

## RESISTANCE AND DIFFERENTIAL SUSCEPTIBILITY OF *Bidens pilosa* AND *B. subalternans* BIOTYPES TO ALS-INHIBITING HERBICIDES

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**ABSTRACT:** The frequent application of herbicides in agricultural areas may select resistant biotypes in weed populations, whose biological characteristics influence the speed and patterns of resistance. This research aims to characterize, simultaneously, resistance patterns and differential susceptibility of *Bidens pilosa* and *B. subalternans* biotypes to ALS-inhibiting herbicides of the imidazolinone and sulfonyleurea chemical groups. Six hairy beggarticks biotypes, four suspected resistant and two known susceptible, were treated with eight rates of chlorimuron-ethyl or imazethapyr, in greenhouse conditions. Percent control and percent fresh weight of the plants were evaluated at 28 days after the application. *B. subalternans* is less susceptible to ALS-inhibiting herbicides than *B. pilosa*; *B. subalternans* biotypes were more resistant than *B. pilosa* biotypes; there are *B. pilosa* and *B. subalternans* biotypes with cross resistance to the ALS-inhibiting herbicides of the sulfonyleurea and imidazolinone groups; there are different patterns of cross resistance to the diverse groups of ALS-inhibiting herbicides.

**Key words:** imidazolinone, sulfonyleurea, hairy beggarticks, soybean

## RESISTÊNCIA E SUSCETIBILIDADE DIFERENCIAL DE BIÓTIPOS DE *Bidens pilosa* E *B. subalternans* AOS HERBICIDAS INIBIDORES DA ALS

**RESUMO:** A freqüente aplicação de herbicidas em áreas agrícolas pode selecionar resistência em biótipos de plantas daninhas, cujas características biológicas influenciam na velocidade e nos padrões da resistência. Este trabalho foi conduzido com o objetivo de caracterizar, simultaneamente, o padrão de resistência e a susceptibilidade diferencial de biótipos de *Bidens pilosa* e *B. subalternans* aos herbicidas inibidores da ALS dos grupos químicos das imidazolinonas e sulfoniluréias. Para tanto, seis biótipos de picão-preto, quatro supostamente resistentes e dois sabidamente suscetíveis, foram submetidos à aplicação de oito doses de chlorimuron-ethyl ou imazethapyr, em condição de casa-de-vegetação. Avaliou-se o controle e a massa fresca percentual das plantas aos 28 dias após a aplicação. *B. subalternans* é menos suscetível aos herbicidas inibidores da ALS que *B. pilosa*; os biótipos de *B. subalternans* mostraram-se mais resistentes que o biótipo de *B. pilosa*; há ocorrência de biótipos de *B. pilosa* e *B. subalternans* com resistência cruzada aos herbicidas inibidores da ALS do grupo das sulfoniluréias e imidazolinonas; há padrões diferenciados de resistência cruzada de picão-preto aos diversos grupos de herbicidas inibidores da ALS.

**Palavras-chave:** imidazolinona, sulfoniluréia, picão-preto, soja

### INTRODUCTION

Acetolactate synthase (ALS)-inhibiting herbicides are among the most effective and widely used herbicides (Tranel & Wright, 2002), and the selection of resistant biotypes by these products (imidazolinones – IMIs, sulfonyleurea – SUL, triazolopyrimidine

sulfonanilides – TPs and pyrimidinylthiobenzoates – PTBs) has been observed in many parts of the world (Heap, 2005; <http://www.weedscience.com>).

Weed resistance to herbicides is defined as a natural and inherited capacity of biotypes to survive and reproduce after the application of an herbicide rate that should be lethal to a normal (susceptible) popula-

tion of the same species (Christoffoleti & López-Ovejero, 2004). The two most notable weeds that have gained resistance to ALS-inhibiting herbicides in Brazilian soybean fields are *Bidens pilosa* and *B. subalternans* (Christoffoleti, 2002; Gelmini et al., 2002).

