PLANTA DANINHA
SOCIEDADE BRASILEIRA DA CIÊNCIA DAS PLANTAS DANINHAS
<http://www.sbcpd.org>
ISSN 0100-8358 (print)
1806-9681 (online)

Article

Identification and Mapping of Cross-Resistance Patterns to ALS-Inhibitors in Greater Beggarticks (*Bidens* spp.)

*Identificação e Mapeamento de Padrões de Resistência Cruzada a Inibidores da Acetolactato Sintase (ALS) em Picão-Preto (**Bidens** spp.)*

MENDES, R.R.[1*]
OLIVEIRA JR., R.S.[1]
CONSTANTIN, J.[1]
SILVA, V.F.V.[1]
HENCKS, J.R.[1]

ABSTRACT - Greater beggarticks (*Bidens pilosa* and *Bidens subalternans*) biotypes have been under selection pressure of ALS-inhibitors since early 90's in Brazil. The objectives of this work were to investigate whether there are different cross-resistance patterns among ALS-inhibitors herbicides in *Bidens* spp. biotypes; to understand the geographic distribution of resistance patterns in grains producing regions in Brazil; and evaluate the possibility of multiple resistance to ALS-inhibitors, EPSPs inhibitor and photosystem II inhibitors. Dose-response experiments were carried out with imazethapyr, chlorimuron and diclosulam in three populations. Sensibility to others 34 populations both from Paraná State (PR) and from others Brazilian regions were also evaluated. The dose-response assay revealed cross-resistance with different patterns. One population was resistant to all three herbicides, the second population was tolerant to both imazethapyr and chlorimuron, but not to diclosulam, while a third population was resistant merely to imazethapyr. The results exhibited different cross-resistance patterns, since they can be found in other *Bidens* spp. populations. However, no relationship was observed between geographic areas where samples were collected and resistance patterns. Conclusively, the most frequent resistance pattern was R2 (resistance to imazethapyr, chlorimuron and diclosulam).

Keywords: imazethapyr, chlorimuron, diclosulam, *Bidens subalternans*, *Bidens pilosa*.

*RESUMO - Biótipos de picão-preto (**Bidens pilosa** e **Bidens subalternans**) têm estado sob pressão de seleção de herbicidas inibidores da ALS desde a o início da década de 90 no Brasil. Os objetivos deste trabalho foram de investigar se existem padrões de resistência cruzada diferentes entre os herbicidas inibidores da ALS em biótipos de **Bidens** spp.; entender a distribuição geográfica dos padrões de resistência nas regiões produtoras de grãos do Brasil; e avaliar a possibilidade de haver resistência múltipla a inibidores da ALS, inibidor da EPSPs e inibidores do fotossistema II. Experimentos de dose-resposta foram realizados com herbicidas do grupo das imidazolinonas (imazethapyr), sulfonilureias (chlorimuron) e triazolopirimidinas (diclosulam) em três populações consideradas resistentes. Outras 34 populações coletadas no Estado do Paraná e em outras áreas do Brasil foram avaliadas quanto à sensibilidade aos três herbicidas, para verificar se os padrões ocorrem em outras regiões. Os experimentos de dose-resposta revelaram resistência cruzada com características diferentes. Uma das populações (R2) foi resistente aos três herbicidas. Outra população foi resistente a imazethapyr e chlorimuron, mas não a diclosulam (R1), e a terceira população apresentou resistência apenas a imazethapyr (R3). Os resultados evidenciaram que existem*

*** Corresponding author:**
<rafaromero.mendes@gmail.com>

Received: March 5, 2018
Approved: May 21, 2018

Planta Daninha 2019; v37:e019192481

Copyright: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided that the original author and source are credited.

[1] Universidade Estadual de Maringá, Maringá-PR, Brasil.

Doi: 10.1590/S0100-83582019370100117

*padrões diferentes de resistência cruzada, pois estes se repetem em outras populações de **Bidens** spp., porém sem que houvesse relação entre os padrões observados e a localização geográfica das amostras. O padrão de resistência que apresentou mais frequência foi o R2 (resistente a imazethapyr, chlorimuron e diclosulam).*

Palavras-chave: imazethapyr, chlorimuron, diclosulam, *Bidens subalternans*, *Bidens pilosa*.

INTRODUCTION

Weeds species of the genus *Bidens* belong to the Asteraceae family, which is originally from South America and can be found in the entire Brazilian territory, especially in Midwestern, Southeastern and Southern regions. These plants are reproduced strictly by seeds and can reach up to 1.2 m. Such weeds may be found in annual crops, particularly in soybeans, corn, wheat and cotton (Kissmann and Groth, 1999; Lorenzi, 2014).

B. pilosa and *B. subalternans* are the most known weed species among the genus *Bidens*. The distinction between both species has been challenging for farmers, agronomists and weed scientists due to their similar morphology. The main differences between both species are in the achenes structure and their branching. *B. pilosa* has two to three arista in its achenes, whereas *B. subalternans* denotes three to four arista. In *B. pilosa* upper third, the branching is dichotomous, whist it is alternate in *B. subalternans* (Kissmann and Groth, 1999; Grombone-Guaratini et al., 2004).

Biotypes from both species have been confirmed as resistant to herbicides. *B. pilosa* was the first weed reported as resistant to herbicides in Brazil in the mid-1990's, exhibiting cross-resistance to acetolactate synthase (ALS) inhibitors (Christoffoleti, 2002). Posteriorly, similar resistance patterns were also described in *B. subalternans* biotypes (Gelmini et al., 2002). Afterwards, biotypes of both species from grain-producing areas have been observed with multiple resistance to ALS and photosystem II inhibitors (Takano et al., 2016). Recently, one biotype of *B. pilosa* was depicted as resistant to glyphosate in Mexico (Cruz et al., 2016).

