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Selectivity of the herbicide atrazine in Crotalaria species

Ana Laura Fernandes Maciel[1] , Paulo César Timossi[1], Murilo Henrique de Carlo[1], Fenelon Lourenço de Sousa Santos[2], Itamar Rosa Teixeira[3]

[1] Universidade Federal de Jataí, Jataí, Goiás, Brasil. E-mail: analauramaciel@gmail.com, timossi@ufj.edu.br, murilodecarlo@gmail.com

[2] Universidade Estadual de Goiás, Unidade Universitária de Posse, Posse, Goiás, Brasil. E-mail: fenelon.santos@ueg.br

[3] Universidade Estadual de Goiás, Unidade Universitária de Anápolis, Anápolis, Goiás, Brasil. E-mail: itamar.teixeira@ueg.br

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ABSTRACT

One of the limitations of adopting crotalaria in crop rotation is the lack of information on the selectivity of herbicides for controlling volunteer soybean plants. To find alternatives for soybean management, the selectivity of atrazine applied pre-emergently to crotalaria species at a dose of 2.5 kg ha^{-1} of atrazine active ingredient was investigated. The experiment was set up in the experimental area of the Federal University of Jataí, using a randomized block design arranged in a 3x3+1 factorial scheme consisting of three species of crotalaria (*Crotalaria juncea*, *C. spectabilis*, and *C. breviflora*), three periods of application of the herbicide atrazine (20, 10, and 0 days before sowing) and a control treatment (no application of atrazine) with four replications. The density of emerged crotalaria plants 10 days after sowing was assessed. Plant height and fresh and dry biomass were assessed at 30, 60, and 90 days. It can be concluded that the application of atrazine at 0 DAS interferes with the initial development of *Crotalaria juncea*, and the species *Crotalaria spectabilis* and *Crotalaria breviflora* do not show any interference in the establishment and development of the plants when grown in clay soil.

Keywords: *Crotalaria juncea, Crotalaria spectabilis, Crotalaria breviflora,* Crop rotation.

Seletividade do herbicida atrazine em espécies de Crotalária

RESUMO

Uma das limitações na adoção crotalárias na rotação de culturas é a ausência de informações quanto à seletividade de herbicidas para o controle de soja guacho. Visando buscar alternativas para o manejo da soja, foi pesquisada a seletividade de atrazine aplicada em pré-emergência de espécies de crotalária na dose de 2,5 kg ha^{-1} de ingrediente ativo de atrazine. O experimento foi instalado no campo experimental da Universidade Federal de Jataí, adotando-se o delineamento experimental de blocos casualizados no esquema fatorial 3x3+1, composto por três espécies de crotalárias (*Crotalaria juncea*, C. *spectabilis*, e C. *breviflora*), três épocas de aplicação do herbicida atrazine (20, 10 e 0 Dias Anteriores à Semeadura) e um tratamento controle (sem aplicação de atrazine) com quatro repetições. Foi avaliada a densidade de plantas de crotalária emergidas aos 10 dias após a semeadura. Aos 30, 60 e 90 dias avaliou-se a altura, a biomassa fresca e seca das plantas. Conclui-se que a aplicação do atrazine aos 0 DAS interfere no desenvolvimento inicial da *Crotalaria juncea* e as espécies *Crotalaria spectabilis* e *Crotalaria breviflora* não apresentam quaisquer tipos de interferência no estabelecimento e desenvolvimento das plantas quando cultivadas em solo argiloso.

Palavras-chave: *Crotalaria juncea, Crotalaria spectabilis, Crotalaria breviflora,* Rotação de culturas.

1. Introduction

Green manures have been used in Brazilian soils to ensure the sustainability of agricultural systems. Leguminous plants are commonly used as they have high plant mass production, a deep root system, and the ability to establish symbiosis with atmospheric nitrogen-fixing bacteria (Soratto et al., 2012; Ma et al., 2021; Meena et al., 2018; Silva and Menezes, 2007).

