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Effectiveness of terbuthylazine and atrazine in the control of *Bidens subalternans* in three stages of application

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ABSTRACT: The objective was to evaluate the efficacy of terbuthylazine and atrazine doses in controlling greater beggarticks (*Bidens subalternans*) that are resistant to ALS inhibitors and exhibit resistance to atrazine, at three application times. The treatments were arranged in a 2x6 factorial design, in which two herbicides were used: atrazine and terbuthylazine, and six doses: 0; 375; 750; 1,500; 3,000 and 6,000 g ai ha^{-1}. The application was carried out in three stages of *B. subalternans* plant development: early post-emergence (2-4 leaves), late post-emergence (6-8 leaves), and pre-emergence. The control of *B. subalternans* plants was evaluated. Data were analyzed separately for each stage. In early post-emergence, the dose of 375 g ai ha^{-1} was enough to reach almost 100% control, in which terbuthylazine and atrazine were similar. In late post-emergence, the application of atrazine was effective at a dose of 3,000 g ai ha^{-1}, with 95% control. Terbuthylazine at a dose of 375 g ai ha^{-1} was effective with 99.8% control. In pre-emergence, atrazine and terbuthylazine were effective in controlling *B. subalternans*, at doses of 1,500 g ai ha^{-1} (96% control) and 375 g ai ha^{-1} (99% control), respectively, evidencing the superiority of terbuthylazine.
Keywords: greater beggarticks; triazines; photosystem II inhibitors; pre-emergence; post-emergence.

Eficácia de terbutilazina e atrazina no controle de *Bidens subalternans* em três estádios de aplicação

RESUMO: Objetivou-se avaliar a eficácia de doses de terbutilazina e atrazina no controle de picão-preto (*Bidens subalternans*), resistente a inibidores da ALS e com indicativo de resistência a atrazine, em três épocas de aplicação. Os tratamentos foram dispostos em esquema fatorial 2x6, foram utilizados dois herbicidas: atrazina e terbutilazina, e 6 doses: 0, 375, 750, 1.500, 3.000 e 6.000 g ia ha^{-1}. Foi realizada a aplicação em três estádios das plantas de *B. subalternans* (pós-emergência inicial: 2-4 folhas, pós-emergência tardia: 6-8 folhas e pré-emergência). Foi avaliado o controle das plantas de *B. subalternans*. Os dados foram analisados separadamente para cada estádio. Em pós-inicial, a dose de 375 g ia ha^{-1} já foi suficiente para atingir quase 100% de controle, com terbutilazina e atrazina sendo similares. Em pós-tardio, a aplicação de atrazina foi eficaz na dose de 3.000 g ia ha^{-1} propiciando 95% de controle. A aplicação de terbutilazina na dose 375 g ia ha^{-1} foi eficaz com 99,8% de controle. Em pré-emergência, atrazine e terbutilazina foram eficazes no controle de *B. subalternans*, nas doses de 1.500 g ia ha^{-1} (96% de controle) e 375 g ia ha^{-1} (99% de controle), respectivamente, tendo grande superioridade da terbutilazina.
Palavras-chave: picão-preto; triazinas; inibidores do fotossistema II; pré-emergência; pós-emergência.

1. INTRODUCTION

Greater beggarticks (*Bidens subalternans* DC.) and hairy beggarticks (*Bidens pilosa* L.) are broadleaf weeds of the family Asteraceae, commonly found in several crops in Brazil, with significant importance in grain and sugarcane crops (MOREIRA; BRAGANÇA, 2011; HAO et al., 2018). Studies highlight *Bidens* spp. as an important weed in soybean, for example, Ferreira et al. (2015) observed reductions in shoot dry mass and photosynthetic parameters of soybean in competition. Additionally, in corn, this can lead to interference with crop development (BARROS et al., 2017). In addition, cases of resistance to acetolactate synthase (ALS) inhibitor herbicides have been reported in Brazil (LAMEGO et al. 2009; MENDES et al. 2019a). Furthermore, multiple resistance to ALS and atrazine (TAKANO et al., 2016) and multiple resistance to ALS and glyphosate (MENDES et al., 2019b) have also been observed.