Morphology and the ecological conditions of habitats occupied by both *B. pilosa* and *B. subalternans* are quite similar; these are sympatric species infesting soybean fields (Kissmann & Groth, 1999). Their similarity and overlapping of characters have resulted in imprecise taxonomic characterization, difficulty in establishing clear boundaries, and doubts about the material identification (Grombone-Guaratini et al., 2005a; 2005b), even in resistance experiments. These weed species are difficult to control mainly because of their high levels of inbreeding and seed production, and long term survival of seeds on the soil (Grombone-Guaratini et al., 2004).

However, because it is so difficult identify *Bidens* species accurately, many authors could not say with which species they to deal with (Monquero et al., 2000; Carvalho et al., 2004). This fact raises questions about the populations used in several published works. The aims of this research were (i) identify and characterize ALS-resistant *Bidens* biotypes, and (ii) evaluate the degree of resistance and differential susceptibility of *B. pilosa* and *B. subalternans* biotypes to chlorimuron-ethyl and imazethapyr.

## MATERIAL AND METHODS

**Seed origin** - Seeds from resistant biotypes of *Bidens pilosa* and *B. subalternans* were collected from soybean production areas in São Paulo (SP), Paraná (PR), Mato Grosso (MT), and Mato Grosso do Sul (MS) states, on properties that had been sprayed with ALS-inhibiting herbicides for at least six consecutive years. Susceptible seed samples were collected in the open field in Santa Bárbara do Oeste (SP) and Instituto de Botânica de São Paulo (IBt/SMA), which had never

been sprayed with ALS-inhibiting herbicides. For locations and biotype names see Table 1.

### Dose-response curves

**Place** - The experiment was conducted in a greenhouse at Piracicaba - SP, Brazil (22°41'15''S, 47°41'15''W and 560 m of altitude) from December/2004 to February/2005.

### Bioassay testing ALS sensitivity to herbicides

- Seeds were germinated in plastic boxes (0.11 × 0.11 × 0.03 m) filled with commercial substrate, inside a germination chamber with photoperiod 8h-light, 30°C, and 16h-dark, 20°C. When seedlings were at the cotyledon stage, they were transplanted to 0.5-L plastic pots (four plants per pot), also filled with commercial substrate.

When plants averaged, four leaves, post-emergence herbicide treatments were applied. Spraying was done inside closed spray chamber, using a flat jet spray tip (Teejet 80.02E), calibrated at 0.50 m height above the target surface, and with a relative volume of 200 L ha<sup>-1</sup>. After herbicide application, pots were placed in the greenhouse and irrigated on the following day to secure adequate foliar absorption of the molecules.

Trials were set up in randomized block design (n = 4), factorial scheme with six biotypes and eight rates of the herbicide chlorimuron-ethyl (sulfonyleurea), and five biotypes and eight rates of the herbicide imazethapyr (imidazolinone). Sufficient seeds did not allow biotype 3 to receive applications of imazethapyr. Rates applied were multiple values of the recommended rate (R) for each herbicide (0.0R; 0.06R; 0.125R; 0.5R; 1R; 2R; 4R and 16R). The recommended rate adopted was 17.5 g ha<sup>-1</sup> for chlorimuron-ethyl, and 100.0 g ha<sup>-1</sup> for imazethapyr. Herbicide treatments are listed in Table 2; all treatments received the addition of mineral oil at 0.5% v/v concentration.

**Evaluation** - Percent control and percent fresh weight were evaluated at 28 days after the application (DAA). Percent control was rated as 0% when the herbicide effect was absent, and 100% when all plants

Table 1 - Number, suspect, species, and origin locality of *Bidens* biotypes (*B. pilosa* and *B. subalternans*).

