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Article

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Interaction Between Saflufenacil and Other Oxidative Stress Promoting Herbicides to Control Wild Poinsettia

Interação entre Saflufenacil e Outros Herbicidas Promotores de Estresse Oxidativo para o Controle de Leiteiro

ABSTRACT - Synergism is a desired result in the interaction between herbicides because it provides many benefits, among which the reduction of doses and the increment of control efficiency, and because it positively signals for the joint application of herbicides. The objective of this study is to evaluate the effect of the association of saflufenacil with other herbicides that promote oxidative stress on the development of Euphorbia heterophylla as well as to analyze if the resulting interactions are antagonistic, additive or synergistic. The experiment was conducted in pots, in a greenhouse, using a completely randomized design with four replications. Treatments consisted of saflufenacil (0, 1,2, 2,32, 4,47, 8,63, 16,65 g ha⁻¹) alone and associated with clomazone (288 and 504 g ha⁻¹), metribuzin (105.6 and 206.4 g ha⁻¹) and paraquat (20 and 40 g ha⁻¹), as well as these herbicides applied alone at the same doses described above. Twenty-one days after the application (DAA), the shoot dry matter (SDM) of plants was evaluated in each experimental unit. The Limpel-Colby method was used to calculate the expected response of the herbicide interaction. All combinations of saflufenacil with clomazone, metribuzin and paraquat were considered synergistic. Among the treatments associated with saflufenacil, those including metribuzin at the dose of 105.6 g ha⁻¹ were the ones that provided the most promising results, which stood out for the greater reductions of wild poinsettia shoot dry matter, compared to the application of saflufenacil alone.

Keywords: *Euphorbia heterophylla*, synergism, clomazone, metribuzin, paraquat.

RESUMO - O sinergismo é um resultado desejado na interação entre os herbicidas por proporcionar muitos benefícios, entre os quais a redução de doses e o incremento da eficiência de controle, e por sinalizar positivamente para a aplicação conjunta de herbicidas. O objetivo deste trabalho foi avaliar o efeito da associação de saflufenacil com outros herbicidas promotores de estresse oxidativo sobre o desenvolvimento de Euphorbia heterophylla, bem como analisar se as interações resultantes possuem natureza antagônica, aditiva ou sinérgica. O experimento foi realizado em vasos, em casa de vegetação, em delineamento inteiramente casualizado com quatro repetições. Os tratamentos consistiram de doses de saflufenacil (0; 1,2; 2,32; 4,47; 8,63; e 16,65 g ha⁻¹) aplicadas de forma isolada e associadas com clomazone (288 e 504 g ha⁻¹), metribuzin (105,6 e 206,4 g ha⁻¹) e paraquat (20 e 40 g ha⁻¹), além desses últimos herbicidas aplicados isoladamente, nas mesmas doses descritas. Aos 21 dias após a aplicação (DAA), foi avaliada a massa seca parte aérea das plantas em cada unidade experimental. O método de Limpel-Colby foi utilizado para cálculo da resposta esperada da interação entre os herbicidas. Todas as associações de saflufenacil com clomazone, metribuzin e

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paraquat foram sinérgicas. Dos tratamentos associados a saflufenacil, aqueles que incluíram metribuzin na dose de 105,6 g ha¹ foram os que proporcionaram resultados mais promissores, destacando-se pela maior redução da massa seca parte aérea das plantas de leiteiro, em comparação à aplicação isolada de saflufenacil.

Palavras-chave: Euphorbia heterophylla, sinergismo, clomazone, metribuzin, paraquat.

