean	09	San Pedro	23°31'54"S	56°42'09"W	Soybean
04	Alto Paraná	24°44'06"S	54°52'59"W	Oat	10	Canindeyú	24°16'48"S	54°50'44"W	Soybean
05	Canindeyú	24°18'25"S	54°59'24"W	Soybean	11	Canindeyú	24°12'55"S	54°41'51"W	Oat
06	Canindeyú	24°16'48"S	54°23'09"W	Soybean	12	Canindeyú	24°05'34"S	54°23'09"W	Maize

Abbreviations: P - point; Dep. - department.

Supplementary file 2.
Arquivo suplementar 2.
Control (%) of *D. insularis* at 28 days after herbicide application.

P	clethodim	haloxyfop	glyphosate	glufosinate	P	clethodim	haloxyfop	glyphosate	glufosinate
01	100	100	25	99	34	99	90	20	100
02	100	100	27	100	35	100	100	20	100
03	97	90	20	100	36	100	100	25	100
04	100	98	18	100	37	100	95	25	99
05	100	100	15	100	38	96	94	25	100
06	95	90	25	100	39	100	100	33	100
07	100	100	30	100	40	100	90	15	100
08	100	100	33	100	41	99	100	20	100
09	100	92	25	100	42	100	100	20	100
10	100	100	28	100	43	100	100	33	100
11	97	100	30	100	44	100	92	15	99
12	100	100	30	100	45	98	100	35	100
13	100	100	35	100	46	100	100	20	100
14	100	93	25	99	47	100	100	22	100
15	100	100	18	100	48	99	95	25	100
16	100	95	33	100	49	100	95	20	100
17	97	90	35	100	50	97	93	18	100
18	100	95	40	100	51	100	100	15	100
19	100	100	30	100	52	100	100	15	100
20	100	100	33	100	53	100	100	34	100
21	90	100	30	99	54	95	92	22	98
22	100	100	30	100	55	100	100	25	100
23	97	95	25	100	56	99	95	25	100
24	100	100	28	99	57	92	85	35	100
25	100	100	27	100	58	100	80	25	99
26	95	90	18	100	59	100	99	27	100
27	97	95	15	100	60	99	83	30	100
28	100	100	25	100	61	93	80	28	100
29	100	97	25	100	62	90	97	20	100
30	100	100	22	100	63	100	100	22	100
31	98	100	30	100	64	88	85	18	100
32	100	100	35	100	65	100	82	17	99
33	98	92	22	100					

Control (%) of *C. elata* at 28 days after herbicide application.

P	clethodim	haloxyfop	glyphosate	P	clethodim	haloxyfop	glyphosate
01	100	100	100	26	93	100	90
02	100	78	97	27	100	100	100
03	98	98	100	28	100	100	100
04	100	100	95	29	98	100	90
05	100	100	98	30	100	100	100
06	100	100	100	31	100	98	90
07	100	97	90	32	100	100	100
08	100	100	100	33	100	90	95
09	100	100	99	34	100	100	100
10	100	100	100	35	100	97	100
11	99	100	100	36	100	100	100
12	100	100	100	37	100	100	96
13	100	95	100	38	100	100	100
14	100	100	100	39	100	100	100
15	99	92	90	40	100	92	97
16	100	87	100	41	100	100	100
17	100	100	100	42	95	99	98
18	95	100	93	43	100	100	100
19	100	100	100	44	100	100	98
20	100	95	96	45	100	100	93
21	100	100	100	46	100	100	100
22	100	100	100	47	100	100	99
23	100	100	100	48	99	100	100
24	100	95	92	49	95	95	93
25	100	100	97	50	100	100	100

Control (%) of *B. subalternans* at 28 days after herbicide application.

P	glyphosate	chlorimuron	imazethapyr	lactofen	P	glyphosate	chlorimuron	imazethapyr	lactofen
01	25	55	70	99	17	35	40	55	99
02	97	100	100	100	18	30	75	85	100
03	68	90	100	99	19	25	40	65	100
04	45	35	80	100	20	95	90	95	100
05	97	100	100	100	21	28	30	65	99
06	90	20	35	99	22	95	100	100	100
07	25	30	70	100	23	98	95	80	100
08	65	95	100	100	24	90	57	37	100
09	28	60	75	100	25	85	55	45	100
10	95	90	90	100	26	97	60	65	99
11	78	100	100	100	27	99	75	70	100
12	20	30	45	99	28	75	40	35	100
13	25	65	60	100	29	100	55	47	100
14	100	100	100	100	30	98	72	68	100
15	42	80	100	99	31	70	40	35	100
16	98	100	100	100	32	65	45	40	100

Control (%) of *A. hybridus* at 28 days after herbicide application.

P	glyphosate	chlorimuron	imazethapyr	lactofen	P	glyphosate	chlorimuron	imazethapyr	lactofen
01	100	65	70	100	07	75	32	45	100
02	100	92	98	100	08	80	65	58	100
03	80	30	35	99	09	95	56	60	100
04	99	35	60	100	10	85	90	95	100
05	90	30	40	100	11	72	28	32	99
06	70	28	35	99	12	99	97	98	100