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Effect of straw and rainfall on *Amaranthus hybridus* control with Imazethapyr + Flumioxazin

Efeito da palha e da precipitação pluviométrica no controle de *Amaranthus hybridus* com Imazetapir + Flumioxazina

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Highlights

Imazethapyr + flumioxazin was effective against *A. hybridus,* even applied on straw.
The herbicide was effective even after periods of drought or heavy rainfall.
Straw weighing ≥ 6 Mg ha^{-1} suppressed the emergence of *A. hybridus.*

Abstract

The use of herbicides applied at pre-emergence is an important measure for managing *Amaranthus hybridus.* However, the presence of straw and rainfall regime after application may alter the efficacy of these herbicides. This study was carried out with the objective of evaluating the effect of different straw mass on the soil surface and different rainfall regimes on the efficacy of imazethapyr + flumioxazin in controlling *Amaranthus hybridus.* Three greenhouse experiments were conducted and replicated for two years. Experiment 1 consisted of five *Brachiaria humidicola* straw mass (0, 2, 4, 6 and 8 Mg ha^{-1}) with 20 mm rainfall simulation after herbicide application; in experiment 2, six times to the first rainfall (0, 3, 6, 9, 12 and 15 days) after herbicide application were assessed; and experiment 3 involved four rainfall volumes (0, 10, 30 and 50 mm) after herbicide application. The presence of straw did not alter the efficacy of imazethapyr + flumioxazin, even at the highest mass assessed (8 Mg ha^{-1}) and rainfall of 20 mm. In the absence of herbicide, the highest *B. humidicola* weight reduced emergence of *A. hybridus* by 63% when compared to the straw-free treatment. The herbicide imazethapyr + flumioxazin demonstrated flexibility in response to rainfall regime, with total control of *A. hybridus* even after15 days of drought and up to 50 mm of rain after spraying. The herbicide imazethapyr + flumioxazin was effective against *A. hybridus* in all straw and rainfall situations evaluated.

Key words: Cover crop. No-tillage. Pre-emergent. Smooth amaranth. Weed control.

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Resumo

O uso de herbicidas aplicados em pré-emergência é uma medida importante para o manejo do *Amaranthus hybridus.* Porém, a presença de palha e o regime de chuvas após a aplicação podem alterar a eficácia desses herbicidas. Este estudo foi realizado com o objetivo deoi avaliar o efeito de diferentes massas de palha na superfície do solo e diferentes regimes de chuvas na eficácia de imazetapir + flumioxazina no controle de *Amaranthus hybridus*. Três experimentos em casa de vegetação foram conduzidos e replicados por dois anos. O Experimento 1 constou de cinco massas de palha de *Brachiaria humidicola* (0, 2, 4, 6 e 8 Mg ha^{-1}) com simulação de 20 mm de chuva após a aplicação do herbicida; no experimento 2, foram avaliados seis períodos até a primeira chuva (0, 3, 6, 9, 12 e 15 dias) após a aplicação do herbicida; e o experimento 3 envolveu quatro volumes de chuva (0, 10, 30 e 50 mm) após aplicação do herbicida. A presença de palha não alterou a eficácia do imazethapyr + flumioxazina, mesmo na maior massa de palha avaliada (8 Mg ha^{-1}) e chuva de 20 mm. Na ausência do herbicida, a maior massa de *B. humidicola* reduziu a emergência de *A. hybridus* em 63% quando comparada ao tratamento sem palha. O herbicida imazetapir + flumioxazina demonstrou flexibilidade em resposta à ocorrência de chuvas, com controle total mesmo após 15 dias de estiagem e até 50 mm de chuva após a pulverização. O herbicida imazetapir + flumioxazina foi eficaz sobre *A. hybridus* em todas as situações avaliadas de palha e ocorrência de chuvas.

Palavras-chave: Plantas de cobertura. Plantio direto. Pré-emergente. Caruru. Controle de plantas daninhas.