For the control of *Bidens* spp. and other broadleaf weeds in corn crops, atrazine is widely used in pre- or post-emergence applications, with an effect on weeds in pre-emergence or early post-emergence stages (GIRALDELI et al., 2019; BARNES et al., 2020; LANGDON et al., 2021). This helps to explain cases of resistance to this herbicide. Another herbicide that can be used is terbuthylazine, a triazine with a photosystem II inhibitor mode of action, similar to atrazine, and effective in controlling weeds in corn (PANACCI; ONOFRI 2016). However, it has some distinct characteristics that can also differentiate its control.

In addition to cases of *Bidens* spp. resistance, triazines are herbicides that are susceptible to leaching into the soil, which can lead to reduced effectiveness in weed control and even contaminate groundwater (XIAO; PIGNATELLO, 2015; PORTOCARRERO et al., 2019). In the comparison between terbuthylazine and atrazine, regarding leaching, the latter had a higher leaching potential (FENOLL et al., 2012). Due to this and other aspects, some herbicides from this group are no longer authorized for use in some countries; for example, atrazine is not authorized for use in the European Union, while terbuthylazine is (EUROPEAN COMMISSION, 2024).

In this context, studies on the effectiveness of terbuthylazine in controlling *B. subalternans* are needed to advance, considering its potential as a substitute for atrazine when its use is not authorized, or even in controlling atrazine-resistant populations. In addition to identifying whether atrazine-resistant *Bidens* spp. populations are also resistant to terbuthylazine. Therefore, the objective was to evaluate the efficacy of atrazine and terbuthylazine doses in controlling *B. subalternans*, a species resistant to ALS inhibitors and known to exhibit resistance to atrazine, across three application stages.

2. MATERIALS AND METHODS

2.1. Site description and experimental design

Three experiments were conducted in a greenhouse (24°17'36.2"S 53°50'27.5"W) located in Palotina, state of Paraná (PR), Brazil, in 2021. A completely randomized design was used with five repetitions. The experimental units were composed of 200 mL pots. The treatments were arranged in a 2 x 6 factorial design, two herbicides used: atrazine (Atrazina Nortox®) and terbuthylazine (Click®), and six doses: 0; 375; 750; 1,500; 3,000, and 6,000 g ai ha^{-1}.

The application was carried out in three development stages of *B. subalternans* plants. In early post-emergence, at the 2-4 leaf stage; in late post-emergence, at the 6-8 leaf stage; and in pre-emergence. For experiments involving post-emergence applications, pots were filled with a suitable substrate. For the pre-emergence experiment, pots were filled with medium-textured soil (14.3% clay, 22% silt, 63.7% sand, and 2.7% organic matter). In post-emergence experiments, each pot had two plants at the time of application. In pre-emergence, five seeds were sown per pot (1 - 2 cm deep) and then treated with the tested herbicides. Assist® adjuvant (0.5 L ha^{-1}) was added to the herbicide application for post-emergence experiments.

Herbicides were applied on September 16, 2021, under a temperature of 28 ºC, RH of 66%, and wind of 1.4 km h^{-1}. A CO_2 pressurized backpack sprayer equipped with six nozzles (AIXR 110.015, Teejet®) was used, with a pressure of 2 kgf cm^{-2} and a speed of 3.6 km h^{-1}, which provided an application volume of 150 L ha^{-1}.

2.2. Data collection and analysis

The control of *B. subalternans* was evaluated at 7, 14, 21, and 28 days after application (DAA). The experiment was conducted in pre-emergence from 14 DAA due to the low emergence of seedlings at 7 DAA. Visual scores were assigned to each experimental unit, where 0 represents no damage and 100% plant death (VELINI et al., 1995).

Data were analyzed using analysis of variance (ANOVA) and the F-test ($p \leq 0.05$), separately for each experiment (application stage). Mean levels of the herbicide factor and factor interaction were compared by an F-test ($p \leq 0.05$). Mean doses were subjected to regression analysis ($p \leq 0.05$) to consider biological explanations, significant and non-significant regression deviations, and the coefficient of determination. If no model was applied to the data, mean doses were compared using standard deviation. For the ANOVA, the Sisvar 5.6 software (FERREIRA, 2011) was used. For the regression analysis, SigmaPlot 15 (Systat Software Inc.) was used, while Microsoft Excel 365 (Microsoft Corp.) was used to construct the figures.