N <sup>1</sup>	Biotype <sup>2</sup>	Species	Locality		
			State	City	Latitude / Longitude
1	Resistant	<i>Bidens subalternans</i>	Mato Grosso	Rondonópolis	16°22'30"S / 54°37'30"W
2	Resistant	<i>Bidens subalternans</i>	São Paulo	Presidente Prudente	22°11'15"S / 51°26'15"W
3	Resistant	<i>Bidens subalternans</i>	Mato Grosso do Sul	São Gabriel do Oeste	19°26'15"S / 54°33'45"W
4	Resistant	<i>Bidens pilosa</i>	Paraná	Castro	24°48'45"S / 50°03'45"W
5	Susceptible	<i>Bidens subalternans</i>	São Paulo	Santa Bárbara do Oeste	22°48'45"S / 47°26'15"W
6	Susceptible	<i>Bidens pilosa</i>	São Paulo	Piracicaba	22°41'15"S / 47°41'15"W

<sup>1</sup>N<sup>o</sup> - number; <sup>2</sup>Suspect ALS resistant or susceptible biotype.

Table 2 - Treatments with multiples of the recommended rates of the herbicides applied on *Bidens pilosa* and *B. subalternans* biotypes.

Treatments	Multiples of the recommended rate (R)							
	0R	0.06R	0.125R	0.5R	1.0R*	2.0R	4.0R	16.0R
Herbicides	Rate (g ha <sup>-1</sup> )							
chlorimuron-ethyl	0.0	1.09	2.19	8.75	17.50	35.00	70.00	280.00
imazethapyr	0.0	6.25	12.50	50.00	100.00	200.00	400.00	1600.00

\*R is the recommended rate to each herbicide.

died. Percent fresh weight was obtained based on the pots that received the application rate 0D (check), as 100% of possible weight. Fresh weights were corrected based on the checks within each respective block. Percent correction was used to make up for differences in biotypes growth rates.

**Data analysis and elaboration of dose-response curves** - Data were initially submitted to ANOVA ("F" test). Treatment showing significant, qualitative effects (biotypes), were compared by the application of Tukey's test ( $\alpha = 0.05$ ); quantitative effects (rates), were adjusted to a log-logistic model, proposed by Seefeldt et al. (1995),

$$y = a + \frac{b}{\left[1 + \left(\frac{x}{c}\right)^d\right]}$$

where:  $y$  is the variable,  $x$  is the herbicide rate (g i.a. ha<sup>-1</sup>); and  $a$ ,  $b$ ,  $c$  and  $d$  are parameters of the curve, that  $a$  is the minimum limit of the curve,  $b$  is the difference among the maximum and minimum point of the curve,  $c$  is the rate that promotes 50% of response from the variable and  $d$  is the curve slope around  $c$ .

The log-logistic model presents an advantage once one of the parameters of the equation ( $c$ ) is an estimative of  $C_{50}$  or  $GR_{50}$  value (Christoffoleti, 2002).  $C_{50}$  (control by 50%) or  $GR_{50}$  (growth reduction by 50%) are the herbicide rate (g i.a. ha<sup>-1</sup>) that promotes 50% of control or weight reduction on plants, respectively (Christoffoleti, 2002; Christoffoleti & López-Ovejero, 2004). From the equations obtained it was also possible to re-calculate mathematically the values of  $C_{50}$  or  $GR_{50}$ , which were considered during the discussion (Carvalho et al., 2005). Using the  $C_{50}$  or  $GR_{50}$  values, the resistance factor was obtained to all biotypes and all herbicides tested. The resistance factor ( $C_{50}$  resistant /  $C_{50}$  susceptible;  $GR_{50}$  resistant /  $GR_{50}$  susceptible or simply R/S) is an adimensional number that expresses how many times the necessary rate to control 50% of resistant biotype is superior than the rate that controls 50% of susceptible biotype, of the same species (Christoffoleti, 2002; Hall et al., 1998).

## RESULTS AND DISCUSSION

*Bidens pilosa* and *B. subalternans* show morphological differences mainly in the cypselas: in *Bidens pilosa* the majority of cypselas have three awns, while in *B. subalternans* they have four. The angle between the awns and the cypselas is about 135° in *B. pilosa*, while in *B. subalternans* the angle is about 180°. In *B. pilosa*, the fruit surface is scabrous and minutely tuberculate in almost all its length, with trichomes arising from the tubercles. In *B. subalternans*, the surface of the cypselas is scabrous, lacks tubercles and has trichomes concentrated in the apex. Both species are widely distributed in agricultural areas and along roadsides.