Introduction

Weeds are undesirable plant species because they compete directly with crops for water, light, nutrients, carbonic gas and physical space (Pitelli, 2015). They can also interfere in commercial crops indirectly by hosting pests and diseases, releasing allelopathic substances, interfering in crop treatments and harvesting (Ramos et al., 2019). Among the species that restrict crop development is *Amaranthus hybridus* (smooth amaranth), a widespread species in production fields around the world, primarily in the summer growing season (Dellaferrera et al., 2018). It is a C4 weed with high vegetative growth that rapidly reaches the reproduction stage, exhibiting high fecundity and seed longevity (Barroso et al., 2012).

Integrated weed management is recommended for grain production. The use of preventive, mechanical, crop and chemical methods is essential for effective weed control in the field (Heap & Duke, 2017). Chemical control with herbicides is currently the most widely used in large-scale production (Vieira et al., 2018). However, in Brazil, there are cases of *A. hybridus* with multiple resistance to acetolactate synthase (ALS) inhibitors and 5-enol pyruvyl shikimate 3-phosphate synthase (EPSPs) (Mendes et al., 2022; Sulzbach et al., 2024; Heap, 2024), which hinders its management. Thus, diversifying control methods, including the use of herbicides applied at pre-emergence, rotating the mechanisms of action of herbicides and applying mixtures, is recommended as a measure against resistance (Lagator et al., 2013).

In this respect, industry has provided herbicide mixtures, such as imazethapyr + flumioxazin. This mixture is broadly applied to control weeds in pre- and post-emergence in the pre-sowing burndown of soybean and peanut. These products can also be sprayed in the pre-emergence of crops in a so-called plant-apply system. Imazethapyr is an anionic water soluble imidazolinone that is persistent in soil and inhibits ALS, with leaching inversely proportional to the amount of organic matter in the soil. It leaches more rapidly through sandy soil, in proportion to rainfall volumes after application (Pertile et al., 2020). Flumioxazin inhibits protoporphyrinogen oxidase (PROTOX), the chemical group of N-phenyl-phthalimides, is nonionic and exhibits poor water solubility. This compound shows strong adsorption, mainly to the organic and clay fractions of soil. These traits make the herbicide a low leaching risk. However, the shorter interval between application and occurrence of the first rainfall increases the availability of flumioxazin in the soil solution (Alister et al., 2008).

In the case of pre-emergent, no-till systems hinder contact between the sprayed drops and target plants. Straw prevents herbicide contact with the soil, especially under dry climate conditions and when products have poor water solubility, limiting straw transposition (Christoffoleti et al., 2008). Even if it reaches the soil, the herbicide often remains in the surface layer, while some weed seeds are found at depth and, when exposed to suitable environmental conditions, germinate and emerge (Silva et al., 2019).

Rainfall in adequate periods and amounts after pre-emergent spraying improves the control of target species. However, excess water may cause surface runoff of the product and/or leaching to deeper soil layers, below the weed seed bank, resulting in low efficacy and a decline in residual herbicide, like observed by Silva et al. (2019). On the other hand, low rainfall may be insufficient for the herbicide to cross the straw layer and reach the seed bank. As such, this study was carried out with the objective to evaluate the effect of different straw weights on the soil surface and different rainfall regimes on the efficacy of imazethapyr + flumioxazin in the control of *A. hybridus.*

Material and Methods

Study site and plant material

Three greenhouse experiments were carried out at the Universidade Estadual de Londrina (UEL) (23º 19′ 44″ S and 51º 12′ 13″ W, altitude of 560 meters), in November and December 2021, and repeated between January and March 2022. The experiments were conducted in 8.0 L pots with a diameter and depth of 0.23 and 0.24 m respectively. The soil used in the experiments was collected on the UEL Teaching Farm at a weed-free depth. Soil chemical analysis in the 0-20 cm layer obtained the following data: pH ($CaCl_2$) = 4.6; P = 21.1 mg dm^{-3}; K = 0.48 $cmol_c$ dm^{-3}; Ca = 3.3 $cmol_c$ dm^{-3}; Mg = 1.21 $cmol_c$ dm^{-3}; Al = 0.14 $cmol_c$ dm^{-3}; H+Al = 4.96 $cmol_c$ dm^{-3}; CTC = 10.09 $cmol_c$ dm^{-3} and V = 50.15%. For correction, 2.5 g of NPK 05 20 20 fertilizer and 7.5 g of dolomitic limestone kg^{-1} soil were mixed.