INTRODUCTION

The wild poinsettia (*Euphorbia heterophylla*) is one of the dicot species with the highest incidence in crops over Brazil, standing out for its short cycle and high growth rates, multiplication and competitiveness (Voll et al., 2002; Meschede et al., 2004). A period of mutual coexistence between *E. heterophylla* and soybean, between 11 and 68 days after emergence, resulted in a daily loss of 6.45 kg ha⁻¹, and in a 38% loss of soybean grains throughout the cycle (Meschede et al. al., 2004). In the bean crop, the daily loss for each wild poinsettia plant per m² depends on the crop sowing time in relation to the emergence of the weed species, being 2.4 kg ha⁻¹ day⁻¹ for the simultaneous sowing of the species, and 5.5 kg ha⁻¹ day⁻¹ when the sowing of wild poinsettia plants occurs 12 days before sowing beans (Machado et al., 2015).

The association of herbicides in the same crop is a common practice in weed control (Kruse et al., 2006). The use of herbicide combinations may result in synergistic, antagonistic or additive interactions (Colby, 1967). Synergism, as a response to the associated use of herbicides, is desired because it provides a greater spectrum of weed control, while helping to prevent the development of resistance, allowing dose reduction, increasing application efficiency, and reducing time and labor (Hatzios and Penner, 1985; Gressel, 1990; Green and Owen, 2011). The synergistic response of the interaction between herbicides is often associated with the use of herbicides with different mechanisms of action, by the complementary physical and/or biochemical action of the simultaneous use (Matthews, 1994).

Saflufenacil is a Protox-inhibiting herbicide recommended for crop desiccation and weed control in pre-sowing and pre-emergence of the recommended crops, especially dicot weeds. It has an acidic ionization constant (pka) and an octanol/water partition coefficient (kow), which determine its translocation via xylem and phloem, differentiating it from the other Protox-inhibiting herbicides (Grossmann et al., 2011).

Clomazone is a carotenoid biosynthesis inhibiting herbicide, recommended to be used in the pre-emergence of grass and broadleaf weeds (Dan et al., 2011). Metribuzin belongs to the group of photosystem II inhibitors, and is recommended for pre- and post-emergence use, with a broad control spectrum of broadleaf weeds (Carvalho et al., 2010). Both clomazone and metribuzin are selective to the recommended crops and have a residual effect (Inoue et al., 2011; Walperes et al., 2015). Paraquat is a photosystem I inhibitor, non-selective and non-systemic, and is therefore indicated for desiccation (Hawkes, 2014).

Saflufenacil, clomazone, metribuzin and paraquat are similar in their mode of action, promoting oxidative stress, and share the direct or indirect dependence from light (Hess, 2000). Positive effects have been reported by the association of oxidative stress-promoting herbicides (Kruse et al., 2001, 2006; Trezzi et al., 2016); however, there are few studies relating the interaction between herbicides with similar modes of action.

The objective of this work was to evaluate the effect of the association of saflufenacil with other herbicides that promote oxidative stress on the development of *E. heterophylla*, as well as to analyze if the resulting interactions are antagonistic, additive or synergic.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse, using pots in a completely randomized design with four replications. Soil samples classified as Distroferric Red Latosol were collected from the 0 to 20 cm depth layer in the Experimental Area of the UTFPR (latitude 26°17' S and longitude of 52°69' E). The soil samples were sieved and placed in pots with 9 dm³ of capacity.



Five susceptible *E. heterophylla* seeds were arranged per pot, approximately 2 cm deep. About 20 days after sowing, thinning was done, leaving two plants per pot. When plants reached the stage of 4 to 6 fully expanded leaves, herbicides were applied. The treatments consisted of saflufenacil (0; 1.2; 2.32; 4.47; 8.63; and 16.65 g ha⁻¹) alone and associated with clomazone (288 and 504 g ha⁻¹); metribuzin (105.6 and 206.4 g ha⁻¹) and paraquat (20 and 40 g ha⁻¹), and also the latter herbicides applied alone, at the same described doses. The aromatic polyglycol ether adjuvant was added to all treatments (Dash HC® 0.5% v/v).

