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Multiple resistance to atrazine and imazethapyr in hairy beggarticks (*Bidens pilosa*)

Resistência múltipla a atrazina e imazethapyr em picão-preto (*Bidens pilosa*)

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ABSTRACT

Resistance to herbicides is a serious threat to crop production worldwide, especially in agronomic crops and cereals. This research evaluated the possible occurrence of *Bidens pilosa* resistant to imazethapyr and atrazine in Brazil. The resistant biotype was collected from an area with a history of repeated application of photosystem II (PSII) and ALS inhibitor herbicides. The susceptible biotype was collected from an area with no history of herbicide application. Resistance verification experiments were carried out in the greenhouse. The treatments were arranged in a 3 x 8 factorial scheme, where the first factor was populations [susceptible (S), parent resistant (PR), and resistant F1 (RF1)]; and the second factor was herbicide dose (0, 375, 750, 1500, 3000, 6000, 12000 and 24000 g ha^{-1} for atrazine; or 0, 12.5, 25, 50, 100, 200, 400 and 800 g ha^{-1} for imazethapyr). The resistance factor to atrazine was 2.83 for PR and 5.55 for RF1. This population was more resistant to imazethapyr (>21-fold) than it was to atrazine. The recommended maximum dose of the herbicides did not control this *B. pilosa* population adequately. The data support the claim that *B. pilosa* population from this field in Quarto Centenário, Parana is resistant to two herbicide modes of action - PSII inhibitor (i.e. atrazine) and ALS inhibitor (i.e. imazethapyr). This is the first report of such case for this species, globally. Cross-resistance to other ALS inhibitors and other PS II inhibitors as well as the respective mechanisms of resistance to each herbicide are being investigated.

Index terms: Photosystem II; resistance level; herbicide resistance.

RESUMO

A resistência de plantas daninhas à herbicidas constitui uma das principais ameaças para a agricultura no mundo, especialmente para a produção de grãos. O presente trabalho objetivou verificar a possível existência de *Bidens pilosa* resistente a imazethapyr e atrazina. Dois experimentos foram realizados em casa-de-vegetação, sendo um para cada herbicida. O biótipo resistente foi proveniente de área com histórico de aplicações de herbicidas inibidores do fotossistema II e da ALS, enquanto que o biótipo suscetível foi coletado em área sem histórico de aplicação de herbicidas. Os tratamentos foram dispostos em esquema fatorial 3 x 10, em que o primeiro fator foi composto por diferentes populações [suscetível (S), resistente parental (RP) e resistente F1 (RF1)], e o segundo fator constituiu de doses de cada herbicida (0, 375, 750, 1500, 3000, 6000, 12000 and 24000 g i.a. ha^{-1} para atrazina e 0; 12,5; 25; 50; 100; 200; 400 e 800 g e.a. ha^{-1} para imazethapyr). O fator de resistência variou entre 2,83 e 5,55 para atrazina e >21 para imazethapyr. Além disso, a dose máxima de registro dos herbicidas não controlou as plantas do biótipo resistente. Os resultados revelaram que a população de B. pilosa proveniente de Quarto Centenário, Paraná, apresenta resistência múltipla a dois mecanismos de ação: inibidores da ALS (atrazine) e do fotossistema II (imazethapyr). Este é o primeiro relato deste tipo de resistência nesta espécie no mundo. A resistência cruzada a outros herbicidas destes dois mecanismos, bem como o mecanismo de resistência estão sendo investigados.

Termos para indexação: Fotossistema II; fator de resistência; resistência a herbicidas.

INTRODUCTION

Resistance to herbicides has become an important problem in crop production worldwide over the last two decades. In Brazil, 36 unique cases have been reported so far on weed species with resistance to at least one mechanism of action; 61.8% of these cases are related to biotypes with resistance to acetolactate synthase (ALS) inhibitors, and 11.8% to inhibitors of photosystem II (PSII) (Heap, 2016).

Species with confirmed resistance to herbicides, two of the most widespread in Brazil are *Bidens pilosa* (BIDPI) and *Bidens subalternans* (BIDSU). Both species, locally known as "*picão-preto*", are very similar morphologically and are distributed widely over agricultural areas and along roadsides, in tropical and subtropical regions of the country (Grombone-Guaratini; Solferini; Semir, 2004). The main morphological differences between BIDPI and BIDSU are in the cypselae, the typical Asteraceae achene-like fruit: most cypselae in BIDPI have three aristae, as compared to four in BIDSU. Also, the angle between the aristae and the cypselae in BIDPI is about 135º, while in BIDSU is 180°. The corolla of the marginal flowers is white in BIDPI and yellow or orange in BIDSU. BIDPI also has broader leaf segments, while BIDSU has narrower leaf segments (Grombone-Guaratini; Solferini; Semir, 2004; Lopez-Ovejero et al., 2006).