According to Kissmann & Groth (1999), *B. pilosa* and *B. subalternans* are sympatric, soybean crop pests. However, in this research, we identified by morphological seed and seedling characters, all resistant seeds from Paraná as *B. pilosa*, and all resistant seeds from Mato Grosso, Mato Grosso do Sul and São Paulo as *B. subalternans*.

For both herbicides, in all evaluations, there were significant interactions between the rates applied and *Bidens* biotypes, justifying factorial decomposition. Table 3 presents the parameters of log-logistic model of two variables studied for the herbicides chlorimuron-ethyl and imazethapyr. These parameters allowed fitting plant control or fresh weight (%) as function of herbicides rates, as presented on Figures 1 to 4. Least significant differences (LSD) of Tukey's test ( $\alpha = 0.05$ ) comparing biotypes, also are presented in the figures.

Control results indicated that the *B. subalternans* susceptible biotype is more tolerant than the *B. pilosa* susceptible biotype, and that *B. subalternans* resistant biotypes display higher level of resistance than *B. pilosa* resistant biotypes. Neither chlorimuron-ethyl nor imazethapyr was effective in controlling resistant biotypes of *B. pilosa* and *B. subalternans* (Figures 1 and 2). However, both herbicides were effective in controlling the susceptible biotypes of *B. pilosa* and *B. subalternans* at recommended rates.  $C_{50}$  and  $GR_{50}$  values showed differences between

Table 3 - Log logistic model<sup>1</sup> parameters to *Bidens* biotypes for all variables evaluated, when treated with chlorimuron-ethyl or imazethapyr.

N°	State	Species	Variable	Parameters				
				a	b	c	d	R <sup>2</sup>
chlorimuron-ethyl								
1	MT	<i>B. subalternans</i>	Control % - 28 DAA	-1.249	93.427	234.410	-1.971	0.980
			Fresh weight (%)	7.081	79.694	76.360	1.372	0.975
2	SP	<i>B. subalternans</i>	Control % - 28 DAA	-2.067	85.697	87.220	-0.979	0.972
			Fresh weight (%)	11.209	81.950	69.211	0.591	0.859
3	MS	<i>B. subalternans</i>	Control % - 28 DAA	-0.822	86.038	169.380	-1.215	0.961
			Fresh weight (%)	16.668	76.899	209.040	1.897	0.960
4	PR	<i>B. pilosa</i>	Control % - 28 DAA	-0.556	102.410	75.066	-1.752	0.983
			Fresh weight (%)	-1.638	99.543	46.461	1.489	0.970
5	SP	<i>B. subalternans</i>	Control % - 28 DAA	-0.646	102.932	5.695	-1.225	0.998
			Fresh weight (%)	-5.988	106.568	4.391	0.641	0.969
6	SP	<i>B. pilosa</i>	Control % - 28 DAA	-0.431	103.964	3.654	-0.952	0.991
			Fresh weight (%)	-0.027	102.246	1.454	0.988	0.950
imazethapyr								
1	MT	<i>B. subalternans</i>	Control % - 28 DAA	2.035	106.650	73.585	-0.476	0.964
			Fresh weight (%)	-5.065	104.400	16.556	0.490	0.972
2	SP	<i>B. subalternans</i>	Control % - 28 DAA	-2.807	102.100	140.160	-1.516	0.967
			Fresh weight (%)	-5.808	100.910	124.410	0.641	0.964
4	PR	<i>B. pilosa</i>	Control % - 28 DAA	1.780	102.020	20.048	-1.027	0.970
			Fresh weight (%)	-0.307	100.570	7.739	1.260	0.993
5	SP	<i>B. subalternans</i>	Control % - 28 DAA	0.132	103.214	14.803	-0.874	0.999
			Fresh weight (%)	0.263	101.303	6.926	0.834	0.956
6	SP	<i>B. pilosa</i>	Control % - 28 DAA	0.175	102.298	10.101	-0.900	0.998
			Fresh weight (%)	4.105	97.759	5.367	1.076	0.939

<sup>1</sup>Model:  $y = a + (b/(1+(x/c)^d))$ .