Herbicides were applied using a ${\rm CO}_2$ pressurized backpack sprayer, maintained at constant pressure, equipped with XR 110.02 fan-type nozzles, spaced 0.50 m apart on a 1.5 m wide bar, totaling a spraying volume of 200 L ha⁻¹. The application started at 5 pm, with a relative air humidity of 78%, wind speed of 1.2 m s⁻¹, and finished at 6:45 pm.

Twenty-one days after application (DAA), the shoot dry matter (SDM) of the plants per pot was evaluated. Plants were sectioned close to the soil, and their shoot was inserted in paper bags, identified and placed for drying in a forced air circulation oven at 60 °C, until reaching constant weight. Subsequently, the shoot dry matter was determined through a precision scale.

Data about the shoot dry matter of the single treatments were submitted to the Colby method (1967). The equation used to calculate the expected response in the interaction between saflufenacil and clomazone, metribuzin and paraquat was:

$$E = 100 - ((100 - X) * (100 - Y)) / 100$$

where E = estimated effect; X = effect observed by herbicide X; and Y = effect observed by herbicide Y.

Effect differences (estimated and observed) were compared by t test at 5% significance (p<0.05). SDM data were initially subjected to analysis of variance by F test and complemented by regression analysis. Data were adjusted to the three parameter logistic equation:

$$y = a/(1+(x/c)^b)$$

where: y = dependent variable, x = herbicide concentration, a = mean control treatment response, b = curve slope, and c = concentration providing a 50% reduction of the dependent variable value (GR_{so}).

RESULTS AND DISCUSSION

In order to calculate the expected response of the associations, it is necessary to determine the effect of the herbicides alone (Figure 1). The application of the lowest dose of clomazone (288 g ha⁻¹) did not result in a dry matter reduction (SDM) of *E. heterophylla*, while the highest dose (504 g ha⁻¹) resulted in a 56% SDM reduction (Figure 1A). As for metribuzin, SDM reductions were 15% and 59%, respectively, for the lowest (105.6 g ha⁻¹) and the highest dose (206.4 g ha⁻¹), respectively (Figure 1B). Compared with clomazone and metribuzin, the used doses of paraquat resulted in a greater SDM reduction, 43% with the use of the lowest dose (20 g ha⁻¹) and 79% with the use of the highest dose (40 g ha⁻¹). The application of saflufenacil alone resulted in a shoot dry matter reduction (SDM) of *E. heterophylla* by 71% and 88%, at the lowest and highest dose (1.2 and 16.65 g ha⁻¹), respectively (Figure 2).

The saflufenacil doses recommended by the manufacturer in Brazil to control susceptible *E. heterophylla* are between 24.5 and 70 g ha⁻¹ for the different crops and use modalities for which the herbicide is indicated. The use of doses that are lower than those used in this experiment is justified because higher doses impair association effects (Dalazen et al., 2015); the use of sub-doses is recommended to evaluate the interaction between herbicides (Green et al., 1997; Streibig et al., 1998; Hugie et al., 2008). Although clomazone and metribuzin are not registered in Brazil to control *E. heterophylla*, the results of this experiment indicate, as with saflufenacil, that the application of doses that are lower than those recommended to control other weeds (Brazil, 2017) is enough to expressively reduce the SDM of *E. heterophylla* plants.

The lowest dose of clomazone (288 g ha⁻¹) associated with the lowest dose of saflufenacil (1.2 g ha⁻¹) resulted in a 50% SDM reduction of *E. heterophylla*, while when associated to the highest dose of saflufenacil (16.65 g a.i. ha⁻¹), the reduction was 85% (Figure 2A). The associations