A total of 90 *A. hybridus* var. *patulus* seeds were sown per pot, deposited on the soil surface and lightly incorporated. Seeds were collected in an experimental area used for herbology tests in the municipality of Eldorado do Sul, Rio Grande do Sul state. The biotype used in this research does not show resistance to herbicides.

Experimental design and treatments

Experiment 1- Different straw weights

The experiment was conducted using a completely randomized design (CRD) with four repetitions and a 5 x 2 factorial scheme. Factor A consisted of five *Brachiaria humidicola* straw weights (0, 2, 4, 6 and 8 Mg ha^{-1}), uniformly distributed on the soil surface. Factor B was applying or not imazethapyr + flumioxazin (Zethamaxx® SC, 200 g a.e. L^{-1} of imazethapyr + 100 g a.i. L^{-1} of flumioxazin). The dose used was equivalent to 0.6 L ha^{-1} of the commercial product, corresponding to 120 g a.e. ha^{-1} of imazethapyr + 60 g a.i. ha^{-1} of flumioxazin, according to the manufacturer's recommendation. The different straw weights were measured on a precision balance and converted in to Mg ha^{-1} for the pots that contained soil cover.

The pots were irrigated by sprinkler after the herbicide treatments were applied, with the equivalent of 20 mm of rainfall applied to each pot. After the first irrigation, the pots were periodically irrigated, maintaining soil moisture near field capacity.

Experiment 2 - Time to first rainfall after herbicide application

The experiment was conducted using a CRD with four repetitions and treatments arranged in a 6 x 2 factorial scheme. Factor A consisted of six periods between *A. hybridus* sowing and the occurrence of the first rainfall, namely 0, 3, 6, 9, 12 and 15 days; and factor B of applying or not imazethapyr + flumioxazin (Zethamaxx® SC, 200 g a.e. L^{-1} of imazethapyr + 100 g a.i. L^{-1} of flumioxazin), at the same dose used in experiment 1.

In order to simulate the no-till system, after *A. hybridus* sowing, all the experimental units were added with an amount equivalent to 4 Mg ha^{-1} of *B. humidicola* straw. The first rainfall was simulated according to that established in factor A. The amount of water was previously calculated, according to the pot area, to distribute rain equivalent to 20 mm, by manual sprinkler. After this first irrigation, the pots were periodically watered, maintaining soil moisture near field capacity.

Experiment 3 - Different rainfall volumes after herbicide application

The experiment was conducted in a CRD with four repetitions and the treatments arranged in a 4 x 2 factorial scheme. Factor A consisted of four rainfall volumes after herbicide application, namely 0, 10, 30 and 50 mm, and factor B of applying or not imazethapyr + flumioxazin (Zethamaxx® SC, 200 g a.e. L^{-1} of imazethapyr + 100 g a.i. L^{-1} of flumioxazin), at the same dose used in the other experiments.

A fixed amount of *B. humidicola* straw was used on the soil surface, equivalent to 4,0 Mg ha^{-1} for all treatments. This volume of straw was used because it is common prior to soybean sowing in the no-tillage system. The amount of rain (Factor A) was simulated using a manual sprinkler, immediately after herbicide application. After the first irrigation, the pots were periodically watered, maintaining soil moisture near field capacity.

Herbicide application

The herbicide was applied using a CO_2 pressurized backpack sprayer, equipped with a boom containing two AXI 110.02 spray nozzles spaced 0.5 m apart. The sprayer was regulated to 30 psi, with displacement speed of 1 m s^{-1}, resulting in a constant flow of 150 L ha^{-1}. During application, the boom was maintained 0.5 m above the soil in the pots.

Assessments

In all the experiments, *A. hybridus* control was assessed at 7, 14, 21, 28 and 35 days after sowing (DAS), counting the number of emerged plants. At 42 DAS the remaining plants were collected and placed in a forced air oven at 65° C for 72 hours, for subsequent measurement of shoot dry mass (SDM) on a precision balance.