Examples of green manures include species of the genus *Crotalaria*, which has been associated with phytonematode control (Cruz et al., 2020; Wang et al., 2002) and weed suppression (Araújo et al., 2021; Timossi et al., 2011; Timossi et al., 2021), and is an alternative in crop rotation systems which consist of an alternation of crops planted in the same area throughout the harvest (e.g. soybeans, corn, crotalaria, soybeans, sorghum) or in intercropping such as crotalaria and corn (Mohr et al., 2022).

Crotalaria cultivation can cause changes in weed populations due to allelopathic effects and competition for light, water, oxygen, and nutrients, suppressing some of them (Adler and Chase, 2007; Monquero et al., 2009). Timossi et al. (2021) highlight evidence of the allelopathic effect of *Crotalaria juncea* in suppressing bittergrass (*Digitaria insularis*). However, there is a notorious scarcity of research aimed at phytosanitary management of green manure species (Ramos et al., 2015), especially chemical control of weeds in coexistence with cover crops.

One challenge in adopting crotalaria in crop rotation is the infestation of volunteer soybean plants during their establishment. The presence of soybeans in the area makes it unfeasible to grow crotalaria in the off-season since the presence of the crop in the area is prohibited due to the sanitary void to reduce the incidence of Asian rust (*Phakopsora pachyrhizi*) (Almeida Júnior et al., 2021). The only way to solve this problem is to find solutions to control soybeans in areas where green manure species are grown.

Despite the importance of green manure for agriculture, studies on weed control in coexistence with green manures are still scarce (Dias et al., 2017). Brazil has no record of specific herbicides for controlling weeds in coexistence with crotalaria species (Agrofit, 2023). Therefore, for control to be efficient, the selectivity of herbicides for green manure species must be known. Another way to manage the presence of volunteer soybean plants in areas cultivated with green manure species would be to anticipate control by adopting weed management programs in the production system. This study aimed to assess the selectivity of the herbicide atrazine applied at different times before sowing *Crotalaria juncea*, *Crotalaria spectabilis*, and *Crotalaria breviflora*.

2. Material and Methods

The experiment was carried out in the experimental area of the Federal University of Jataí, campus of Jatobá, located at 17º52'53" S, 51º42'52" W, and an altitude of 670 m. The region has an altitude of around 670 meters and annual rainfall between 1,650 and 1,800 millimeters, distributed between September and April, with an average annual temperature of 25 ºC.

The soil in the area was classified as Latossolo Vermelho Distroférrico (Santos et al., 2018), and the results of the physical and chemical analysis of the soil are shown in Table 1. The data from rainfall and maximum and minimum temperature recorded by the Meteorological Station of Jataí (INMET) during the period in which the experiment was conducted are shown in Figure 1.

The randomized block design arranged in a 3x3+1 factorial scheme was used. The treatments consisted of three crotalaria species (*Crotalaria juncea*, *C. spectabilis*, and *C. breviflora*), three periods of application of the herbicide atrazine (20, 10, and 0 days before sowing) and a control treatment (control without application of atrazine) with four replications. The experimental plots were 4 m x 7 m, totaling 28 m².

The herbicide was applied using a customized research sprayer (Timojet) pressurized by CO_2 with an application boom using AD IA 110015 D tips and a spray volume of 150 L ha^{-1}. The applications were made 20, 10, and 0 days before the crotalaria were sown in the area. The crotalaria was sown on 03/11/2019 with the seeds distributed manually, followed by light incorporation by a closed harrow (zero opening angle) after the last application of the herbicide atrazine (0 days).

Table 1. Chemical and textural characterization of the soil (0-20 cm soil layer) in the experimental area when the experiment was set up. Jataí, GO, 2019.