Resistance to ALS inhibitors in BIDPI was first reported in soybean fields from Mato Grosso do Sul in 1993. Although this biotype was selected under a 6-year imazaquin/chlorimuron rotation, it was also cross-resistant to other ALS inhibitors in the imidazolinone (imazethapyr), sulfonylurea (nicosulfuron), and pyrimidinylthiobenzoate (pyrithiobac-sodium) chemical groups. The resistance in BIDPI was due to target site mutation in the *ALS* gene (Heap, 2016). Resistance to ALS inhibitors in BIDSU was subsequently confirmed in 1996 for the herbicides chlorimuron, imazethapyr and nicosulfuron also in soybean fields from Mato Grosso do Sul. Afterwards, in 1996, multiple resistance to ALS (foramsulfuron and iodosulfuron methyl sodium) and PSII inhibitors (atrazine) was reported for BIDSU biotypes in corn (Heap, 2016). Resistance to herbicides that inhibit the ALS enzyme has been attributed to amino acid alteration(s) in the ALS protein. Lamego et al. (2009) demonstrated that resistance to ALS inhibitors in BIDSU was correlated to one of the following amino acid substitutions in the resistant biotype: Trp_{574}Leu, Phe_{116}Leu or Phe_{149}Ser.

Many grain-producing farmers of West-Central and Southern regions of Brazil practice soybean-corn double cropping. Before glyphosate-resistant (GR) soybean was introduced in Brazil in 2006, the recurrent use of ALS inhibitors in soybean and of PSII inhibitors in corn was the primary driver for resistance selection.

Once selected, resistant biotypes of BIDPI tend to spread quickly, due to its rapid growth, short life cycle, and to the special structures on the aristae that facilitate adhesion to surfaces, increasing its capacity to colonize new areas (Baio; Pires; Tomquelsk, 2013). Despite its high tendency to spread, the predicted increase in importance of the resistant biotypes of BIDPI did not occur, probably due to the gradual substitution of conventional soybeans with GR varieties and to the high sensitivity of *Bidens* spp. to glyphosate (Braz et al., 2011).

Ten years after the introduction of GR soybean in Brazil, at least seven species have evolved resistance to glyphosate and "traditional" herbicides such as imidazolinones, sulfonylureas, triazolopyrimidines and triazines are again widely used both for in-crop and off-season weed management (Oliveira Neto et al., 2010). All the eight cases of multiple resistance reported in Brazil so far (Heap, 2016) include resistance to ALS inhibitors, PSII inhibitors or both.

Economic losses due to the reduced effectiveness of herbicides on BIDPI are evident, both in terms of reduced yield volumes and reduction of grain quality (Freitas et al., 2006; Faria; Barros; Tuffi Santos, 2014). Within this context, this research aimed to confirm the resistance of *Bidens pilosa* from Parana to imazethapyr and atrazine.

MATERIAL AND METHODS

Seeds of the putative resistant (R) population were obtained from a farm located at Quarto Centenário, Paraná (PR), Brazil (24º17'19" S; 53º05'24" W) with a history of at least six years of applications of PSII and ALS inhibitor herbicides in a continuous soybean-corn double cropping rotation. Seeds of the susceptible (S) population were collected from an isolated urban area in Paranavaí, PR, (23º05'46" S; 53º27'14" W) with no history of herbicide application. Plants from the field-collected R population were also grown separately in a greenhouse to produce seeds. Henceforth, the field-collected seeds will be referred to as the 'parent resistant' (PR) population and the progeny of these resistant, field-collected plants will be referred to as the 'resistant F1 (RF1) population. Due to the morphological similarities of BIDPI to other species such as BIDSU, a voucher specimen was collected and deposited at the Herbarium of the State University of Maringá, where it was positively identified as *Bidens pilosa* L. under the record HUEM 29220.

Several commercial formulations of atrazine and imazethapyr are recommended for the control of BIDPI in Brazil. Among the atrazine formulations, more than 80% have a label dose of 1600 to 3000 g a.i ha^{-1} for post-emergence control of BIDPI. For imazethapyr, all labels in Brazil recommend a dose range of 85.6 to 100 g a.e ha^{-1}. For the purposes of this study, the highest recommended dose for each herbicide (3000 g ha^{-1} for atrazine and 100 g ha^{-1} for imazethapyr) was used, and the range for the dose-response assays was established based on these doses.