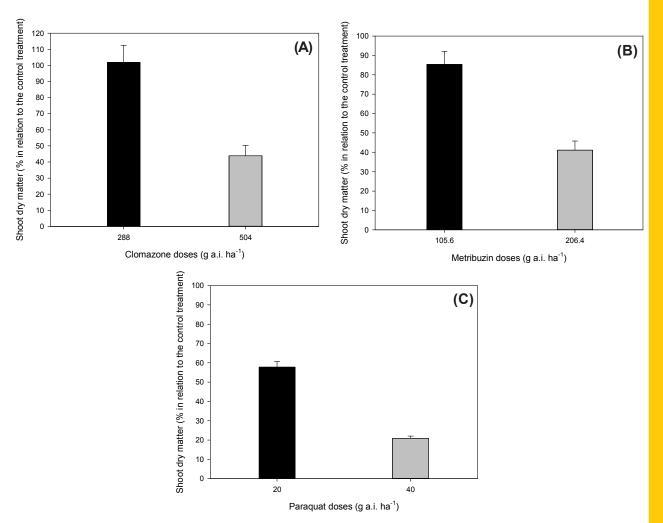


Figure 1 - Shoot dry matter (SDM) in relation to the control treatment (%) of Euphorbia heterophylla, in response to the application of two doses of clomazone (A), metribuzin (B) and paraquat (C) alone.

with both clomazone doses (288 and 504 g ha⁻¹) presented a similar response. Generally speaking, it is possible to observe that the application of saflufenacil alone was more efficient in reducing SDM than the association of this herbicide with clomazone. It is worth highlighting the effect produced with the lowest dose of saflufenacil alone (1.2 g ha⁻¹), which produced greater efficiency and great disparity in comparison to the associated treatments. The lowest dose of saflufenacil resulted in a 71% SDM reduction, whereas its association with the two doses of clomazone (288 and 504 g ha⁻¹) resulted in a 50% and 42% SDM reduction, respectively.

Protox-inhibiting herbicides result in rapid necrosis and may result in the inhibition of absorption and translocation of other herbicides that are associated (Eubank et al., 2013). The use of the Protox lactofen inhibitor at lower doses facilitates the simplistic movement of glyphosate in the plant, maximizing the capture of glyphosate into the cellular interior (Wells and Appleby, 1992). This would partially explain the more pronounced effect of the lowest clomazone dose when this herbicide is associated with the lowest saflufenacil dose (Figure 2A). That is, in this combination, the effect of clomazone may be benefited by the use of the low dose of Protox inhibitor, which does not occur with the combination of clomazone at higher doses of saflufenacil.

The associations of the lowest and highest dose of saflufenacil with metribuzin at 105.6 g ha⁻¹ caused reductions in the SDM of *E. heterophylla* by 74% and 87%, respectively. Generally speaking, combinations of saflufenacil with the lowest dose of metribuzin (105.6 g ha⁻¹) were more efficient than the association of saflufenacil at the highest dose (206.4 g ha⁻¹) and also than saflufenacil alone (Figure 2B). It is worth highlighting the association of saflufenacil (2.32 ha⁻¹) + metribuzin (105.6 g ha⁻¹), which reduced 85% of SDM, differing significantly from the association of saflufenacil (2.32 g ha⁻¹) with the highest dose of metribuzin (66% reduction) and the application of saflufenacil



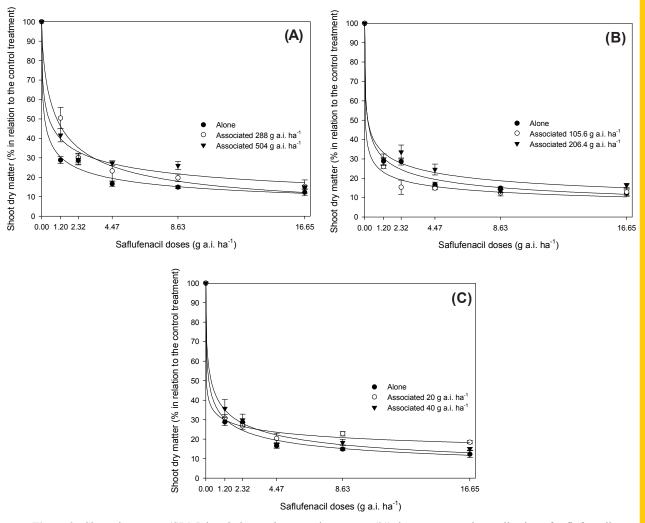


Figure 2 - Shoot dry matter (SDM) in relation to the control treatment (%), in response to the application of saflufenacil associated to two doses of clomazone (A), metribuzin (B) and paraquat (C).