Particle size analysis (g kg^{-1})									
Sand		Silt		Clay		Textural Classification			
285		150		565		Clayey			
Chemical analysis									
pH	P	K	Al	Ca	Mg	H+Al	CEC	BS	OM
(H_2O)	(mg/dm^3)	------------------- ($cmol_c/dm^3$) -------------------						%	mg/dm^3
5.2	34.6	230	0.0	4.12	2.23	4.4	11.3	61.4	34.6

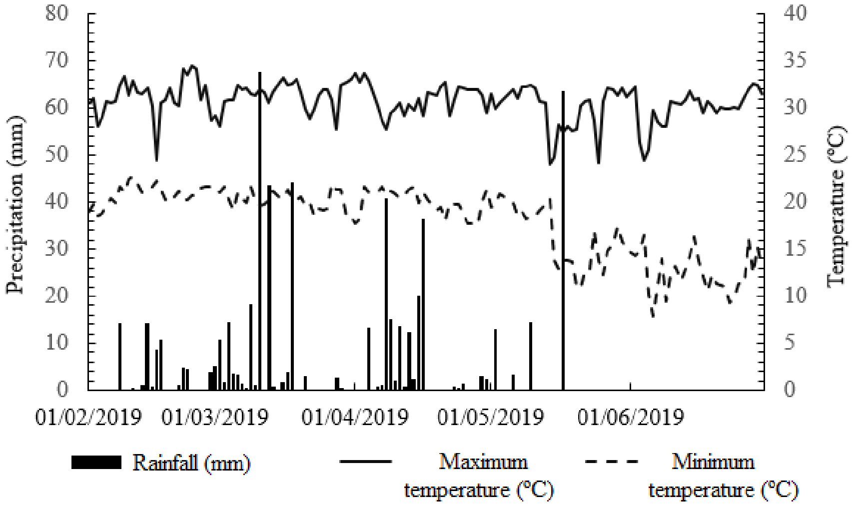

Figure 1. Rainfall (mm) and maximum and minimum temperature (°C) from February to June 2019. Source: INMET.

The quantity of seeds used followed the recommendations for each species of crotalaria. To obtain the density of crotalaria plants, a 0.5 x 0.4 m metal frame was randomly placed five times in each plot 10 days after sowing (DAS). At 30, 60, and 90 DAS, 10 plants taken at random from the useful area of each plot were collected to determine height and fresh biomass. After determining the fresh biomass, the material was packed in paper bags and taken to an oven at 65 ºC for 72 hours to obtain the dry biomass.

The data was submitted to analysis of variance (ANOVA), and the Scott Knott test was used to group the means, adopting a 5% significance level using the AgroEstat® software (Barbosa and Maldonado Junior, 2015).

3. Results and Discussion

Applying the herbicide atrazine on the day the *Crotalaria juncea* was sown reduced the plant stand in the evaluation 10 days after sowing (Figure 2). It is, therefore, possible to state that adopting the herbicide in a possible "plant-and-apply or apply-and-plant" situation could compromise the establishment of the *C. juncea* species. In this condition, using the herbicide reduced the plant population by 35% compared to the control without applying atrazine. When determining the height of *C. juncea* plants at 30 DAS, it can be seen that applying the herbicide atrazine under plant-and-apply conditions (0 DAA) inhibited plant growth (Table 2).

In this evaluation, the herbicide application on the day the crotalaria was sown reduced plant growth by 23% compared to the control without the herbicide application. Even in the plant-and-apply condition (0 DAA), in which there was a reduction in plant size at 30 DAS, the values observed are higher than those reported by Diogo et al. (2023), which was probably due to the time *C. juncea* was grown. From 60 DAS onwards, there was no difference between the treatments evaluated, showing that the plants had recovered from the possible toxic effects caused by the herbicide. This result characterizes plant compensation, regardless of the density established at sowing (Figure 2).

The accumulation of fresh biomass of *Crotalaria juncea* plants shows a reduction at 30 DAS when sown on the same day as the herbicide application and the recovery of the plants in the following evaluations (Table 3). This behavior is consistent with that shown for plant height (Table 2), demonstrating that the plants could detoxify and develop normally after 30 DAS, regardless of the time of herbicide application. Souza et al. (2023) observed that growing *C. juncea* increases bacterial activity in the soil and can mitigate the effects of some herbicides. Similarly, the accumulation of dry biomass of *Crotalaria juncea* plants was affected by the application at 0 DAA and showed a lower mean at 30 DAS (Table 3).