ALS inhibitors comprise five chemical groups of herbicides: imidazolinones (IMI), sulfonylureas (SU), triazolopyrimidines (TP), pyrimidinyl-benzoates (PYB) and sulfonylamino-cabonyl-triazolinones (SCT). Nevertheless, commercial products are available only from the four first groups in Brazil. These herbicides inhibit the enzyme that catalyzes the first stage in the synthesis of branched-chain amino acids, such as leucine, isoleucine and valine.

The reaction catalyzed by ALS involves the decarboxylation of a pyruvate molecule, producing an enzyme-bound hydroxyethyl (HE) intermediate that reacts with either a second molecule of pyruvate or with a 2-ketobutyrate to produce (*S*)-2-acetolactate or (*S*)-2-aceto-2-hydroxybutyrate, respectively (Garcia et al., 2017). ALS inhibitor are selective for several crops and requires minimal doses for weed control, thus presenting low toxicity to mammals. However, the intense use of these herbicides has frequently led to the selection of more resistant weeds worldwide (Liu et al., 2015).

In weed resistant species, different patterns of cross-resistance may be seen among chemical groups exhibiting the same mechanism of action, signifying that resistance can be related to one single herbicide, to all herbicides of a single chemical group or to herbicides from two or more different chemical groups (Deng et al., 2014). Typically, target site mechanisms related to point mutations in ALS gene are responsible for providing such different patterns. Certain mutations may cause exclusive resistance to IMI, others could affect merely SU, while others may result in tolerance to all chemical groups (Beckie and Tardif, 2012; Yu and Powles, 2015; Tranel et al., 2018).

ALS inhibitors utilization from IMI and TRI groups are currently recommended in soybeans to provide control of glyphosate-resistant weeds, such as fleabane (*Conyza* spp.), sourgrass (*Digitaria insularis*) and goosegrass (*Eleusine indica*) (Peterson et al., 2018). In those areas, greater beggarticks escapes have been reported, presumably due to the remaining seedbank of ALS-resistant biotypes.

The objectives of this research were: (a) to characterize and map different patterns of cross-resistance to ALS inhibitors in biotypes of *Bidens* spp. and (b) to evaluate whether reported field control failures are related to the occurrence of multiple resistance cases to ALS inhibitors + atrazine and/or to ALS inhibitors + glyphosate in biotypes collected from soybean-producing areas in Brazil.

MATERIAL AND METHODS

Dose-response assays

One susceptible (SUS) biotype and three putative resistant biotypes (R1, R2 and R3) were used in this study. SUS biotype seeds were collected from a site in Maringá (PR) with no previous history of herbicide use. Seeds of R1 and R3 biotypes were collected, in Assis Chateaubriand (PR) and in Nova Aurora (PR), respectively. Both biotypes were collected from soybean and corn farms without past reports of ALS herbicides application in the last 10 years. Seeds of biotype R2 were sampled in Balsas (MA), from a farm cultivated with conventional (non-RR) soybeans, in which ALS inhibitor herbicides, such as diclosulam and chlorimuron, are frequently used for pre-emergent weed control.

Seeds from five to ten plants that survived to herbicide application throughout soybean cycle were collected from each site in January 2015. These seeds were then combined into a single bulk composite sample, subsequently packed in paper bags, identified and kept at room temperature for further greenhouse experiments.

Seeds were sown at approximately 0.5 cm depth in plastic trays (10 x 6 x 2 cm) filled with substrate for germination. After emergence, seedlings were transplanted to 1.0 L pots containing soil (21% clay, 68% sand, 2.1% organic matter, CEC = 9.8 mg dm^{-3} and pH_{H2O} = 6.4).

Three independent experiments, one for each herbicide from a distinct chemical group (imazethapyr – IMI, chlorimuron – SU and diclosulam – TP), were carried out. All greenhouse experiments respected a 8 x 4 factorial scheme, where the first factor was composed by eight herbicide doses and the second by the four biotypes (S, R1, R2 and R3). For each experiment, herbicide dosages were equivalent to 0, 1/8, 1/4, 1/2, 1, 2, 4 and 8 times the labeled doses recommended by each herbicide manufacturer for *Bidens* spp. control (AGROFIT, 2018). All experiments had four replicates per biotype, in a completely randomized design. The "x" doses for each herbicide were 106 g ha^{-1} of imazethapyr, 20 g ha^{-1} of chlorimuron and 26.2 g ha^{-1} of diclosulam.

Every herbicide was applied post-emergence at the two-to-three leaves per plant stage (≈10 cm). Herbicides were employed with a CO_2-pressurized backpack sprayer equipped with a 1.5 m long boom containing three XR 110.02 spray nozzles (0.5 m between nozzles). The application speed was 1.0 m s^{-1} and the pressure was 2.1 kgf cm^{-2}, providing a spray volume equivalent to 200 L ha^{-1}.

Twenty-eight days after application (DAA), plant shoots were harvested, placed in paper bags, dried at 65 °C for 48 h and weighed (g per pot). Data were subjected to ANOVA and F test (p<0.05) as well as to a regression analysis using a nonlinear dose-response through the logistic Gompertz model with three parameters (Equation 1) for each herbicide:

$$Y = a * \exp\{-\exp[-b * (x - c)]\} \quad \text{(eq. 1)}$$

where: Y is the shoot biomass at 28 DAA (% in relation to untreated check); x is herbicide dose (g ha^{-1}); a is the amplitude between the maximum and the minimum range of the variable; b is the dose which provides 50% response; and c is the curve slope around b.

One of the equation integral terms for the logistic model (b) is an estimate of the herbicide dose that reduces shoot dry weight by 50% (GR_{50}). Although one of the parameters of the logistic model (b) is a GR_{50} estimate, a mathematical solution was appraised for these values using the inverse equation, as proposed by Carvalho et al. (2005). Based on GR_{50}, the resistance factor (RF) was calculated as GR_{50} of the putative resistant population/GR_{50} of the susceptible standard. Doses for acceptable control (≥85%) (GR_{85}) were also determined.