Each experiment was set in a 3x8 factorial scheme in randomized complete blocks with four replications. The first factor was population (S, PR, and RF1) and the second factor was herbicide dose [0, 375, 750, 1500, 3000, 6000, 12000 and 24000 g a.i ha^{-1} for atrazine (Primoleo® SC, 400 g a.i L^{-1}, Syngenta Brazil); and 0, 12.5, 25, 50, 100, 200, 400 and 800 g a.i ha^{-1} for imazethapyr (Pivot® CS, 100 g a.i L^{-1}, BASF Brazil)].

The experimental units were composed of 1.0 dm^3 pots, filled with a commercial substrate (Plantmax HA®). Thirty seeds were planted per pot and seedlings were thinned to three per pot. The herbicides were applied when plants had four to six leaves. All applications were made with a CO_2 backpack sprayer and a 1.5-m boom fitted with three AI 110.02 nozzles (0.5 m between tips) that were calibrated to deliver 200 L ha^{-1} at 245 kPa. During herbicide application, the relative humidity was 65%, air temperature was 25 °C and wind velocity was ≤ 0.33 m s^{-1}.

Weed control was evaluated visually at 28 days after application (DAA) using a scale of 0 to 100%, where 0% represents no control and 100% represents death. Also at 28 DAA, the remaining shoots were harvested, placed in paper bags, dried (65 °C for 72 h) and weighed (g per pot). Data were subjected to analysis of variance and regression, and adjusted for the nonlinear logistic regression model proposed by Streibig (1988) (Equation 1):

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

Where: y is the percentage control; x is the dose of herbicide (g ha^{-1}); and a, b and c are estimated parameters of the equation: a is the amplitude between the maximum and the minimum point of the variable; b is the dose which provides 50% response; and c is the slope of the curve around b.

One of the integral terms of the equation for the logistic model (b) is an estimate of the value of C_{50} or GR_{50}. C_{50} and GR_{50} are the herbicide doses which reduced, respectively, weed control or shoot dry weight by 50%. Although one of the parameters of the logistic model (b) is a C_{50} or GR_{50} estimate, a mathematical solution of these values was estimated using the inverse equation, as proposed by Carvalho et al. (2005) (Equation 2):

$$x = b\left(\left|\frac{a}{y} - 1\right|\right)^{\frac{1}{c}} \quad (2)$$

Based on C_{50} and GR_{50}, the resistance factor (RF) was calculated as C_{50} or GR_{50} of the resistant population / C_{50} or GR_{50} of the susceptible standard). This value expresses the number of times that the dose necessary to control 50% of the resistant population is greater than the dose which controls 50% of the susceptible (Burgos et al., 2013). In addition to C_{50} and GR_{50}, the values for C_{80} and GR_{80} were also estimated with the inverse equation because these values have practical implication in gauging potential economic impact of the resistant population under field conditions (SBCPD, 1995).

For the purpose of this work, the populations were considered resistant when RF≥2.0 and simultaneously satisfied two additional conditions: C_{80} and GR_{80} values > highest recommended dose to control this species (Francischini et al., 2014; Santos et al., 2014).

RESULTS AND DISCUSSION

Resistance to atrazine

The model proposed by Streibig (1988) adequately described % control and shoot dry weight response of BIDPI to atrazine. Based on the fitted models, doses of atrazine required for 50% and 80% control, as well as for the shoot dry weight of each population were estimated (Table 1).

The recommended dose of atrazine postemergence for BIDPI is within the range of 1 to 1.5 kg ha^{-1} (Rodrigues; Almeida, 2011). With this dose, the susceptible population should be adequately controlled by atrazine, which is reflected by the C_{80} and GR_{80} values. For instance, by using the lowest recommended dose (1 kg ha^{-1}), the predicted response of the S population would be 92.9% control or 99.7%, dry matter reduction.

Based on C_{50} values, it would take 4.6 times as much atrazine to control the field-collected (PR) than the S population and about 7 times more atrazine to achieve 80% control of PR than it would for the S population. The offsprings of plants that survived atrazine application in the field (RF1) required similar amount of atrazine to attain 50% or 80% control as PR. Similar response pattern was also observed with biomass reduction between PR and RF1 relative to the S biotype. All together, this showed that the acquired ability of PR to tolerate atrazine is heritable and that PR is resistant to atrazine.