There was no difference in crotalaria biomass accumulation at 60 and 90 DAS, regardless of the interval between herbicide application and crotalaria sowing (Table 3). The effect observed at 30 DAS is probably due to the greater susceptibility of *C. juncea* to the herbicide. Atrazine is an inhibitor of photosystem II, which acts by altering the flow of electrons in photosynthesis (Gao et al., 2019), justifying the reduction in biomass observed at 30 DAS, but the amount absorbed was not capable of killing the plants, allowing them to detoxify and not affecting the accumulation of biomass at 60 and 90 DAS.

According to the results presented regarding the selectivity of atrazine, it can be seen that in the plant-and-apply condition (0 DAA), the herbicide interferes with the establishment and initial development of the *Crotalaria juncea* species.

These results corroborate those reported by Braz et al. (2015), who found that the herbicide atrazine applied in pre-emergence on the same day as sowing was not selective for *Crotalaria juncea*, as it caused high percentages of intoxication when grown in clay soil. For the *C. spectabilis* species, there was no reduction in plant density assessed at 10 days after sowing (Figure 3). These results corroborate those reported by Ikeda et al. (2021), who observed no reduction in the *C. spectabilis* population with the application of atrazine (500 g ha^{-1}) in pre-emergence.

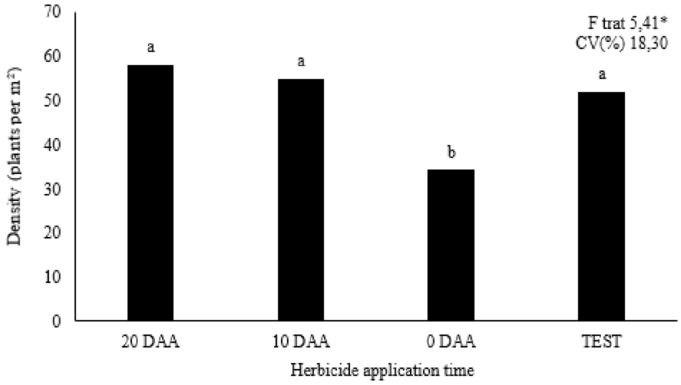

Figure 2. Density of *Crotalaria juncea* plants at ten days after sowing according to the time of atrazine application in pre-emergence.

Table 2. Plant height of *Crotalaria juncea* at 30, 60, and 90 days after sowing (DAS) according to the time of atrazine application in pre-emergence. Jataí GO, 2019.

Variables	Treatments	Plant Height (cm)		
		30 DAS[2]	60 DAS	90 DAS
Season	20 DAA[3]	118.13 a[1]	435.25 a	389.25 a
	10 DAA	117.13 a	382.75 a	376.50 a
	0 DAA	91.50 b	357.75 a	365.75 a
	Control	118.75 a	409.75 a	362.75 a
F	Blocks	18.50**	0.57^{NS}	3.93*
	Season	3.99*	1.19^{NS}	1.21^{NS}
CV (%)		5.54	15.49	5.85

[1] Means followed by the same letter in the same column belong to the same group by the Scott Knott test; [2] Days after *C. juncea* sowing; [3] Days before *C. juncea sowing.*

Table 3. Biomass accumulation of *Crotalaria juncea* at 30, 60, and 90 days after sowing (DAS) according to the time of atrazine application in pre-emergence. Jataí GO, 2019.