Screening of additional biotypes from different sites

After identifying different levels of resistance, this study evaluated the response patterns of additional biotypes to ALS application.

Seeds of *B. pilosa* and *B. subalternans* were collected from soybean-producing areas along the 2016/2017 season, respecting the same procedure described for dose-response experiments. Seeds were collected from 33 sites in Brazil, but mostly from the state of Paraná (Table 1). Geographical coordinates were recorded for all sampling sites.

Sowing and transplanting was analogous to the procedure previously described, except by the fact that plants were placed in 0.2 L pots. One experiment was carried out for each herbicide, with post-emergent applications at three leaves-stage for 37 biotypes (33 collected from different

Table 1 - Identification of biotypes and sampling sites information greater beggarticks seeds (*Bidens* spp.), Maringá (PR), 2018

Biotype code	Species	City	State	Geographical coordinates	
				Latitude	Longitude
SUS	*B. subalternans*	Maringá	PR	23º 23' 47" S	51º 57' 14" W
R1	*B. subalternans*	Assis Chateaubriand	PR	24º 17' 23" S	53º 32' 18" W
R2	*B. subalternans*	Balsas	MA	08º 30' 23" S	46º 44' 59" W
R3	*B. subalternans*	Nova Aurora	PR	24º 34' 29" S	53º 13' 23" W
101_17	*B. subalternans*	Cascavel	PR	24º 53' 55" S	33º 28' 15" W
141_17	*B. subalternans*	Campo Mourão	PR	24º 11' 46" S	52º 16' 00" W
144_17	*B. subalternans*	Engenheiro Beltrão	PR	23º 46' 42" S	52º 16' 24" W
145_17	*B. subalternans*	Boa Esperança	PR	24º 14' 35" S	52º 49' 38" W
147_17	*B. subalternans*	Mamborê	PR	24º 20' 14" S	52º 35' 36" W
15_17	*B. subalternans*	Peabiru	PR	23º 52' 43" S	52º 19' 08" W
184_17	*B. subalternans*	Floresta	PR	23º 34' 51" S	52º 04' 16" W
20_17	*B. subalternans*	Engenheiro Beltrão	PR	23º 51' 05" S	52º 18' 37" W
236_17	*B. subalternans*	São Jorge do Ivaí	PR	23º 27' 27" S	52º 17' 31" W
242_17	*B. subalternans*	Ourizona	PR	23º 24' 55" S	52º 13' 53" W
261_17	*B. subalternans*	Jaguapitã	PR	23º 09' 12" S	51º 31' 24" W
343_17	*B. subalternans*	Maripá	PR	24º 24' 14" S	53º 51' 29" W
352_17	*B. subalternans*	Bela Vista do Paraíso	PR	23º 05' 06" S	51º 10' 59" W
358_17	*B. subalternans*	Braganey	PR	24º 51' 02" S	53º 06' 09" W
37_17	*B. subalternans*	Peabiru	PR	23º 24' 07" S	52º 19' 53" W
395_17	*B. subalternans*	Nova Aurora	PR	24º 23' 51" S	53º 21' 55" W
407_16	*B. subalternans*	Uberlândia	MG	19º 02' 04" S	48º 12' 57" W
413_17	*B. subalternans*	Assis Chateaubriand	PR	24º 33' 03" S	53º 35' 34" W
415_17	*B. subalternans*	Assis Chateaubriand	PR	24º 21' 05" S	53º 30' 09" W
425_17	*B. subalternans*	Bandeirantes	PR	23º 05' 38" S	50º 25' 22" W
431_17	*B. subalternans*	Prado Ferreira	PR	23º 02' 30" S	51º 22' 10" W
532_16	*B. subalternans*	Araguari	MG	18º 33' 14" S	48º 23' 52" W
548_16	*B. subalternans*	Guarapuava	PR	25º 27' 22" S	51º 30' 32" W
567_16	*B. subalternans*	São Desidério	BA	12º 51' 24" S	45º 57' 49" W
568_16	*B. subalternans*	Formosa do Rio Preto	BA	11º 30' 42" S	46º 10' 53" W
59_17	*B. subalternans*	Medianeira	PR	25º 18' 36" S	53º 58' 03" W
81_17	*B. subalternans*	São Miguel do Iguaçu	PR	25º 25' 52" S	54º 16' 11" W
MS35_17	*B. subalternans*	Laguna Carapã	MS	22º 34' 46" S	55º 09' 59" W
MS41_17	*B. subalternans*	Laguna Carapã	MS	22º 36' 34" S	55º 13' 05" W
PG1_17	*B. pilosa*	Ponta Grossa	PR	25º 05" 23" S	50º 03' 29" W
PG31_17	*B. pilosa*	Palmeira	PR	25º 22' 37" S	50º 00' 54" W
PG33_17	*B. pilosa*	Lapa	PR	25º 39' 30" S	49º 50' 10" W
PG36_17	*B. pilosa*	Lapa	PR	25º 44' 10" S	49º 45' 53" W

sites in Brazil and four screened in the dose-response experiment - SUS, R1, R2 and R3). Herbicides and respective dosages for each experiment were: Exp. 1: imazethapyr (212 g ha^{-1}); Exp. 2: chlorimuron (40 g ha^{-1}); Exp. 3: diclosulam (52 g ha^{-1}); Exp. 4: atrazine (1,500 g ha^{-1}) and Exp. 5: glyphosate (960 g a.e. ha^{-1}). Doses of ALS inhibitors were equivalent to twice of "x" dosage used in the dose response assays. These values were established as a resistance threshold, wherein the biotypes that survived to this treatment were classified as resistant. Experiments with atrazine and glyphosate were added to investigate eventual multiple resistance to alternative mechanisms of action.

All experiments were performed in quadruplicates, respecting a completely randomized design for each biotype. Herbicide application was performed as described for the dose response assays. At 28 DAA, control (0-100%, where 0 = no injury and 100 = plant death) of each biotype was evaluated, comparing each herbicide treatment with the respective non-sprayed check of each biotype.