alone (reduction of 71%). Thus, the effect of this association is significant, as it resulted in an SDM reduction that is very close to that of the most efficient treatment observed in this experiment, which resulted in an 88% SDM reduction (saflufenacil 16.65 ha⁻¹).

It is important to consider that saflufenacil has a 2.6 octanol/water partition coefficient (kow) and pKa of 4.4, which inserts it as systemic in the phloem through the ion trap mechanism, according to Kleier's prediction model (Kleier et al., 1998). The metabolic stability of the molecule in some dicot species, together with the physico-chemical characteristics mentioned above, contribute to the systemic characteristic of the herbicide and, therefore, it is considered an exception among Protox-inhibiting herbicides (Grossmann et al. 2011). Metribuzin and clomazone are herbicides that translocate via xylem and are also generators of free radicals, and therefore can cause lipid peroxidation and cellular disruption (Fleck and Vidal, 2001), being able to reduce saflufenacil translocation when applied at higher doses.

The application of saflufenacil + 40 g ha⁻¹ of paraquat resembled the application of saflufenacil alone, achieving a range of SDM reduction between 64% and 85% (Figure 2C). Among the associations, it is worth mentioning saflufenacil 4.47 g ha⁻¹ + paraquat 40 g ha⁻¹, resulting in an 83% SDM reduction, close to the maximum reduction in association with the highest dose of saflufenacil applied alone. The association of saflufenacil with 20 g ha⁻¹ of paraquat resulted in less expressive SDM reductions than with 40 g ha⁻¹.

Contact herbicides, such as paraquat, act rapidly through the production of free radicals and membrane lipid peroxidation (Hess, 2000; Hawkes, 2014), which, in theory, cause a faster negative effect on the translocation of systemic herbicides. Therefore, it would be expected that the



efficiency of saflufenacil would be reduced with increasing doses of paraquat, which in fact did not occur. Waggoner et al. (2011), while evaluating mixtures of saflufenacil and other herbicides in a field experiment to desiccate glyphosate-resistant *Conyza canadensis*, reported that the addition of 702 g a.i. ha⁻¹ of paraquat to saflufenacil (25 and 50 g a.i. ha⁻¹) increased control levels, compared to saflufenacil alone.

The saflufenacil dose resulting in the 50% reduction of SDM (GR_{50}) was 0.1935 ha⁻¹, while GR_{50} saflufenacil in combination with the two tested clomazone doses were higher than that of saflufenacil alone (Table 1). As for metribuzin and paraquat, the lowest doses of these herbicides (105.6 and 20 g ha⁻¹, respectively), associated with saflufenacil, resulted in lower GR_{50} (0.044 and 0.037 g ha⁻¹) than those of saflufenacil alone (Table 1).

Table 1 - Parameters of the equation, determination coefficient (R²) and probability of the equation for the shoot dry matter variable (SDM) of *Euphorbia heterophylla*

Treatment		R^2	n		
Treatment	a	b	c(GR ₅₀)	K	p
Saflufenacil alone	99.9925	0.4528	0.1935	0.9950	0.0004
Saflufenacil + Clomazone 288 g ha ⁻¹	100.2023	0.6930	0.9605	0.9897	0.0014
Saflufenacil + Clomazone 504 g ha ⁻¹	100.0110	0.4399	0.4677	0.9908	0.0009
Saflufenacil + Metribuzin 105.6 g ha ⁻¹	100.0134	0.3630	0.0440	0.9950	0.0004
Saflufenacil + Metribuzin 206.4 g ha ⁻¹	99.9723	0.3912	0.2015	0.9848	0.0019
Saflufenacil + Paraquat 20 g ha ⁻¹	100.0050	0.2458	0.0374	0.9969	0.0002
Saflufenacil + Paraquat 40 g ha ⁻¹	100.0293	0.4872	0.3391	0.9934	0.0005