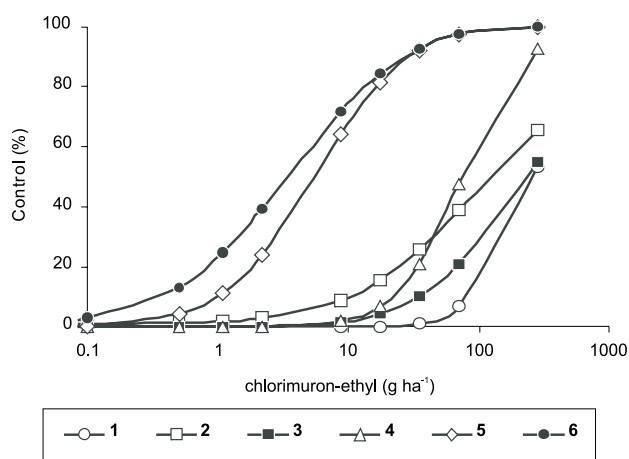


Figure 1 - Dose-response curves of resistant (R) and susceptible (S) biotypes of *Bidens pilosa* and *B. subalternans* for the herbicide chlorimuron-ethyl, at 28 DAA. 1 - *B. subalternans* R - MT; 2 - *B. subalternans* R - SP; 3 - *B. subalternans* R - MS; 4 - *B. pilosa* R - PR; 5 - *B. subalternans* S - SP; 6 - *B. pilosa* S - SP. LSD<sub>(biotypes)</sub> = 4.23 at Tukey test ( $\alpha = 0.05$ ).

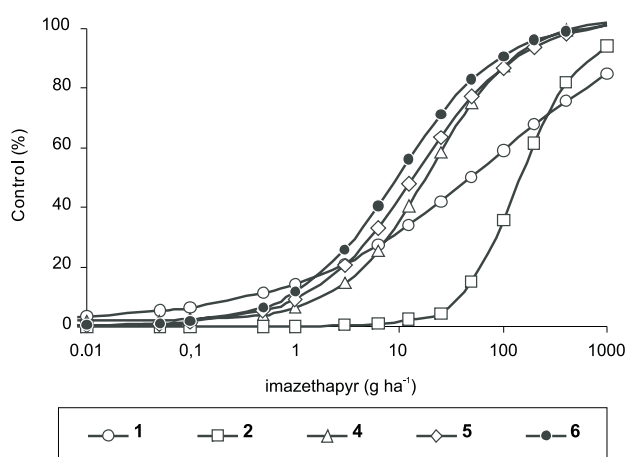


Figure 2 - Dose-response curves of resistant (R) and susceptible (S) biotypes of *Bidens pilosa* and *B. subalternans* for the herbicide imazethapyr, at 28 DAA. 1 - *B. subalternans* R - MT; 2 - *B. subalternans* R - SP; 4 - *B. pilosa* R - PR; 5 - *B. subalternans* S - SP; 6 - *B. pilosa* S - SP. LSD<sub>(biotype)</sub> = 3.54 at Tukey Test ( $\alpha = 0.05$ ).



Table 4 -  $C_{50}$ ,  $GR_{50}$  and resistance factor (R/S) for resistant and susceptible biotypes of *Bidens pilosa* and *B. subalternans*.