The fact that the progenies had similar resistance level as the parents is expected in autogamous plants such as those of the genus *Bidens*. Although autogamous, BIDPI and BIDSU are genetically compatible and can cross-pollinate at low levels (up to 9%), depending on the presence of pollinators (Grombone-Guaratini; Solferini; Semir, 2004; Vidal et al., 2006; Huang; Kao, 2014). Conversely, the findings in our work raise the hypothesis that resistance in BIDPI could also spread to BIDSU.

The dose of atrazine required to control or reduce shoot dry weight by 80% was 4,194 and 3,901 g ha^{-1} in PR and RF1, respectively, indicating that the maximum dose recommended in commercial formulations of atrazine (1.5 kg ha^{-1}) is no longer effective (41.6 to 56.6%) on this field population and its progenies. Conversely, the doses to control or reduce dry weight at least by 80% of the S population were 547 and 479 g ha^{-1}, respectively, indicating the expected level of performance of the commercial label dose (Figures 1 and 2). To the best of our knowledge, this is the first report of resistance in BIDPI to a photosystem II inhibitor anywhere in the world.

Atrazine-susceptible BIDPI have been controlled (100%) with doses ranging from 1500 to 1600 g ha^{-1} applied both pre and postemergence, including those reported as ALS-resistant (Dan et al., 2010; Guerra et al., 2011).

Resistance to Imazethapyr

The response of PR and RF1 to imazethapyr in terms of % control did not fit the Streibig model, although the model describes the response of S population well (Table 2). Even the highest dose used (800 g ha^{-1}), which is ≈ 8 x the recommended dose, was less than 50% effective on the putative resistant population and its progeny. The model was adjusted for the dry matter data and all parameters were significantly; however, the estimated GR_{50} and GR_{80} were outside the tested dose range (>800 g ha^{-1}). Given that the highest dose tested (800 g ha^{-1}) was not enough to provide at least 50% control, and that the C_{50} for the S population was 37 g ha^{-1}, we could say that the PR and RF1 plants had >21 times the capability of the susceptible plants to tolerate the commercial dose of imatzethapyr. Therefore, we concluded that PR is also highly resistant to imazethapyr besides being resistant to atrazine.

Table 1: Estimated parameters of the Streibig model and resistance factor (RF) for % control or reduction of shoot dry matter accumulation in the susceptible (S), parent resistant (PR) and resistant F1 (RF1) populations, and estimated doses of atrazine to provide 50% or 80% of *Bidens pilosa* control or shoot dry matter reduction.

	R^2	*a*	*b* (C_{50} or GR_{50})	*c*	RF	C_{80} or GR_{80}
% Control S	0.97	101.64	C_{50} = 260	-1.76	-	C_{80} = 547
% Control PR	0.94	108.22	C_{50} = 1,626	-1.10	5.6	C_{80} = 4,194
% Control RF1	0.95	111.55	C_{50} = 1,450	-0.94	4.6	C_{80} = 3,901
Dry matter reduction S	0.96	2.75	GR_{50} = 381	6.05	-	GR_{80} = 479
Dry matter reduction PR	0.89	3.03	GR_{50} = 1,084	1.01	2.9	GR_{80} = 4,437
Dry matter reduction RF1	0.93	2.89	GR_{50} = 1,117	1.13	2.8	GR_{80} = 3,884

$C_{50;}$ C_{80} or GR_{50}; GR_{80}: doses of atrazine which provides 50 or 80% of control or dry weigh reduction.

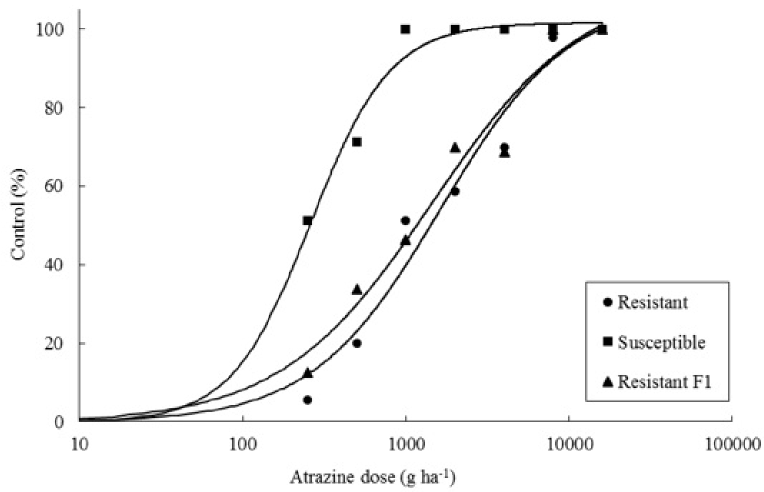

Figure 1: Response (% control) of *Bidens pilosa* populations to increasing doses of atrazine 28 days after application, Maringá, PR, Brazil 2016.