Statistical analysis

The data collected in duplicate were submitted to exploratory analyses, where residue homogeneity (Bartlett) and normality (Shapiro-Wilk) were tested to determine the analysis of variance (ANOVA) assumptions. Next, ANOVA ($p<0.05$) was conducted and the interaction between factors determined. The consequences of each factor were then analyzed, generating the adjusted polynomial regression for the quantitative parameters, selecting the second-order equation. For the qualitative parameters, means were compared by Tukey's test at 5% probability. All the analyses were jointly carried out, using the R program, version 4.0.5. (R Core Team [R], 2021).

Results

Experiment 1- Different straw weights

Applying imazethapyr + flumioxazin resulted in 100% *A. hybridus* control at all the assessment times, irrespective of straw weight (Table1). In other words, pre-emergent weed control was total, in both the absence and presence of up to 8 Mg ha^{-1} of straw. By contrast, in the herbicide-free treatments, weed emergence was observed from the start of the assessment period. Although *A. hybridus* emerged in the herbicide-free treatments in the first week post herbicide application, straw weight showed no significant effect, with an average emergence of 1.9 plants per pot (data not presented).

Table 1
Number of emerged plants and shoot dry mass (SDM) of *Amaranthus hybridus* per pot as a response to applying or not imazethapyr + flumioxazin and different rainfall volumes after application

Assessment	Herbicide	Rainfall volume (mm)			
		0	10	30	50
		Number of emerged plants per pot			
7 DAS	Without	0.13 aC	1.25 aBC	4.63 aA	2.63 aAB
	With	0.00 aA	0.00 bA	0.00 bA	0.00 bA
14 DAS	Without	12.63 aA	9.25 aA	10.25 aA	8.50 aA
	With	0.00 bA	0.00 bA	0.00 bA	0.00 bA
21 DAS	Without	18.86 aA	14.00 aA	13.75 aA	11.13 aA
	With	0.00 bA	0.00 bA	0.00 bA	0.00 bA
28 DAS	Without	18.88 aA	15.13 aA	14.13 aA	12.38 aA
	With	0.00 bA	0.00 bA	0.00 bA	0.00 bA
35 DAS	Without	19.00 aA	15.25 aA	14.13 aA	12.50 aA
	With	0.00 bA	0.00 bA	0.00 bA	0.00 bA
		g pot^{-1}			
SDM	Without	20.24 aA	19.50 aA	22.01 aA	20.64 aA
	With	0.00 bA	0.00 bA	0.00 bA	0.00 bA

*The same lower-case letters in the column and upper case in the row do not differ according to Tukey's test at 5% probability.

From 14 DAS onwards, in the absence of herbicide the increase in straw weight with 4,0 Mg ha^{-1} reduced *A. hybridus* emergence (Figure 1). In the last assessment, at 35 DAS, the largest amount of straw (8,0 Mg ha^{-1}) decreased weed emergence by 63% (Figure 1D), whereas 2,0 Mg ha^{-1} resulted in greater *A. hybridus* emergence when compared to the straw-free treatment, with a 24% increase in emergence.