3. RESULTS

3.1. Experiment in early post-emergence

ANOVA evidenced a significant effect ($p \leq 0.05$) for herbicides in controlling B. subalternans only at 7 DAA (Table 1). Superiority was observed for terbuthylazine in terms of efficacy, with a control of 82.2%, compared to atrazine, which achieved a control of 81.5% (Table 2). In the other evaluations, no differences were detected between herbicides. Additionally, no significant interaction effect was observed in any of the assessments. While for doses, a significant effect was observed in all control evaluations (Table 1).

Table 1. Results of the F-test by ANOVA (experiment early post-emergence).
Tabela 1. Resultados do teste F pela ANAVA (experimento em pós-emergência inicial)

	Control			
Source	7 DAA	14 DAA	21 DAA	28 DAA
Herbicide (H)	4.8*	2.7^{ns}	1.3^{ns}	1.4^{ns}
Dose (D)	9,552*	26,620*	79,491*	153,417*
H x D	1.2^{ns}	0.6^{ns}	0.1^{ns}	0.3^{ns}
CV (%)	1.6	0.9	0.6	0.4

DAA = days after application.
* $p \leq 0.05$, means differ from each other.
ns $p > 0.05$, means they do not differ from each other.

Table 2. *Bidens subalternans* control at 7 days after application of herbicides (early post-emergence experiment).
Tabela 2. Controle de *Bidens subalternans* aos 7 dias após a aplicação de herbicidas (experimento em pós-emergência inicial)

Herbicide	Control (%)
Atrazine	81.5 b
Terbuthylazine	82.2 a

Means with different letters differ from each other by the F-test ($p \leq 0.05$).

Despite the significant effect of doses on controlling *B. subalternans*, the regression model could not be used. The control scores were high (≥ 96.8%), even for the 375 g ai ha^{-1} dose application. By comparing the means considering the standard deviation, no differences were observed between the doses. Still, all of them were superior in efficacy compared to the zero dose (no application) (Figure 1) and a trend towards superiority for terbuthylazine.

3.2. Experiment in late post-emergence

ANOVA indicated a significant effect ($p \leq 0.05$) for herbicide, dose, and interaction in all evaluations of *B. subalternans* control (Table 3). For 7 DAA, logarithmic adjustment was possible for the doses of atrazine and terbuthylazine, with terbuthylazine demonstrating superior efficacy at all doses. The highest control score, in absolute value, for atrazine and terbuthylazine was achieved with the application of 3,000 g ai ha^{-1}, resulting in 79.6% and 96.6% control, respectively (Figure 2).

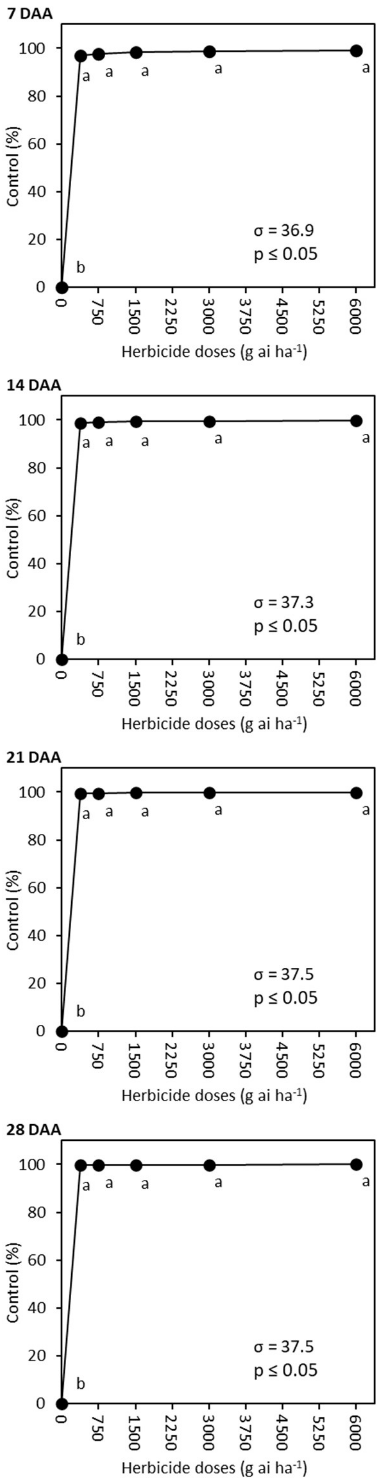

Figure 1. *Bidens subalternans* control at 7, 14, 21, and 28 days after application (DAA) of herbicide doses (experiment early post-emergence).