Variables	Treatments	Fresh biomass accumulation (g)		
		30 DAS[2]	60 DAS	90 DAS
Season	20 DAA[3]	308.00 a[1]	724.00 a	715.00 a
	10 DAA	333.00 a	746.00 a	557.00 a
	0 DAA	248.50 b	727.00 a	756.00 a
	Control	361.50 a	748.00 a	595.00 a
F	Blocks	2.22^{NS}	1.49^{NS}	1.45^{NS}
	Season	3.93*	0.01^{NS}	2.06^{NS}
CV (%)	---	15.52	31.47	20.14
Variables	Treatments	Dry biomass accumulation (g)		
		30 DAS[2]	60 DAS	90 DAS
Season	20 DAA[3]	78.13 a[1]	258.50 a	312.00 a
	10 DAA	85.89 a	171.50 a	283.50 a
	0 DAA	66.59 b	257.50 a	331.75 a
	Control	92.89 a	253.50 a	297.50 a
F	Blocks	2.05^{NS}	1.75^{NS}	0.12^{NS}
	Season	5.01*	1.55^{NS}	0.29^{NS}
CV (%)	---	12.45	22.07	24.96

[1] Means followed by the same letter in the same column belong to the same group by the Scott Knott test; [2] Days after *C. juncea* sowing; [3] Days before *C. juncea sowing.*

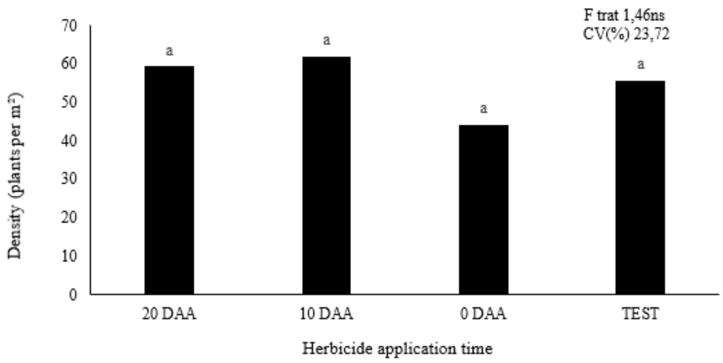

Figure 3. Density of *Crotalaria spectabilis* plants at ten days after sowing according to the time of atrazine application in pre-emergence. Jataí, 2019.

There was no influence of atrazine application on plant height of *Crotalaria spectabilis* plants (Table 4). De Paula et al. (2020) reported a reduction in *C. spectabilis* growth with the post-emergence application of different atrazine doses. According to the authors, *C. spectabilis* is intolerant to the application of atrazine, indicating that it is not selective to this herbicide post emergence. In the case of this study, the selectivity can be explained by its use in pre-emergence, in which case the adsorption and availability of the herbicide atrazine can be altered depending on the soil characteristics (Rodrigues and Almeida, 2018). Table 1 shows the soil characterization of the experimental area, which shows that the soil texture is very clayey, corroborating the authors information. There was no influence of atrazine application on fresh and dry biomass accumulation of *Crotalaria spectabilis* (Table 5). The greater amount of biomass can result in benefits for crops in succession, such as an increase in soil organic matter, an increase in the availability of nutrients, and suppression of the development of the weed community (Timossi et al., 2011; Soratto et al., 2012). The behavior of the *C. spectabilis* plants observed in this study corroborates those reported by Ikeda et al. (2021), who, when evaluating the behavior of crotalaria species subjected to pre emergence herbicide application, observed no phytotoxic effects and reduction in population and dry biomass when atrazine was used at a dose of 500 g ha^{-1}. However, it is important to note that in this study the dose used was increased by 500% (five times) the dose researched by the authors.

Table 4. Plant height of *Crotalaria spectabilis* at 30, 60, and 90 days after sowing (DAS) according to the time of atrazine application in pre-emergence. Jataí GO, 2019.

Variables	Treatments	Plant height (cm)		
		30 DAS[2]	60 DAS	90 DAS
Season	20 DAA[3]	39.75 a[1]	209.25 a	210.50 a
	10 DAA	34.88 a	211.00 a	232.50 a
	0 DAA	28.38 a	187.00 a	203.75 a
	Control	35.00 a	231.25 a	215.50 a
F	Blocks	0.91^{NS}	0.31^{NS}	1.95^{NS}
	Season	4.37*	1.29^{NS}	1.42^{NS}
CV (%)	---	12.96	15.21	9.57

[1] Means followed by the same letter in the same column belong to the same group by the Scott Knott test; [2] Days after *C. spectabilis* sowing; [3] Days before *C. spectabilis* sowing.