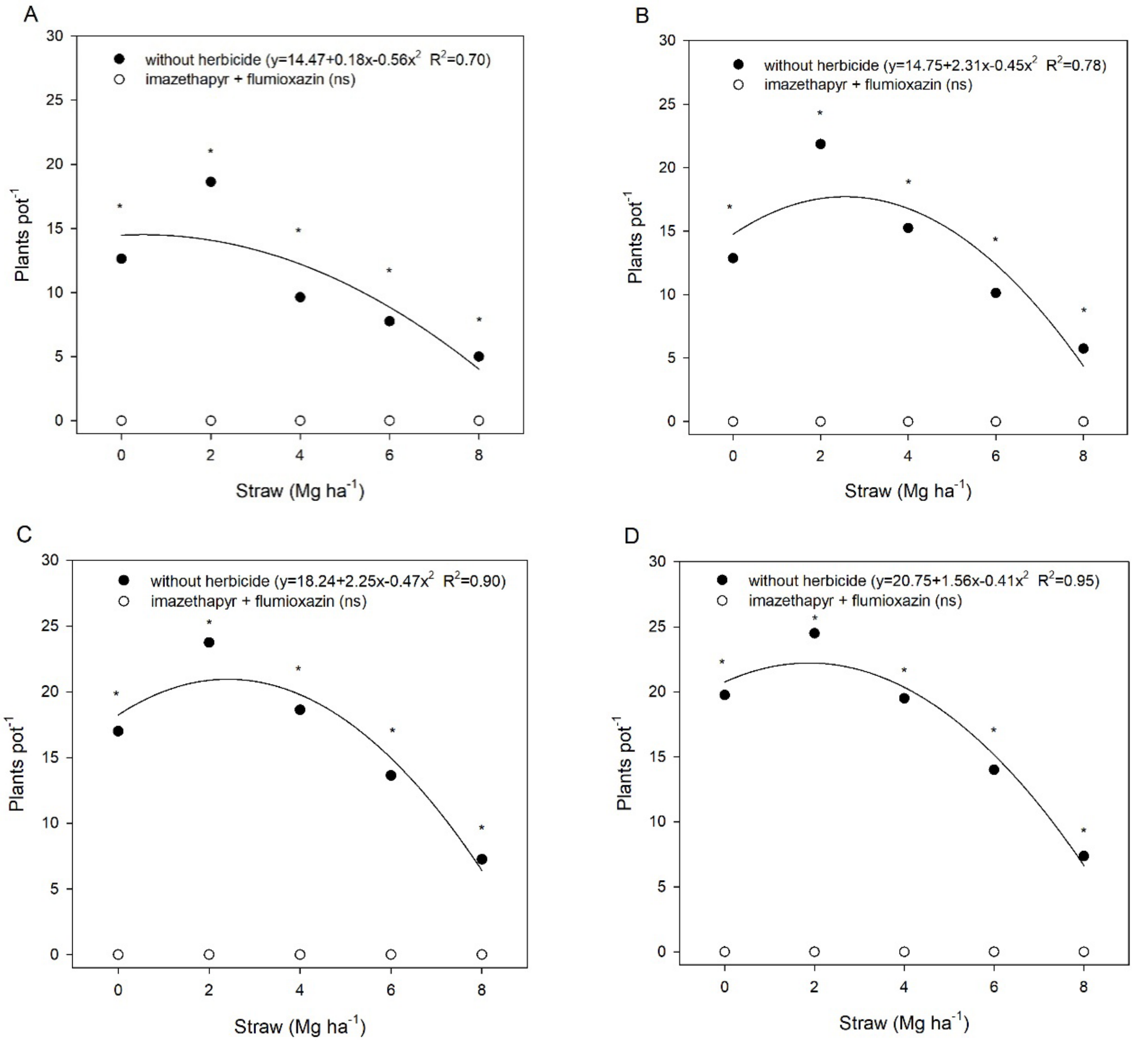

Figure 1. Number of *Amaranthus hybridus* plants emerged per pot in response to applying imazethapyr + flumioxazin or not on different *Brachiaria humidicola* straw weights at 14 (A), 21 (B), 28 (C) and 35 (D) DAS. Asterisks (*) within each straw amount straw indicate a significant effect of herbicide use according to Tukey's test ($p < 0.05$).

Since no plant emergence occurred in the treatments containing imazethapyr + flumioxazin, shoot dry mass (SDM) was null, differing from herbicide-free treatments (data not presented). However, in herbicide-free treatments, even with a marked difference in *A. hybridus* emergence between treatments, SDM did not differ when submitted to different straw weights, with an average of 16.42 g pot^{-1}. The smaller number of plants per pot promoted greater individual growth, occupying space in the absence of other plants. This caused higher dry mass per plant, equivalent to treatments with greater emergence, resulting in similar values between the five straw weights.

Experiment 2 - Time to first rainfall after herbicide application

Imazethapyr + flumioxazin promoted 100% *A. hybridus* control, regardless of the interval between application and the first rainfall, for all the assessment times (Figure 2). This demonstrates the continued efficacy of the herbicide mixture, even at 15 days between application and the first irrigation.