Control data were subjected to analysis of variance ($p \leq 0.05$) and the means of significant variables were grouped by the Scott-Knott test ($p \leq 0.05$), using SISVAR statistical package. Maps presenting the coordinates C of greater beggarticks biotypes were elaborated using Qgis.2012 software. A color scale based on control rates was used for expressing the final control results in this study (red = resistant (R); yellow = moderately resistant (MOR); green = non-resistant (NR).

RESULTS AND DISCUSSION

Dose-response assays

Dose-response experiments demonstrated three different patterns of cross-resistance for imazethapyr, chlorimuron and diclosulam (Table 2). Biotype R1 was considered resistant to both imazethapyr (RF 13.8) and chlorimuron (RF 3.9). However, its GR_{85} for diclosulam was 28.5 g ha^{-1}, which is within the labeled doses range and, therefore, the biotype was classified as susceptible. Biotype R2 portrayed elevated FR values for all three herbicides: >29.9 for imazethapyr, 15.7 for chlorimuron and >271.6 for diclosulam. In contrast, biotype R3 exhibited resistance only to imazethapyr (RF 11.4), whilst further chlorimuron and diclosulam provided satisfactory control levels once respecting the labeled doses recommended for each herbicide (Table 2).

In Brazil, previous research with *B. pilosa* and *B. subalternans* biotypes resistant to ALS inhibitors have brought to light higher resistance levels for chlorimuron (López Ovejero et al., 2006) and for imazethapyr (Monquero et al., 2000). Lamego et al. (2009) acquired preeminent levels of cross-resistance for imazethapyr (IMI), chlorimuron (SUL) and cloransulam (TP) in a

Table 2 - Model parameters, doses for 50% (GR_{50}) and 85% (GR_{85}) reduction of biomass in relation to non-treated check and resistance factors (RF) for greater beggarticks (*Bidens subalternans*) biotypes resistant to ALS inhibitors. Maringá (PR), 2018

Herbicide	Biotype	a	b	c	R^2	GR_{50}	GR_{85}	RF
Imazethapyr	SUS	99.78	30.5	0.59	0.84	28.3	570	-
	R1	80.74	513.74	1.82	0.91	392	1155	13.8
	R2	NF	NF	NF	NF	>848	>848	>29.9
	R3	96.69	359.5	0.66	0.91	324	4680	11.4
Chlorimuron	SUS	99.99	1.17	1.47	0.85	1.49	3.8	-
	R1	97.23	106.07	0.98	0.94	5.88	595	3.9
	R2	98.13	25.34	0.53	0.92	23.5	640	15.7
	R3	99.36	1.82	0.59	0.87	1.78	34	1.19
Diclosulam	SUS	99.91	0.7385	0.89	0.9	0.74	5.2	-
	R1	99.16	5.98	1.1	0.94	5.85	28.5	7.9
	R2	NF	NF	NF	NF	>201	>201	>271.6
	R3	99.78	1.52	0.77	0.94	1.5	14.4	2.02

RF = GR_{50}SUS/GR_{50}R. Non-linear model fitted: Gompertz, three parameters. NF not fitted by Gompertz model. SUS: susceptible biotype; R1: resistant 1; R2: resistant 2; R3: resistant 3.

biotype from Goiás State, which is an outcome similar to the pattern found in this work for biotype R2.

Screening of additional biotypes from different sites

After describing patterns SUS, R1, R2 and R3, seed samples were analyzed from biotypes collected from other areas to evaluate if they presented similar patterns of cross-resistance. Groups provided by Scott-Knott test were used to rank each biotype according to their control level (Table 3).

Table 3 - Classification of resistance levels in biotypes of greater beggarticks (*Bidens subalternans and Bidens pilosa*) based on groups formed by Scott-Knott (5% probability). Maringá (PR), 2018

Class	Color	Imazethapyr		Chlorimuron		Diclosulam	
		Control (%)	Scott-Knott group	Control (%)	Scott-Knott group	Control (%)	Scott-Knott group
Non-resistant (NR)	green	>84	a	>90	a	>90	a
Moderately resistant (MOR)	yellow	13.4 - 83	b, c, d	8.9 - 89	b, c, d	21-89	b
Resistant (R)	red	<13.3	e	<8.8	e	<20	c

The largest quantity of biotypes classified as green (non-resistant - NR) was found for treatments with diclosulam (48.6%), followed by those comprising chlorimuron (37.8%) and imazethapyr (18.9%). For the yellow group, representing intermediate levels of control (moderately resistant - MOR), chlorimuron was the most frequent herbicide (48.6%), followed by imazethapyr (37.8%) (Table 4). A relatively low frequency (8.1%) was perceived in biotypes submitted to diclosulam. When comparing samples to the lowest control levels (red) to assess the uppermost resistance levels (resistant - R), imazethapyr and diclosulam stood out as the herbicides with highest frequencies (43.2%), whilst chlorimuron denoted the nethermost occurrence (13.5%) (Table 4). All biotypes were susceptible to both atrazine and glyphosate, indicating that no multiple resistance was found (data not shown).