These results evidence the potential use of the herbicide atrazine for managing areas intended to cultivate *Crotalaria spectabilis*, corroborating the results obtained by Dias et al. (2017). Concerning the establishment of the crop in the area, *C. breviflora* showed similar behavior to *C. spectabilis*, with no population reduction due to the application of the herbicide atrazine (Figure 4). Information on the chemical management of crotalaria is scarce, especially for the *C. breviflora* species, demonstrating research importance. It can be seen that the application of atrazine, even at a dosage of 2500 g ha^{-1}, did not compromise the establishment of *C. breviflora* in very clayey soils. As for the height of *C. breviflora* plants, the evaluations carried out at 30 and 60 DAS showed no statistical differences between the treatments. However, at 90 DAS, *C. breviflora* grew less when sown on the day the herbicide was applied (0 DAA) and when sown 10 DAA apart, showing statistical differences (Table 6).

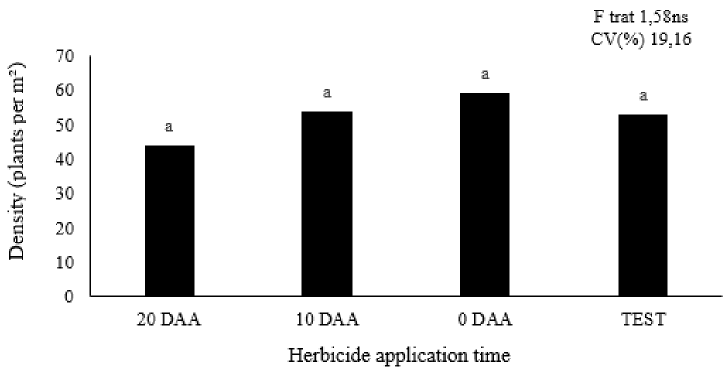

Figure 4. *Crotalaria breviflora* plant density at 10 days after sowing as a function of the time of application of atrazine in pre emergence. Jataí, 2019.

Table 5. Biomass accumulation of *Crotalaria spectabilis* at 30, 60, and 90 days after sowing (DAS) according to the time of atrazine application in pre-emergence. Jataí GO, 2019.

Variables	Treatments	Fresh biomass accumulation (g)		
		30 DAS[2]	60 DAS	90 DAS
Season	20 DAA	195.50 a[1]	475.00 a	701.50 a
	10 DAA	196.00 a	533.00 a	809.00 a
	0 DAA[3]	155.50 a	535.00 a	895.00 a
	Test	191.50 a	627.00 a	704.50 a
F	Blocks	1.56^{NS}	3.89*	1.27^{NS}
	Season	1.39^{NS}	0.96^{NS}	0.65^{NS}
CV (%)	---	5.54	15.49	21.94
Variables	Treatments	Dry biomass accumulation (g)		
		30 DAS[2]	60 DAS	90 DAS
Season	20 DAA	39.00 a[1]	127.00 a	231.00 a
	10 DAA	37.86 a	127.50 a	209.50 a
	0 DAA[3]	31.56 a	152.50 a	261.00 a
	Test	33.64 a	140.00 a	169.75 a
F	Blocks	0.68^{NS}	3.12^{NS}	0.67^{NS}
	Season	1.75^{NS}	0.50^{NS}	2.07^{NS}
CV (%)	---	14.84	25.09	24.74

1 Means followed by the same letter in the same column belong to the same group by the Scott Knott test; 2 Days after *C. spectabilis* sowing; 3 Days before *C. spectabilis* sowing.