N <sup>o1</sup>	State	Species	$C_{50}$ or $GR_{50}$			
			chlorimuron-ethyl		imazethapyr	
			Control	Fresh Weight	Control	Fresh weight
----- % -----						
1	MT	<i>B. subalternans</i>	258.765	68.228	48.167	13.230
2	SP	<i>B. subalternans</i>	136.309	82.906	146.664	89.225
3	MS	<i>B. subalternans</i>	229.079	240.737	--	--
4	PR	<i>B. pilosa</i>	73.989	44.178	18.022	7.733
5	SP	<i>B. subalternans</i>	5.549	3.747	13.704	7.232
6	SP	<i>B. pilosa</i>	3.432	1.518	9.536	6.013
Resistance factor (R/S)						
1	MT	<i>B. subalternans</i>	46.63	18.21	3.51	1.83
2	SP	<i>B. subalternans</i>	24.57	22.12	10.70	12.32
3	MS	<i>B. subalternans</i>	41.28	64.24	--	--
4	PR	<i>B. pilosa</i>	21.56	29.09	1.89	1.29

<sup>1</sup>N<sup>o</sup> - Number.

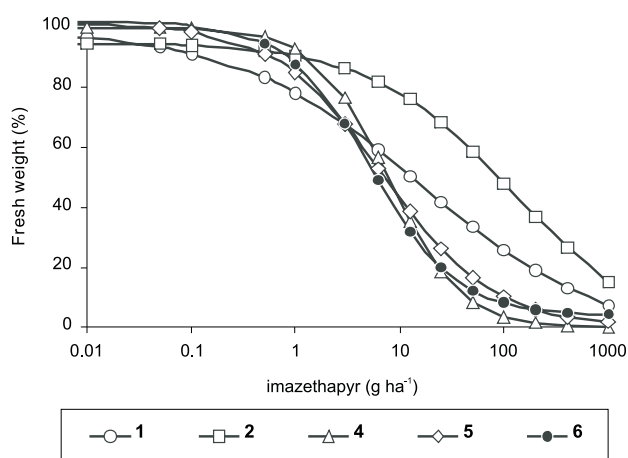


Figure 3 - Fresh weight (%) of resistant (R) and susceptible (S) biotypes of *Bidens pilosa* and *B. subalternans* for the herbicide chlorimuron-ethyl, at 28 DAA. 1 - *B. subalternans* R - MT; 2 - *B. subalternans* R - SP; 3 - *B. subalternans* R - MS; 4 - *B. pilosa* R - PR; 5 - *B. subalternans* S - SP; 6 - *B. pilosa* S - SP.  $LSD_{(biotypes)} = 32.76$  at Tukey Test ( $\alpha = 0.05$ ).

the resistant (R) and susceptible (S) biotypes of both species (Table 4). According to Kissmann & Groth (1999), *B. subalternans* has higher levels of tolerance to herbicides than *B. pilosa*.

*B. subalternans* from Mato Grosso and Mato Grosso do Sul had highest levels of resistance to chlorimuron-ethyl. Rates up to 70 g ha<sup>-1</sup> of this herbicide were used to obtain controls of 50% in these populations. To reach control of 50% in *B. pilosa* population, rates of the same herbicide used were lower (Table 4). Control tests with imazethapyr showed that *B. subalternans* from São Paulo displayed highest lev-

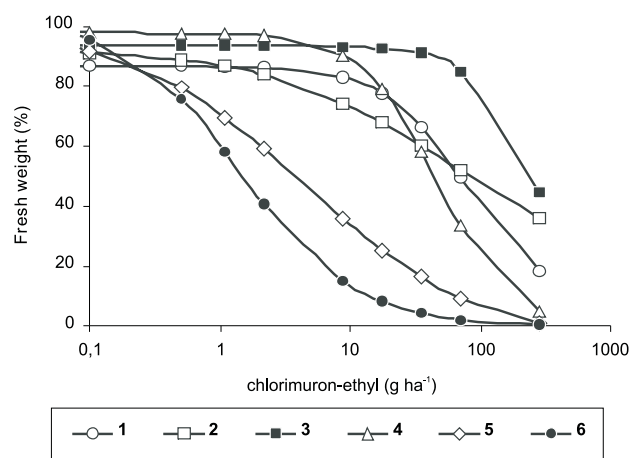


Figure 4 - Fresh weight (%) of resistant (R) and susceptible (S) biotypes of *Bidens pilosa* and *B. subalternans* for the herbicide chlorimuron-ethyl, at 28 DAA. 1 - *B. subalternans* R - MT; 2 - *B. subalternans* R - SP; 4 - *B. pilosa* R - PR; 5 - *B. subalternans* S - SP; 6 - *B. pilosa* S - SP.  $LSD_{(biotypes)} = 29.66$  at Tukey Test ( $\alpha = 0.05$ ).