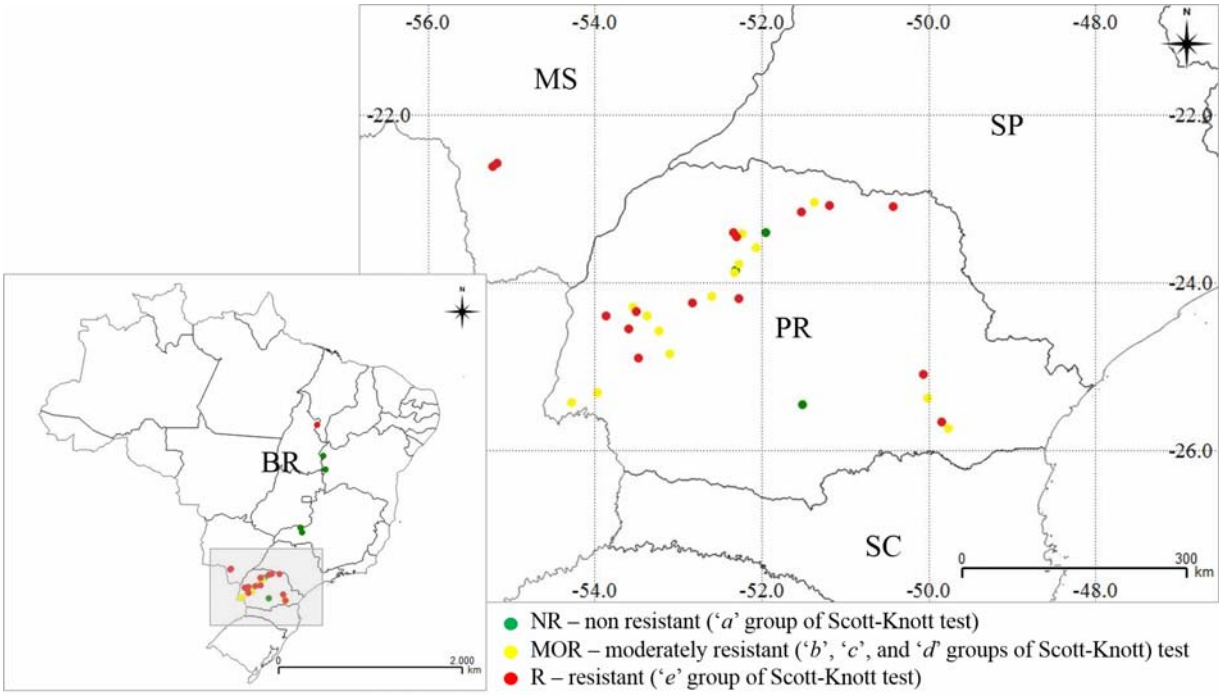
Although differential susceptibility may be present between *B. subalternans* and *B. pilosa* (López-Ovejero et al., 2006), no clear differences were found to separate the two species. This suggests that greater beggarticks sensibility to ALS inhibitor herbicides may depend more on the selection pressure applied to a particular biotype than on the species involved.

A relationship between geographical coordinates and ALS cross-resistance patterns was not observed, since different standards may have occurred in biotypes collected from proximate sites. Meanwhile, biotypes presenting similar patterns may have arisen from spatially distant geographies for imazethapyr (Figure 1), chlorimuron (Figure 2) and diclosulam (Figure 3).

Resistance patterns observed in greater beggarticks biotypes were compared to those found in SUS, R1, R2 e R3 biotypes from the first dose-response experiments. Four biotypes were comparable to pattern R1, ten biotypes exhibited R2 pattern and five biotypes presented R3 pattern. Six biotypes were classified as pattern SUS, while the remaining (12) were resistant to at least one of the ALS herbicides. Nevertheless, these biotypes portrayed distinct patterns from those previously identified (Figure 4).

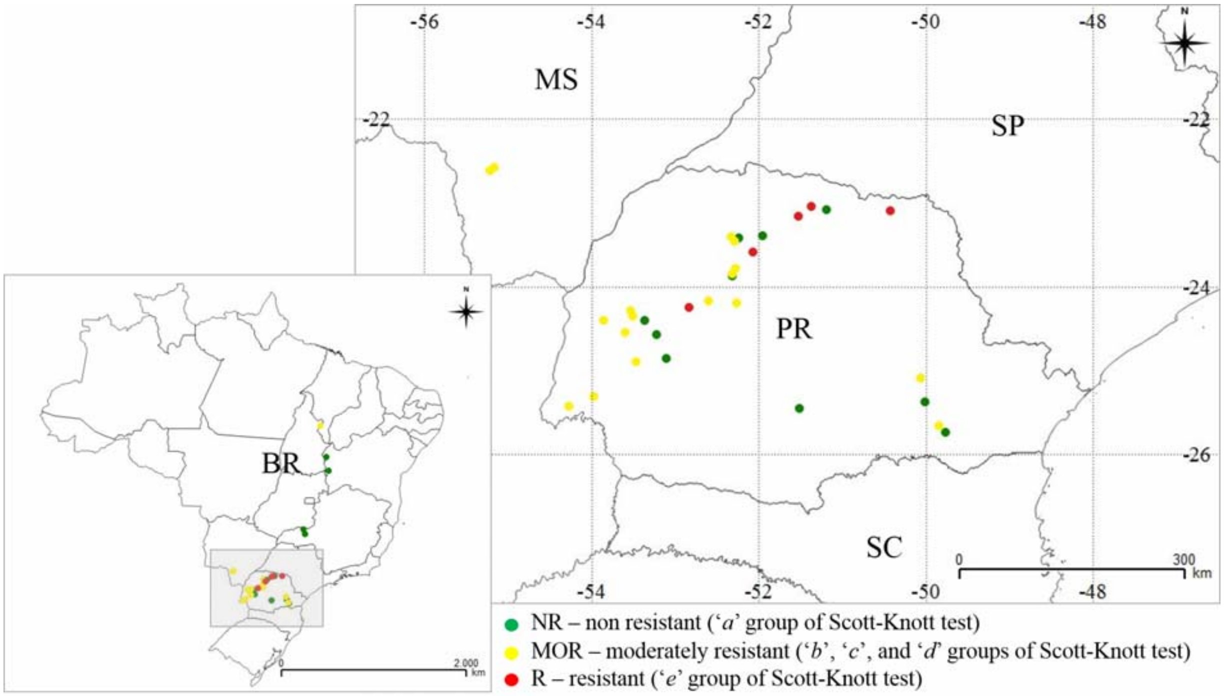
Table 4 - Number (nº) and frequency (%) of *Bidens subalternans and Bidens pilosa* biotypes with different resistance class to ALS inhibitors in Maringa (PR), Brazil, 2018