Figura 1. Controle de *Bidens subalternans* aos 7, 14, 21 e 28 dias após a aplicação (DAA) de doses de herbicidas (experiment early post-emergence).

Means with the same letter do not differ from each other by ± standard deviation (σ), p ≤ 0.05.

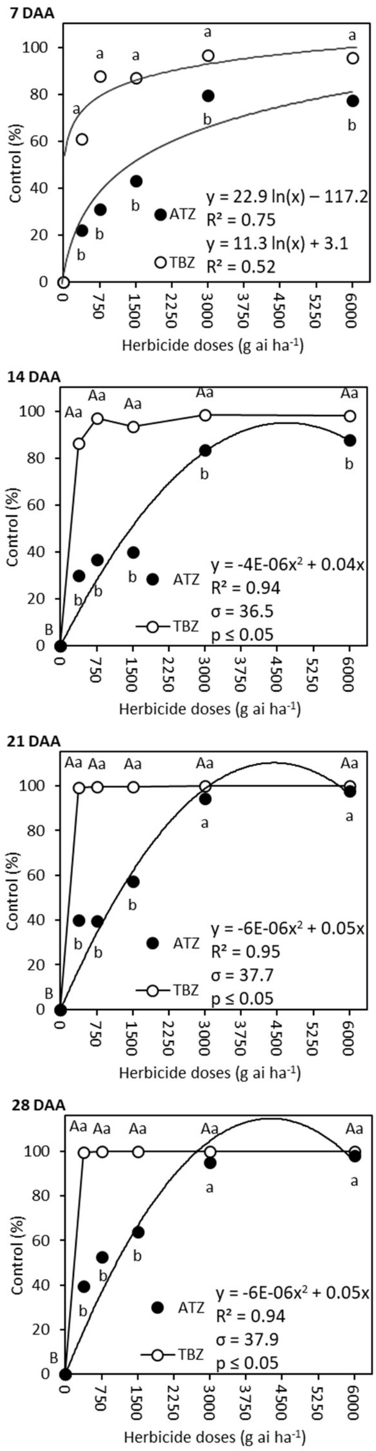

Figure 2. *Bidens subalternans* control at 7, 14, 21, and 28 days after application (DAA) of terbuthylazine (TBZ) or atrazine (ATZ) doses (late post-emergence experiment).

Figura 2. Controle de *Bidens subalternans* aos 7, 14, 21 e 28 dias após a aplicação (DAA) de doses de terbutilazina (TBZ) ou atrazina (ATZ) (experimento em pós-emergência tardia).

Means, with the same lowercase letter (herbicide factor), do not differ from each other by the F-test, p ≤ 0.05.

Means, with the same uppercase letter (dose factor), do not differ from each other by ± standard deviation (σ), p ≤ 0.05. (14, 21, and 28 DAA).

For doses of terbuthylazine at 14, 21, and 28 DAA, the regression model could not be used. In comparison, considering the standard deviation, differences between the doses were detected, where all were higher than the zero dose. Terbuthylazine showed control ≥99.2% even for the dose 375 g ai ha^{-1} at 28 DAA. For the application of atrazine in these evaluations, a polynomial adjustment was possible, with maximum effectiveness observed at a dose of 3,000 g ai ha^{-1}, resulting in a final control of 95%. At 14 DAA, in a dose-to-dose comparison, atrazine was always less effective than terbuthylazine. Equivalence was observed between the herbicides only at 21 and 28 DAA for the two highest doses (Figure 2).