Among the different tested associations, metribuzin was the only herbicide that, associated with saflufenacil, provided a greater SDM reduction than that of saflufenacil alone, when the dose of 105.6 g ha⁻¹ was used (Figure 2B). The application of paraquat at its highest dose (40 g ha⁻¹) associated with saflufenacil showed a very similar behavior to that of saflufenacil alone (Figure 2C). The association of saflufenacil with clomazone at both doses resulted in a lower SDM reduction than that of saflufenacil alone (Figure 2A). This association provided an 85% SDM reduction of *E. heterophylla*, when accompanied by the highest dose of saflufenacil (16.65 g ha⁻¹).

The analysis of the biological behavior of the three herbicides can help in the interpretation of the effects resulting from the associations with saflufenacil. It is possible to state that the associations with the lowest dose of clomazone and metribuzin produced a more pronounced SDM reducing effect than the association with the highest dose of these herbicides. However, unlike the others, when in association with saflufenacil, paraquat was more efficient with the use of the highest dose.

Another aspect to be considered is that the association of herbicides that have low residual action with herbicides that present a longer residual effect allows the development of the crop without the presence of weeds in a longer period, avoiding yield losses related to the interference of weeds with the culture. This is the case of the association saflufenacil + glufosinate (relatively low residual effect) to indaziflam (relatively high residual effect) (Jhala et al., 2013a). In this sense, the interactions of saflufenacil with clomazone and metribuzin are relevant because, in theory, they reduce the interference of weed species controlled by these herbicides for a longer period of time.

The validations of the interactions, performed by the Limpel-Colby method (Colby, 1996), identified that all combinations of saflufenacil with clomazone, metribuzin and paraquat presented synergism for shoot dry matter (SDM), regardless of the used doses (Table 2). This means that the SDM reduction resulting from the interactions between herbicides exceeded the results estimated by the method.

Interactions of saflufenacil with other oxidative stress-generating herbicides have already been evaluated in the *Alternanthera tenella* species, for which the association with metribuzin was more efficient compared to other associations and to saflufenacil alone (Trezzi et al., 2016).



In agreement with this experiment, paraquat in association resulted in considerable SDM reductions, with an emphasis on the highest dose. The associations with clomazone presented lower efficiency compared to saflufenacil alone and associated with metribuzin or paraquat. Similarly to this experiment, all associations appeared to be synergistic by the Limpel-Colby method as for the SDM variable.

Table 2 - Analysis of the interaction of saflufenacil and clomazone, saflufenacil and metribuzin and saflufenacil and paraquat for the shoot dry matter variable (SDM) of *Euphorbia heterophylla*

Saflufenacil + Clomazone (g)	SDM (%)			Saflufenacil +	SDM (%)		P 5%	Saflufenacil +	SDM (%)		P 5%
	Exp.	Obs.		Metribuzin (g)	Exp.	Obs.	1 3/0	Paraquat (g)	Exp.	Obs.	1 3/0
1.2 + 288	89.0	50.4	*	1.2 + 105.6	90.0	26.0	*	1.2 + 20	69.8	30.7	*
2.32 + 288	88.9	30.1	*	2.32 +105.6	90.1	15.4	*	2.32 + 20	69.6	26.8	*
4.47 + 288	86.9	23.3	*	4.47 + 105.6	87.9	14.9	*	4.47 + 20	64.7	20.4	*
8.63 + 288	86.7	19.7	*	8.63 + 105.6	87.5	12.5	*	8.63 + 20	64.0	22.8	*
16.65 + 288	86.4	15.3	*	16.65 + 105.6	87.0	12.9	*	16.65 + 20	63.0	18.5	*
1.2 + 504	59.8	39.9	*	1.2 + 206.4	57.9	29.6	*	1.2 + 40	43.7	32.7	*
2.32 + 504	59.9	29.2	*	2.32 + 206.4	57.9	33.6	*	2.32 + 40	43.5	29.9	*
4.47 + 504	53.6	27.3	*	4.47 + 206.4	51.1	24.6	*	4.47 + 40	34.1	17.1	*
8.63 + 504	52.4	26.0	*	8.63 + 206.4	50.0	13.5	*	8.63 + 40	32.7	19.1	*
17.65 + 504	50.7	14.7	*	16.65 + 206.4	48.4	16.4	*	16.65 + 40	30.6	15.1	*