Class	Imazethapyr		Chlorimuron		Diclosulam	
	nº	%	nº	%	nº	%
Non-resistant (NR)	7	18.9	14	37.8	18	48.6
Moderately resistant (MOR)	14	37.8	18	48.6	3	8.1
Resistant (R)	16	43.2	5	13.5	16	43.2



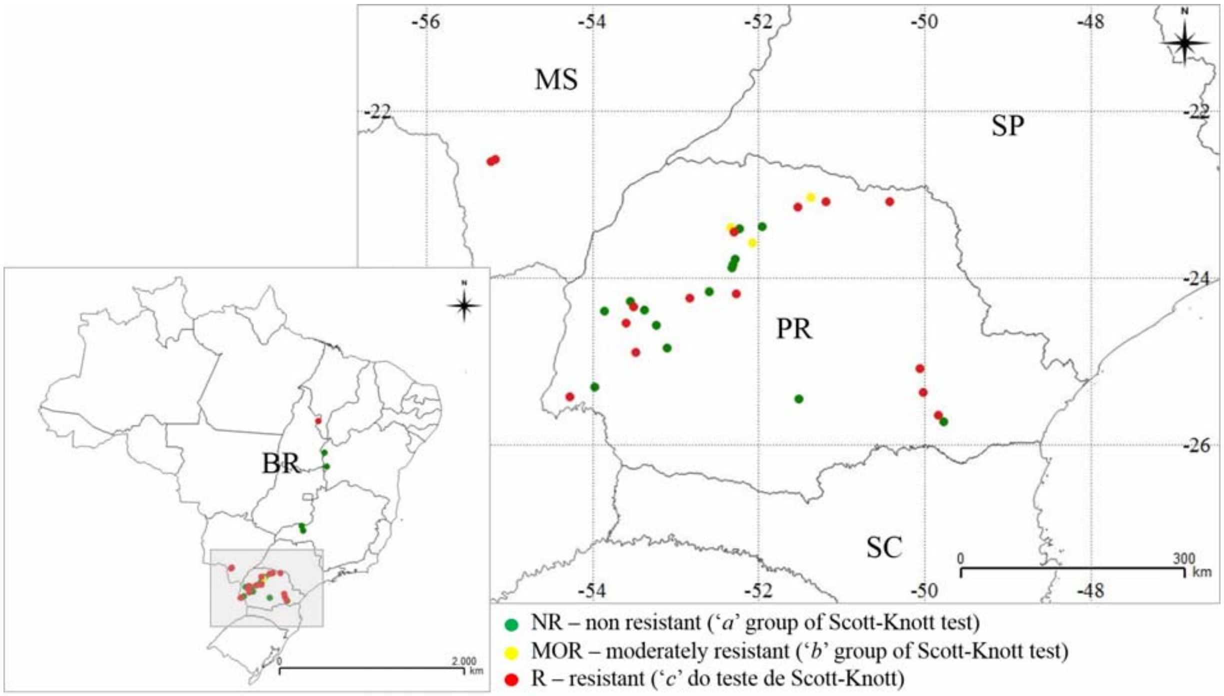
Biotypes grouped as 'a' by Scott-Knott test correspond to >90% control; groups 'b', 'c' and 'd' correspond to control ranging from 13.4% to 83%; group 'e' corresponds to control levels <13.3%.

Figure 1 - Dispersion of greater beggarticks (*Bidens* spp.) biotypes resistant to imazethapyr in Paraná and other regions of Brazil. Maringá (PR), 2018.



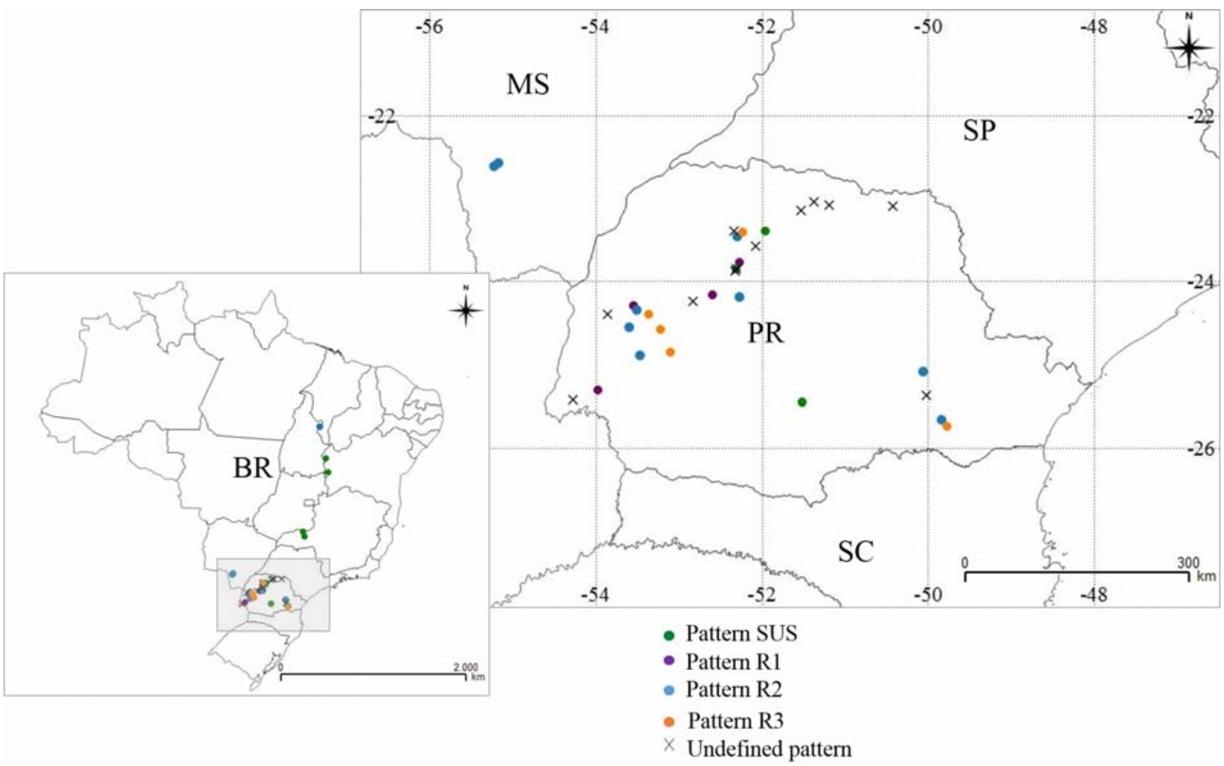
Biotypes grouped as 'a' by Scott-Knott test correspond to >90% control; groups 'b', 'c' and 'd' correspond to control ranging from 8.9% to 89%; group 'e' corresponds to control levels <8.8%.