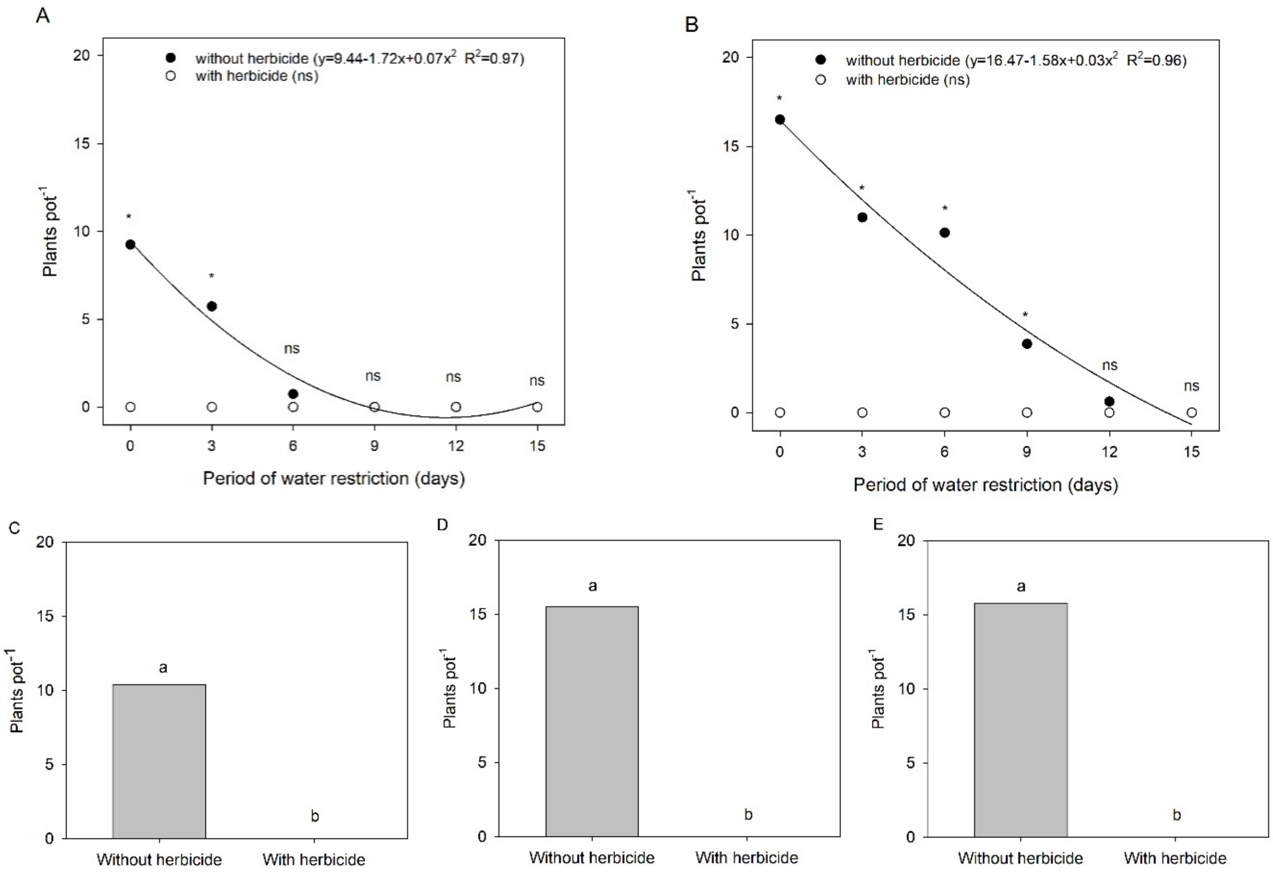

Figure 2. Number of *Amaranthus hybridus* plants emerged per pot as a response to applying imazethapyr + flumioxazin or not after different water restriction periods following application at 7 (A), 14 (B), 21 (C), 28 (D) and 35 DAS. Asterisks (*) within each interval between application and the first irrigation and different lower-case letters indicate a significant effect of herbicide use, according to Tukey's test ($p < 0.05$).