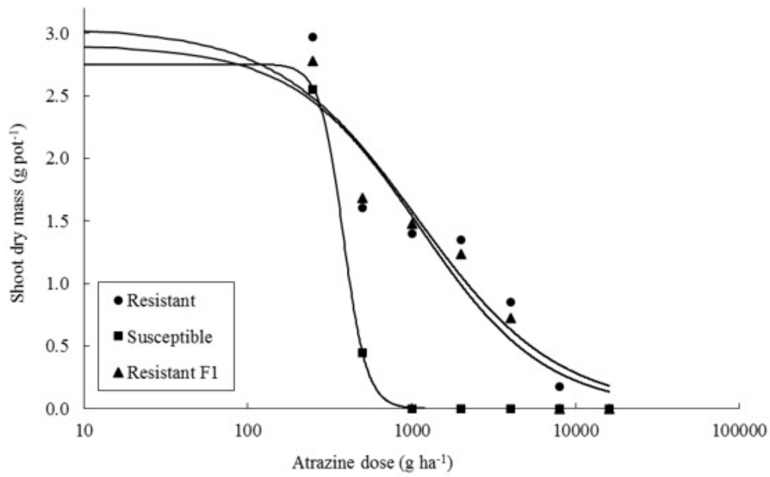

Figure 2: Response (g dry shoot biomass/pot) of *Bidens pilosa* populations to increasing doses of atrazine 28 days after application.

Table 2: Estimated parameters of the Streibig model and resistance factor (RF) for % control or reduction of shoot dry matter accumulation in the susceptible (S), parent resistant (PR) and resistant F1 (RF1) populations, and estimated doses of imazethapyr to provide 50% or 80% control or shoot dry matter reduction of *Bidens pilosa*.

	R^2	*a*	*b* (C_{50} or GR_{50})	*c*	RF	C_{80} or GR_{80}
% Control S	0.97	101.71	37	-1.29	>21	C_{80} = 102
% Control PR	-	NA	NA	NA	>21	NA[1/]
% Control RF1	-	NA	NA	NA	-	NA[1/]
Dry matter reduction S	0.88	2.83	29	1.43	>27	GR_{80} = 77
Dry matter reduction PR	0.69	2.88	935[2/]	0.63	>27	GR_{80} > 800[2/]
Dry matter reduction RF1	0.61	3.11	1944[2/]	0.37	2.8	GR_{80} > 800[2/]

$C_{50;}$ C_{80} or GR_{50}; GR_{80}: doses of imazethapyr which provides 50 or 80% of control or dry weigh reduction; [1/]NA: not adjusted for Streibig model; [2/]Values outside the tested dose range.

The C_{80} and GR_{80} values for the susceptible population were similar to the recommended dose for postemergence control of BIDPI (100 g ha^{-1}), which means that the recommended dose had good activity on this population, although it did not provide 100% control (Figures 3 and 4). It is not unusual for weed populations to show differential tolerance to a herbicide without prior selection (Burgos et al., 2011). It is possible that this population had a higher baseline tolerance to imazethapyr than others. This same dose, however, had little to no effect on the resistant population and its progeny, resulting in minimal shoot dry matter reduction.

Our data demonstrate that the BIDPI population from Quarto Centenário has a high level of resistance to this imazethapyr (RF>21). Similar results were also found for other populations of BIDSU and BIDPI, where doses eight times higher than the recommended dose for imazethapyr and chlorimuron provided less than 50% control, (Lopez-Ovejero et al., 2006). Thus, it is likely that the field population in this current study is also cross-resistant to sulfonylurea herbicides.

The mechanism that confers resistance to ALS inhibitors in resistant biotypes of BIDPI in Brazil is still unknown. Monquero, Christoffoleti and Carrer (2003) demonstrated lower sensitivity of the ALS enzyme to herbicides in resistant individuals, and a similar RF range (>20) was reported for different species. Previous research with ALS-resistant BIDPI have demonstrated cross-resistance to different chemical groups including imidazolinones and sulfonylureas (chlorimuron, metsulfuron, nicosulfuron and imazethapyr) (Christoffoleti, 2002). In BIDSU, the mechanism of resistance to ALS inhibitors involves a point mutation in the *ALS* gene (Trp_{574}Leu, Phe_{116}Leu or Phe_{149}Ser), which confers high resistance level (RF=166) (Lamego et al., 2009).