Figure 2 - Dispersion of greater beggarticks (*Bidens* spp.) biotypes resistant to chlorimuron in Paraná State and other regions of Brazil. Maringá (PR), 2018.



Biotypes grouped as ‘a’ by Scott-Knott test correspond to >90% control; group ‘b’ correspond to control ranging from 21% to 89%; group ‘c’ corresponds to control levels <20%.

Figure 3 - Dispersion of greater beggarticks (*Bidens* spp.) biotypes resistant to diclosulam in Paraná State and other regions of Brazil. Maringá (PR), 2018.



Pattern SUS: non-resistant to imazethapyr, chlorimuron and diclosulam; pattern R1: resistant to imazethapyr and chlorimuron, but non-resistant to diclosulam; pattern R2: resistant to imazethapyr and diclosulam, moderately resistant to chlorimuron; pattern R3: resistant to imazethapyr and non-resistant to both chlorimuron and diclosulam.

Figure 4 - Dispersion of greater beggarticks (*Bidens* spp.) biotypes with different patterns of cross-resistance to ALS inhibitors in Paraná State and other regions of Brazil. Maringá (PR), 2018.

Three distinct patterns of cross-resistance to ALS inhibitors were identified in greater beggarticks: resistance to imazethapyr and chlorimuron (pattern R1), resistance to imazethapyr, chlorimuron and diclosulam (pattern R2) and an exclusive resistance to imazethapyr (pattern R3). Resistance patterns in these herbicides can be likely defined by the point mutations in ALS gene, the major resistance strategy to this mechanism of action (Deng et al., 2014; Yu and Powles, 2015). For other weed species, mutations conferring resistance to all chemical groups (IMI, SU, TP, PYB and SCT) have been found. In addition, the most common substitutions that result in this cross-resistance pattern are Trp-574-Leu, Ala-122-Thr, Asp-376-Glu and Pro-197-Ser (Tranel et al., 2018).

Contrasting with *Bidens* species, the Ala-122-Thr substitution in *Echinochloa crus-galli* may result in resistance to IMIs, but not to TRI (Riar et al., 2013) Similarly, Ser-653-Thr in *Sorghum bicolor* causes tolerance to IMI, but not to SU (Werle et al., 2017). However, in *Conyza canadensis*, the Pro-197-Ser substitution result in resistance to all chemical groups, except for IMI (Zheng et al., 2011). In Brazil, one biotype of *Bidens subalternans* was identified with Trp-574-Leu substitution, thus resistant to four chemical groups of ALS inhibitors (IMI, SU, TP and PYB) (Lamego et al., 2009). Differences of cross-resistance patterns, both those from the present work and those from other studies, suggest that alternative substitutions in the ALS gene related to resistance could occur as well as other non-target-site-based mechanisms, such as metabolization, reduced absorption, translocation and/or vacuolar sequestration (Délye, 2012).

In the early 80's, herbicides from the IMI and SU groups were the first ALS products available for soybeans in Brazil, while diclosulam (TP) was released only in 1993 (Beckie and Tardif, 2012; Deng et al., 2017). Another fact that may have contributed to the relatively decrease in diclosulam use in relation to both imazethapyr and chlorimuron is the quantity of companies manufacturing these commercial herbicides with those actives (eight and nine, respectively), which is significantly superior to a single company producing herbicides based on diclosulam (AGROFIT, 2018). The lack of market competitiveness for this commercial product has led diclosulam to cost relatively more than other options, which also influences on its adoption by farmers. These facts certainly help to explain the more elevated frequency of NR biotypes than diclosulam (Table 4).

Plants belonging to genus *Bidens* have prolific gene recombination due to cross-fertilization rates of up to 10%, which implies that biotypes from relatively close sites may present different features. Concomitantly, the efficient seed dispersal of these species may assist in gene flow and, therefore, in resistance spread (Vidal et al., 2007). Combined with plant biology, the particular history of herbicide application for each field may contribute to the understanding about the geographically scattered distribution patterns in Brazil (Figure 4).

It is likely that other cross-resistance patterns related to regional practices and with the history of each field may occur. The investigation of resistance mechanisms involved in each biotype (R1, R2 and R3) in greater beggarticks is currently under investigation and will provide important scientific knowledge to establish the causes of such distinct cross-resistance patterns to ALS inhibitors.

Biotypes of *Bidens* spp. with cross-resistance to ALS inhibitors are relatively frequent in Brazil, particularly in Paraná. At least three distinct resistance patterns were identified: resistance to imazethapyr and chlorimuron (pattern R1), resistance to imazethapyr, chlorimuron and diclosulam (pattern R2) (the most frequent), and exclusive resistance to imazethapyr (pattern R3). Concisely, patterns were not site-specific but were rather geographically dispersed in different grain producing areas. Cases of multiple resistance to ALS inhibitors + atrazine or ALS inhibitors + glyphosate were not found.