A significant effect was observed for the time between herbicide application and the first irrigation at 7 and 14 DAS, resulting in second-order regression adjustment (Figures 2A and 2B). In these assessments, *A. hybridus* emergence differed between the times to first rainfall, declining between 0 and 15 days, when no emergence occurred. This result was expected, since at 7 and 14 DAS, some treatments had not been irrigated, which precluded germination and weed emergence.

In the other assessments, at 21, 28 and 35 DAS (Figures 2C, 2D and 2E), no significant effect was observed for the interval between herbicide application and the first irrigation. However, a significant effect was observed for the factor herbicide, with total *A. hybridus* control using imazethapyr + flumioxazin, while weed emergence occurred in the herbicide-free treatments.

In relation to SDM, collected at 42 DAS, the results corroborate emergence assessments, when shorter rainfall intervals lead to greater germination and higher dry mass (Figure 3). This result was also expected, given the longer time required for plants to develop after the first irrigation. However, in the presence of the herbicide, SDM was zero due to total weed control by imazethapyr + flumioxazin.

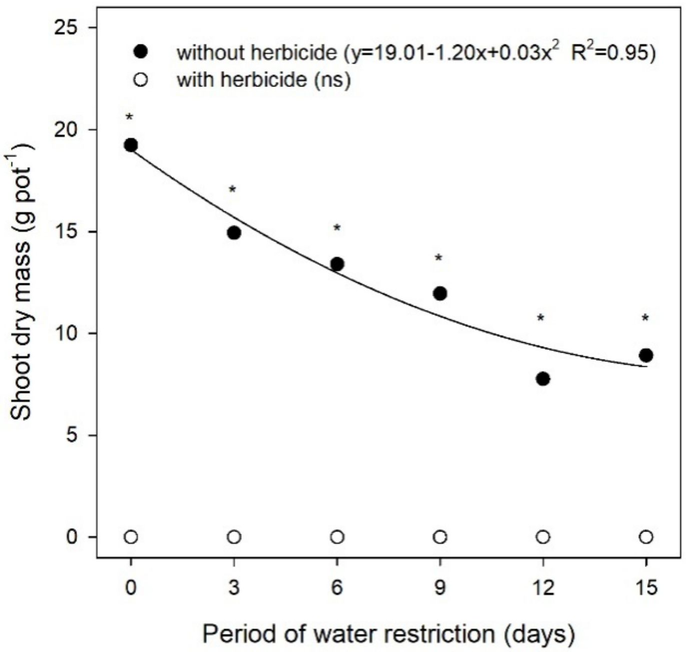

Figure 3. Shoot dry mass of *Amaranthus hybridus* at 42 DAS as a response to different times between imazethapyr + flumioxazin application and the first irrigation. Asterisks (*) within each interval between application and the first irrigation and different lower-case letters indicate a significant effect of herbicide use, according to Tukey's test ($p < 0.05$).

Experiment 3 - Different rainfall volumes after herbicide application

A. hybridus control was total after applying imazethapyr + flumioxazin, regardless of rainfall volume after herbicide spraying. This demonstrated the efficacy of the herbicide even with 50 mm of rain after application. Considering the amount of rain in herbicide-free treatments, the 30 mm simulation resulted in a larger number of emerged *A. hybridus* plants at 7 DAS. Similar behavior was observed when 50 mm was applied, not differing from 30 mm. In treatments consisting of 0 and 10 mm of simulated rain, *A. hybridus* emergence was lower, and almost null with 0 mm. During assessments, as the pots were irrigated periodically, germination of seeds that were waiting for conditions favorable to their development were observed. From 14 DAS onwards, no significant effect was observed for different rainfall volumes (Table 1).

Due to the absence of emerged plants as a function of applying imazethapyr + flumioxazin, SDM was zero in all the treatments that received the herbicide. In the absence of herbicide, no significant difference was observed between rainfall volumes applied to simulate rain.

Discussion

In general, the presence of straw on the soil surface and rainfall regime after application (time to first rainfall after application and the amount of rain) did not affect the efficacy of imazethapyr + flumioxazin in pre-emergent *A. hybridus* control (Figures 1, 2 and 3; Table 1). Carbonari et al. (2010) found that applying flumioxazin on soil promoted good weed control levels, with a declining trend when the herbicide was exposed for 60 days without rain, indicating that the product degraded when submitted to extensive exposure on the soil surface with no rain. Thus, at longer intervals between application and the first rain, the results could be different to those observed in the present study, which assessed a maximum period of 15 days.