els of resistance. Rates up to 140 g ha<sup>-1</sup> of the herbicide were used to obtain control of 50% in these populations (Table 4). These results are similar to those reported by Gelmini et al. (2002), but are in disagreement with Christoffoleti (2002) and Monquero et al. (2000), whose data showed that *B. pilosa* biotype presented higher levels of resistance to imazethapyr.

Cross-resistance between the two ALS-inhibiting herbicides (the imidazolinones - IMIs and the sulfonylurea - SUL) was registered for all *B. pilosa* and *B. subalternans* suspect biotypes studied. Both species had different degrees of cross-resistance to ALS-inhib-

iting herbicides. These data agree with results of Christoffoleti (2002) and Monquero et al. (2003) for *B. pilosa*, and Gelmini et al. (2002) for *B. subalternans*.

There was percent fresh weight reduction with increasing herbicides rates (Figures 3 and 4). Once again, on the supposed resistant biotypes, the rates needed to reduce fresh weight were greater in comparison to the susceptible biotypes. For this variable, the *B. subalternans* biotype from Mato Grosso do Sul was the least sensitive to the herbicide chlorimuron-ethyl (Figure 3), while *B. subalternans* biotype from São Paulo was the least sensitive to the herbicide imazethapyr (Figure 4).

Resistance in many weeds has been attributed to single point mutations, which can occur at multiple sites in the ALS gene (Tan & Medd, 2002). Base changes in at least four protein domains have been associated with *in vivo* resistance in field plants (Wright et al., 1998). The most common mutation in biotypes selected by sulfonylureas is in the highly conserved Domain A, that codes for 13 amino acids, where any alteration of the codon for Pro confers resistance, primarily, to sulfonylureas (SUL) and triazolopyrimidines (TP) (Guttieri et al., 1992). A Trp→Leu mutation in Domain B has been associated with broad cross-resistance to representatives of all four families of ALS-inhibiting chemicals (Bernasconi et al., 1995; Woodworth et al., 1996a). In Domain C, an Ala→Thr mutation appears to confer resistance only to imidazolinones (IMI) (Bernasconi et al., 1995), whilst an Ala→Val substitution in Domain D is reported to confer broad cross-resistance (Woodworth et al., 1996b), as in the case of the mutation in Domain B.

Many mutations have been documented for ALS, conferring different resistance levels to various classes of inhibitors; resistant biotypes to ALS inhibiting herbicides show cross-resistance to members of chemical families with the same action mechanism (Tranel & Wright, 2002). Many works have shown that ALS-resistant weed biotypes have cross-resistance to herbicides that belong to chemical group that selected them, and diverse levels of cross-resistance to other herbicide groups with the same action mechanism (Rizzardi et al., 2002). It is probably a consequence of the selection pressure imposed by the main herbicide used, which selected those mutations especially related with its chemical group. This can, at least in part, explain results of this work and the great diversity of results reported in the literature. There are many ALS amino acid substitutions that confer herbicide resistance (Tranel & Wright, 2002); however we did not find any papers that reported data for the *B. pilosa*-*B. subalternans* complex.

The majority of the resistance cases of hairy beggarticks to ALS-inhibiting herbicides occur in *Bidens subalternans*, not in *B. pilosa*, as previously thought. The confirmation that susceptible biotypes of *B. subalternans* are more tolerant to these herbicides than *B. pilosa* reveals how important species identification is to effective control. The identification of point mutations in the ALS gene domains can clarify the resistance patterns and give insights on the necessity of alternative systems that prevent or delay the emergence of resistance.

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