REFERENCES

Sistema Ministério da Agricultura, Pecuária e Abastecimento - AGROFIT. [acesso em: 28 dez. 2018] Disponível em: http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons.

Beckie HJ, Tardif FJ. Herbicide cross resistance in weeds. Crop Prot. 2012;35:15-28.

Carvalho SJP, Lombardi BP, Nicolai M, López-Ovejero RF, Christoffoleti PJ, Medeiros D. Curvas de dose-resposta para avaliação do controle de fluxos de emergência de plantas daninhas pelo herbicida imazapic. Planta Daninha. 2005;23(3):535-42.

Christoffoleti PJ. Curvas de dose-resposta de biótipos resistente e suscetível de *Bidens pilosa* L. aos herbicidas inibidores da ALS. Sci Agric. 2002;59:513-8.

Cruz RA, Fernández-Moreno PT, Ozuna CV, Rojano-Delgado AM, Cruz-Hipolito HE, Domínguez-Valenzuela JÁ, et al. Target and non-target site mechanisms developed by glyphosate-resistant hairy beggarticks (*Bidens pilosa* L.) populations from Mexico. Front Plant Sci. 2016;7:1-12.

Délye C. Unravelling the genetic bases of non-target-site-based resistance (NTSR) to herbicides: a major challenge for weed science in the forthcoming decade. Pest Manag Sci. 2012;69:176-87.

Deng W, CaoY, Yang Q, Liu M, MeiY, Zheng M. Different cross-resistance patterns to AHAS herbicides of two tribenuron-methyl resistant flixweed (*Descurainia sophia* L.) biotypes in China. Pestic Biochem Physiol. 2014;112:26-32.

Deng W, Yang Q, Zhang Y, Jiao H, Mei Y, Li X , Zheng M . Cross-resistance patterns to acetolactate synthase (ALS)-inhibiting herbicides of fixweed (*Descurainia sophia* L.) conferred by different combinations of ALS isozymes with a Pro-197Thr mutation or a novel Trp-574-Leu mutation. Pestic Biochem Physiol. 2017;136:41-5.

Garcia MD, Nouwens A, Lonhienne TG, Guddat LW. Comprehensive understanding of acetohydroxyacid synthase inhibition by different herbicide families. Proc Natl Acad Sci U S A. 2017;114(7):E1091-E1100

Gelmini GA, Victoria Filho R, Novo MCSS, Adoryan ML. Resistência de *Bidens subalternans* aos herbicidas inibidores da enzima acelolactato sintase utilizados na cultura da soja. Planta Daninha. 2002;20(2):319-25.

Grombone-Guaratini MT, Solferini VN, Semir J. Reproductive biology in species of *Bidens* L. (Asteraceae). Sci Agric. 2004;16(2):185-9.

Kissmann KG, Groth D. Plantas infestantes e nocivas. 2ª ed. São Bernardo do Campo: Basf; 1999.

Lamego FP, Charlson D, Delatorre CA, Burgos NR, Vidal RA. Molecular basis of resistance to ALS-inhibitor herbicides in Greater beggarticks. Weed Sci. 2009;57(5):474-81.

Liu W, Yuan G, Du L, Guo W, Li L, Bi Y, et al. A novel Pro197Glu substitution in acetolactate synthase (ALS) confers broad-spectrum resistance across ALS inhibitors. Pestic Biochem Physiol. 2015;117:31-8.

López-Ovejero RF, Carvalho SJP, Nicolai M, Abreu AG, Grombone-Guaratini MT, Toledo REB, Christoffoleti PJ. Resistance and differential susceptibility of *Bidens pilosa* and *B. subalternans* biotypes to ALS-inhibiting herbicides. Sci Agric. 2006;63(2):139-45.

Lorenzi H. Manual de identificação e controle de plantas daninhas: plantio direto e convencional. 7ª.ed. Nova Odessa: Instituto Plantarum; 2014.

Monquero PA, Christoffoleti PJ, Dias CTS. Resistência de plantas daninhas aos herbicidas inibidores da ALS na cultura da soja (*Glycine max*). Planta Daninha. 2000;18(3):419-25.

Peterson MA, Collavo A, Ovejero R, Shivrain V, Walsh MJ. The challenge of herbicide resistance around the world: a current summary. Pest Manag Sci. 2018;34(10):2246-59.

Riar DS, Norsworthy JK, Srivastava V, Nandula V, Bond JA, Scott RC. Physiological and molecular basis of acetolactate synthase-inhibiting herbicide resistance in barnyardgrass (*Echinochloa crus-galli*). J Agric Food Chem. 2013;61(2):278-89.

Takano HK, Oliveira Jr, RS, Constantin J, Braz GBP, Franchini LHM, Burgos NR. Multiple resistance to atrazine and imazethapyr in hair beggarticks (*Bidens pilosa*). Cienc Agrotec. 2016;40(5):547-54.

Tranel PJ, Wright TR, Heap IM. Mutations in herbicide-resistant weeds to ALS inhibitors. [acesso em: 22 jan 2018]. Disponível em: http://weedscience.org/Mutations/MutationDisplayAll.aspx,

Vidal RA, Nunes AL, Resende LV, Lamego FP, Silva PR. Análise genética de genótipos de *Bidens pilosa* através da técnica RAPD. Sci Agr. 2007;8(4):399-403.

Werle R, Begcy K, Yerka MK, Mower JP, Dweikat I, Jhala AJ, et al. Independent evolution of acetolactate synthase-inhibiting herbicide resistance in weedy sorghum populations across common geographic regions. Weed Sci. 2017;65(1):164-76.

Yu Q, Powles SB. Resistance to AHAS inhibitor herbicides: current understanding. Pest Manag Sci. 2015;70:1340-50.

Zheng D, Kruger GR, Singh S, Davis VM, Tranel PJ, Weller SC, et al. Cross-resistance of horseweed (*Conyza canadensis*) populations with three different ALS mutations. Pest Manag Sci. 2011;67(12):1486-92.