Pre-emergent herbicides with high lipophilicity (high log Kow) and low water solubility tend to be retained in the straw and not reach the soil (Martins et al., 2020). Straw can increase the retention of the products applied, causing volatilization, thermal degradation and photolysis. Soil herbicide mobility depends on the amount of straw, organic matter composition, weather conditions, post-application rainfall and the physicochemical characteristics of the products. Imazethapyr is classified as lipophilic (log Kow of 1.49) and highly water soluble (1400 mg L^{-1}), while the flumioxazin molecule exhibits high affinity for lipophilic partition (log Kow of 2.55) and low water solubility (0.78 mg L^{-1}) (Lewis et al., 2016). Combining these herbicides promotes a complementary effect to molecular action, making control more comprehensive. These characteristics make their application possible even under high straw weight on the soil surface, requiring a lower rainfall volume to penetrate the soil solution. Thus, *B. humidicola* straw weight and the water regime after application did not affect *A. hybridus* control, indicating good imazethapyr + flumioxazin performance in the no-till system, even with straw weight of 8 Mg ha^{-1} and different rainfall patterns.

In the absence of herbicide, the straw resulted in less *A. hybridus* emergence for straw weights from 4 Mg ha^{-1} onwards (Figure 1). Likewise, sugarcane straw also reduced the emergence of *A. hybridus* emergence and other weed species (Hoshino et al., 2017). Martins et al. (2016) found that straw increases *A. hybridus* suppression and decreases plant density and dry mass accumulation, which dispensed with the use of herbicides. However, although the presence of *B. humidicola* straw resulted in less *A. hybridus* emergence, the weeds still emerged even at 6 and 8 Mg ha^{-1}, which would require adopting another control strategy. Thus, there is an evident need to implement integrated management measures for this weed, such as pre-emergence applied herbicides.

The highest straw weight tested reduced the *A. hybridus* population by approximately 63%, demonstrating the suppressor effect of weight on weed emergence, even for neutral photoblastic species such as *A. hybridus.* Although emergence also occurs in the dark, *A. hybridus* emergence is greater in the presence of light (Carvalho & Christoffoleti, 2007). Although this species does not need light to germinate, straw exerts a physical effect, in addition to the possibility of interference by allelopathic compounds that affect plant growth (Toledo et al., 2022).

On the other hand, straw weight equivalent to 2 Mg ha^{-1} increased *A. hybridus* emergence by 24% when compared to the straw-free treatment. Although this amount of straw was insufficient to inhibit *A. hybridus* emergence, it was important in maintaining soil surface moisture, promoting greater emergence when compared to the straw-free treatment. Thus, a no-till system with insufficient straw may favor the occurrence of *A. hybridus.* Similar results were obtained by Correia et al. (2005), where *Chamaesyce* spp. emergence was higher when the soil was covered with millet or sorghum straw when compared to the straw-free treatment.

With an increase in weed resistance to post-emergent herbicides, mainly glyphosate, the use of pre-emergent becomes an important management tool. However, further knowledge of the dynamics of these herbicides in different environments is needed, including the variation in amount and origin (types of soil cover) of the straw, and water regime after herbicide application. As such, more studies are needed to increase understanding and solidify the recommendation and use of pre-emergent herbicides in different cropping systems.

Conclusions

Imazethapyr + flumioxazin was effective in controlling *A. hybridus* in pre-emergence, even when sprayed on 8 Mg ha^{-1} of *B. humidicola* straw with 20 mm of rain after herbicide application.

The herbicide withstood up to15 days in dry conditions after application, exerting total control over *A. hybridus* after the first irrigation. Up to 50 mm of rain after application did not affect *A. hybridus* control, with 100% control in all situations.

Straw exhibited a suppressor effect of up to 63% on *A. hybridus* emergence, and can be considered an ally in the integrated management of this weed.

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