Abbreviations: Exp. Expected; Obs: Observed. * Significant at 5% probability according to the t test.

Studies investigating the interaction of saflufenacil with glyphosate (EPSP inhibitor) were performed on several species, and the results varied between additivity and synergism. For the *Brachiaria decumbens* species, an additive effect was found among these herbicides (Queiroz et al., 2014). For the *Setaria pumila*, *Bidens alba*, *Richardia brasiliensis* and *Eupatorium capillifolium* broadleaf species, a residual additive effect was observed for the mixture of saflufenacil with glyphosate and glufosinate (Jhala et al., 2013b). However, for *Conyza canadensis* (Waggoner et al., 2011), *Conyza bonariensis* (Dalazen et al., 2015) and *Ipomoea hederifolia* (Agostineto et al., 2016), the association of these herbicides was synergistic. According to Dalazen et al. (2015), the systemic characteristic of saflufenacil can explain the synergism observed in their work evaluating the association of saflufenacil with glyphosate to control the glyphosate-resistant biotype of *Conyza bonariensis*. As for the association of saflufenacil with imazethapyr (ALS inhibitor) to control red rice (*Oryza sativa*), additivity was observed (Camargo et al., 2011), a result that was also observed in relation to the association of saflufenacil and sethoxydim to control *Setaria pumila* and *Bidens bipinnata* (Jhala et al., 2013a).

In a study with the species *Chenopodium album* and *Datura stramonium*, it was possible to observe for the mixture acifluorfen (Protox inhibitor) + bentazon (photosystem II inhibitor), synergism to the first species and antagonism for the second species (Green, 1989). This demonstrates that the response of the interaction depends on the target species. In addition to the target species, the result of the interaction depends on the herbicides, being associated with the applied dose (Wesley and Shaw, 1992; Unland et al., 1999; Beyers et al., 2002). It is important to emphasize that the methodology used to analyze the interaction response may also influence the result. The methodology adopted by Limpel-Colby (Colby, 1967) is used in many works involving interactions between agrochemicals, but it is considered less efficient in comparison with the isobolograms method (Streibig et al., 1998). This method, although considered more efficient in the interpretation of the interactions between agrochemicals, involves more complex experiments, with a large number of experimental units and, therefore, a higher cost.

The results of this work and another one analyzing the interaction between herbicides with similar modes of action through the shoot dry matter variable (Trezzi et al., 2016) indicates synergism as a common trend. In the compilation of results about herbicide interactions, Zhang et al. (1995) conclude that the possibility of synergism or antagonism in the association of

herbicides with different mechanisms of action is the same. Furthermore, they indicate a certain synergism trend between herbicides that have similar chemical families. However, for Streibig (1981), the similarity between action modes of herbicides in association does not indicate possible synergism.

Among the herbicides associated with saflufenacil, those including metribuzin at the dose of 105.6 g ha⁻¹ provided more promising results, standing out because they resulted in greater reductions of the SDM of wild poinsettia plants than the application of saflufenacil alone. All combinations of saflufenacil with clomazone, metribuzin and paraquat were synergistic by the Limpel-Colby method.

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