This result was possibly due to competition with weeds in coexistence with crotalaria in control and at 20 DAA, providing greater plant height. Despite the reduction in growth observed at 90 DAS in sowings with less than 10 days between application and sowing, the application of the herbicide, regardless of the time, did not affect the accumulation of raw and dry biomass of *Crotalaria breviflora* (Table 7). The lack of effect on plant development is related to the possible selectivity of this herbicide for this species, which promoted the rapid metabolization of the molecules to non toxic forms, which prevented the plants from dying and continuing to recover (Roman et al., 2007).

It is worth emphasizing that the atrazine molecule is registered for monocotyledonous crops such as sugarcane, corn, and sorghum (Agrofit, 2023).

With the results obtained in the research, it is possible to state that for *C. breviflora*, there is no need for a grace period before sowing crotalaria since the herbicide does not limit its germination and plant development. The results show that the herbicide atrazine has differential selectivity for crotalaria species. Therefore, the management of tiguera soybeans in areas where crotalaria species are planted should be based on the desired species, respecting the grace period between application and sowing.

Table 6. *Crotalaria breviflora* plant height at 30, 60, and 90 days after sowing (DAS) as a function of the time of application of the herbicide atrazine in pre emergence. Jataí GO, 2019.

Variables	Treatments	Average Plant Height (cm)		
		30 DAS[2]	60 DAS	90 DAS
Season	20 DAA[3]	31.86 a[1]	143.50 a	197.50 a
	10 DAA	26.13 a	142.00 a	167.25 b
	0 DAA	20.25 a	128.75 a	173.25 b
	Test	20.13 a	153.75 a	202.00 a
F	Blocks	1.18[NS]	1.56[NS]	1.29[NS]
	Season	2.09[NS]	1.34[NS]	7.21*
CV (%)	---	31.53	12.47	6.96

1 Means followed by the same letter in the same column do not differ by the Scott Knott test; 2 Days after sowing; 3 Days before atrazine application.

Table 7. Biomass accumulation of *Crotalaria breviflora* at 30, 60, and 90 days after sowing (DAS) as a function of the time of application of atrazine in pre emergence. Jataí GO, 2019.

Variables	Treatments	Biomass in Natura reviews (g)		
		30 DAS[2]	60 DAS	90 DAS
Season	20 DAA[3]	79.00 a[1]	261.50 a	610.00 a
	10 DAA	89.50 a	220.00 a	513.25 a
	0 DAA	55.00 a	226.00 a	343.00 a
	Test	79.50 a	298.00 a	464.00 a
F	Blocks	1.04[NS]	4.09*	6.13*
	Season	2.01[NS]	1.39[NS]	3.54[NS]
CV (%)	---	5.54	24.35	24.12
Variables	Treatments	Dry Biomass Reviews (g)		
		30 DAS[2]	60 DAS	90 DAS
Season	20 DAA[3]	23.13 a[1]	71.00 a	138.75 a
	10 DAA	21.77 a	83.00 a	97.25 a
	0 DAA	17.50 a	73.00 a	133.00 a
	Test	22.96 a	61.75 a	145.25 a
F	Blocks	2.78[NS]	2.67[NS]	3.20[NS]
	Season	2.42[NS]	0.74[NS]	0.63[NS]
CV (%)	---	15.83	28.10	42.17

[1] Means followed by the same letter in the same column do not differ by the Scott Knott test; [2] Days after sowing; [3] Days before atrazine application

4. Conclusions

The research results show that the herbicide atrazine has differential selectivity for crotalaria species. *Crotalaria juncea* is the most sensitive species to the herbicide atrazine. The *Crotalaria spectabilis* and *Crotalaria breviflora* species, on the other hand, have no limitations when it comes to using atrazine to manage tiguera soybean plants, regardless of the interval between application and sowing the crotalaria in very clayey soil conditions and up to 2500 g of a.i. ha^{-1}.

Authors' Contribution

Work extracted from the degree's thesis of Ana Laura Fernandes Maciel, under the direction and execution of the research by Paulo Cesar Timossi and the support for the execution of the research by Murilo Henrique de Carlo. Data analysis, writing and review of the manuscript by Fenelon Lourenço de Sousa Santos and Itamar Rosa Teixeira.

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