Table 3. Results of the F-test by ANOVA (experiment late post-emergence)
Tabela 3. Resultados do teste F pela ANAVA (experimento em pós-emergência tardia)

	Control			
Source	7 DAA	14 DAA	21 DAA	28 DAA
Herbicide (H)	131.4*	324.4*	317.7*	277.1*
Dose (D)	110.9*	232.6*	349.3*	391.7*
H x D	11.2*	37.1*	55.6*	50.2*
CV (%)	17.4	11.2	8.9	8.3

DAA = days after application.
* p ≤ 0.05, means differ from each other.

3.3. Experiment in pre-emergence

ANOVA indicated a significant effect (p ≤ 0.05) for herbicide, doses, and interaction in all evaluations of *B. subalternans* control (Table 4).

Table 4. Results of the F-test by ANOVA (experiment pre-emergence).
Tabela 4. Resultados do teste F pela ANAVA (experimento em pré-emergência)

	Control		
Source	14 DAA	21 DAA	28 DAA
Herbicide (H)	62.2*	30.7*	48.1*
Dose (D)	604.5*	4,129*	2,155*
H x D	5.4*	4.3*	8.8*
CV (%)	6.9	2.4	3.3

DAA = days after application.
* p ≤ 0.05, means differ from each other

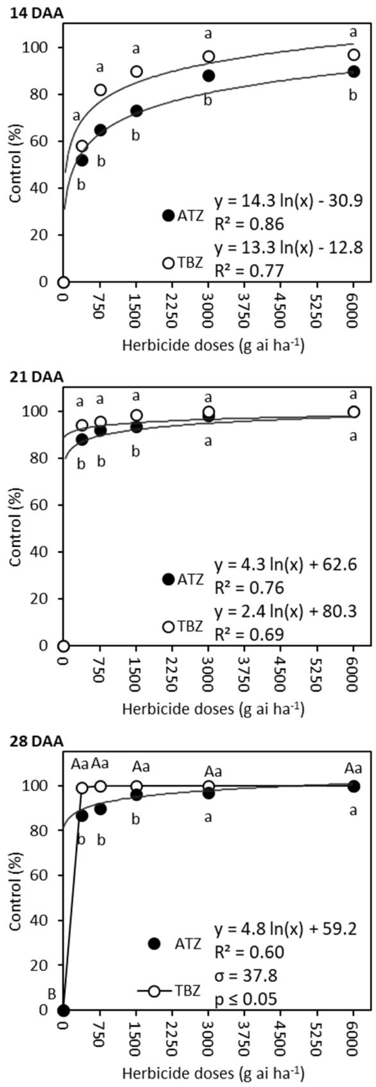

Figure 3. *Bidens subalternans* control at 14, 21, and 28 days after application (DAA) of terbuthylazine (TBZ) or atrazine (ATZ) doses (experiment pre-emergence).
Figura 3. Controle de *Bidens subalternans* aos 14, 21 e 28 dias após a aplicação (DAA) de doses de terbutilazina (TBZ) ou atrazina (ATZ) (experimento em pré-emergência).
Means, with the same lowercase letter (herbicide factor), do not differ from each other by the F-test, p ≤ 0.05.
Means, with the same uppercase letter (dose factor), do not differ from each other by ± standard deviation (σ), p ≤ 0.05. (28 DAA).

Logarithmic adjustment was possible for both herbicides and all evaluations. Except for terbuthylazine at 28 DAA, in which the doses did not differ in efficacy and were higher than the zero dose (Figure 3).

In the dose-to-dose comparison of each herbicide, atrazine was less effective than terbuthylazine in controlling *B. subalternans*; equivalence between the herbicides was observed only for the two highest doses, at 21 and 28 DAA. In the last control evaluation, for the application of atrazine, the efficacy at the 375 g ai ha^{-1} dose was 87%, with 100% efficacy at the highest dose. For terbuthylazine, an application rate of 375 g ai ha-1 resulted in 99% control (Figure 3).

4. DISCUSSION

As for atrazine, the plant development stage affected herbicide efficacy, but for terbuthylazine, this was observed to a lesser extent. Greater susceptibility was found for the early post-emergence application, for which equivalent efficacy was observed between the herbicides. These results indicate that the stage of application affects the effectiveness of herbicides. In general, the application in late post-emergence stages showed less efficacy compared to early post-emergence or pre-emergence stages, with this effect being more pronounced for atrazine.