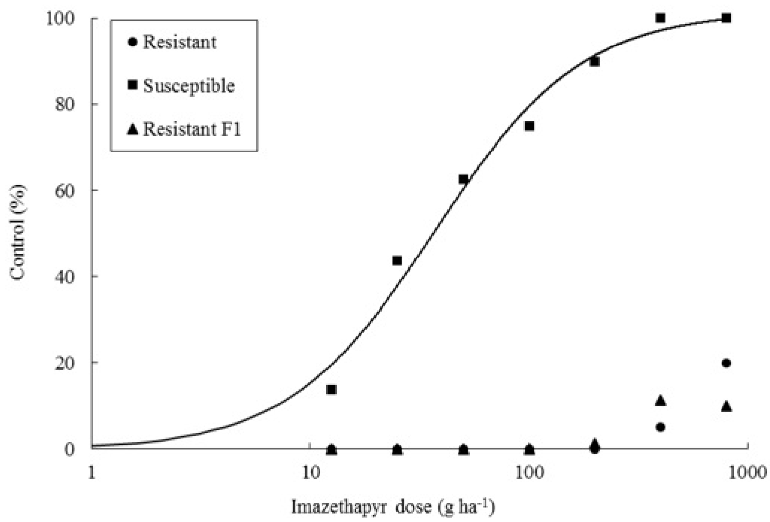

Figure 3: Response (% control) of *Bidens pilosa* populations to increasing doses of imazethapyr 28 days after application.

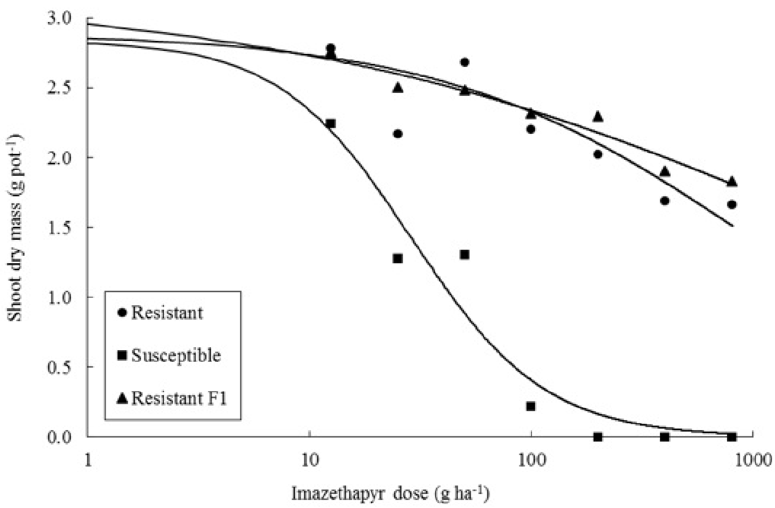

Figure 4: Response (g dry shoot biomass/pot) of *Bidens pilosa* populations to increasing doses of imazethapyr 28 days after application.

Considering that this is the first report of resistance to photosystem II inhibitors in BIDPI, the mechanisms that confer such resistance is still to be investigated. In other species, the resistance level to PSII inhibitors varies with resistance mechanism and species. It is common to find a point mutation in the gene encoding the D1 protein (Ser_{264}Gly), which causes a conformational change in the binding site for plastoquinone; other resistant species are able to metabolize the herbicide by glutathione conjugation or cytochrome P450 monooxygenase activity (Powles; Yu, 2010; Ma et al., 2013). Usually, mechanisms involving mutation of the binding site confer higher resistance level than the other mechanisms (Patzoldt; Tranel, 2007; Aspiazu et al., 2010; Huffman et al., 2015).

Research is necessary to understand the mechanisms of resistance in BIDPI, especially those related to the possibility of cross-resistance to other ALS and PSII inhibitors. The identification of resistance mechanisms could lead to the development of better management strategies for weed resistance.

CONCLUSIONS

We confirmed the multiple resistance in the Bidens pilosa population from Quarto Centenário, Paraná, Brazil. This is the first case confirmed with multiple resistance to a Photosystem II inhibitor, atrazine, and an ALS inhibitor, imazethapyr, for this species.

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