Regarding the dose for effective control, in the early post-emergence stage, 375 g ai ha^{-1} already resulted in a final control of 99.7% regardless of the herbicide used. In the late post-emergence period, a dose of 3,000 g ai ha^{-1} of atrazine was necessary to achieve 95% final control, while a dose of 375 g ai ha^{-1} of terbuthylazine already reached 99.8% control. While in pre-emergence, a dose of 1,500 g ai ha^{-1} of atrazine was necessary to achieve 96% final control. For terbuthylazine, a dose of 375 g ai ha^{-1} already reached 99% control. This evidence shows the influence of the stage of application, dose, and herbicide in the control of *B. subalternans*.

The primary mode of application for triazine herbicides, such as atrazine and terbuthylazine, is through the soil, where they are absorbed by the roots and translocated via the apoplast, resulting in a residual effect on the emergence of susceptible plants. However, they are also effective in controlling weeds in the post-emergence stage, initially, with a loss of effectiveness as the stage advances (TREBST, 2008; GIRALDELI et al., 2024). The optimal stage of application, whether pre- or post-emergence, may vary depending on the species, with reduced effectiveness for applications made in advanced stages, especially in grasses. While for the control of Powell amaranth (*Amaranthus powelli*), equivalent efficacy was observed for the application of atrazine + tolpyralate in pre- or post-emergence; for common ragweed (*Ambrosia artemisiifolia*), no effect was observed from the application in pre-emergence (METZGER et al., 2019).

The application of terbuthylazine or atrazine, combined with glyphosate, was effective in controlling *B. subalternans* in corn, with equivalent efficacy between the herbicides. However, a higher dose was required for atrazine (1,500 g ai ha^{-1}) while a dose of 1,125 g ai ha^{-1} was required for terbuthylazine (BOTTCHER et al., 2022). This reinforces the need for higher doses of atrazine to achieve the same effectiveness as terbuthylazine in controlling this weed.

The effectiveness of terbuthylazine is also reported in other studies, ensuring good levels of weed control (AZZA et al., 2020; CHEPKOECH et al., 2021; NEDELJKOVIĆ et al., 2021; VICENSI et al., 2024), which is particularly important in crops such as corn or sorghum. It is worth noting the performance of terbuthylazine in controlling *B. subalternans* in the three stages of application. Thus, an alternative in the chemical control of this weed is characterized. In Brazil, there are cases of *Bidens* spp. resistant to ALS-inhibiting herbicides (LAMEGO et al., 2009; MENDES et al., 2019a), multiple resistance to ALS and atrazine (TAKANO et al., 2016), and multiple resistance to ALS and glyphosate (MENDES et al., 2019b).

The importance of using pre- and post-emergence herbicides, such as terbuthylazine or atrazine, to control weeds in corn is highlighted (BRANKOV et al., 2021; BOTTCHER et al., 2022; IDZIAK et al., 2022). But in the specific case of *B. subalternans*, our results for the application of terbuthylazine stand out. The population of *B. subalternans* originates from an area with a history of numerous atrazine applications, accompanied by reports of decreased efficacy. This was confirmed by the results obtained, which showed that high doses of atrazine were necessary for effective control (≥3,000 g ai ha^{-1}), primarily during the late post-emergence stage (6-8 leaves). Although terbuthylazine has the same mode of action and chemical group as atrazine, no evidence of resistance was observed in the studied population.

5. CONCLUSIONS

In the early post-emergence stage (2-4 leaves), the 375 g ai ha^{-1} dose was sufficient to achieve nearly 100% control of *B. subalternans* plants, with similar efficacy between terbuthylazine and atrazine, but with a trend toward higher control for terbuthylazine.

In late post-emergence (6-8 leaves), atrazine was effective in controlling *B. subalternans* at a dose of 3,000 g ai ha$^{-1,}$ providing 95% control. The application of terbuthylazine at a dose of 375 g ai ha^{-1} has already been effective in controlling *B. subalternans*, with a 99.8% success rate.

In pre-emergence, atrazine and terbuthylazine were effective in controlling *B. subalternans*, at doses of 1,500 g ai ha^{-1} (96% control) and 375 g ai ha^{-1} (99% control), respectively, evidencing the superiority of terbuthylazine.

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