PLANTA DANINHA

SOCIEDADE BRASILEIRA DA CIÊNCIA DAS PLANTAS DANINHAS

<http://www.sbcpd.org>

ISSN 0100-8358 (print)
1806-9681 (online)

Article

Bioassay for Determining Persistence of the Herbicide Saflufenacil in an Oxisol

Bioensaio para Determinação da Persistência do Herbicida Saflufenacil em Latossolo

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ABSTRACT - Persistence of an herbicide is defined as the ability to maintain the integrity of its molecule and chemical, physical, and phytotoxic properties in the environment where it was applied. Knowledge of persistence has implications both for the toxicity generated on species grown in succession and for the period in which the herbicide controls weeds. This study aimed to assess the persistence of the herbicide saflufenacil in an Oxisol by means of bioassays. Two experiments with the application of 29.4 g ha^{-1} of saflufenacil were carried out under field conditions: the first in the 2011/2012 season and the second in the 2012/2013 season. Soil samples were collected at depths of up to 10 cm at different times after application. Subsequently, two bioassays were carried out in a greenhouse by using beet (*Beta vulgaris* L.) and cucumber (*Cucumis sativus* L.) as bioindicators. The studies were carried out in a completely randomized design with four replications. Treatments consisted of periods after saflufenacil application (0, 5, 10, 15, 25, 35, 50, and 100 days). The crop was also considered as a factor. Stand, height, and phytotoxicity of plants were assessed in both agricultural seasons at 14, 21, and 28 days after sowing (DAS), as well as shoot fresh (SFM) and dry matter (SDM) at 28 DAS. In general, saflufenacil persistence was between 25 and 35 days. Beet was more sensitive to the presence of saflufenacil in the soil when compared to cucumber. Herbicide effects were higher in the 2012/2013 season when compared to the 2011/2012 season.

Keywords: PROTOX inhibitor, *Cucumis sativus* L., *Beta vulgaris* L., residual effect.

*RESUMO - A persistência de um herbicida é definida como a habilidade para manter a integridade da molécula e suas propriedades químicas, físicas e fitotóxicas no ambiente onde ele foi aplicado. O conhecimento da persistência tem implicações tanto sobre a toxicidade gerada sobre espécies cultivadas em sucessão quanto sobre o período em que o herbicida exerce controle de plantas daninhas. O objetivo deste estudo foi avaliar a persistência do herbicida saflufenacil em Latossolo Vermelho distroférrico por meio de bioensaios. Dois experimentos com aplicação de 29,4 g ha^{-1} de saflufenacil foram realizados em campo: o primeiro na safra 2011/2012 e o segundo na safra 2012/2013, de onde foram coletadas amostras de solo na profundidade de até 10 cm, em períodos distintos após a aplicação. Posteriormente, foram realizados dois bioensaios em casa de vegetação, utilizando as plantas bioindicadoras de beterraba (**Beta vulgaris** L.) e pepino (**Cucumis sativus** L.). Os estudos foram efetuados em delineamento inteiramente casualizado com quatro repetições. Os tratamentos foram constituídos pelos períodos de tempo após a aplicação de saflufenacil (0, 5, 10, 15, 25, 35, 50 e 100 dias). Considerou-se também a espécie como fator. Foram avaliados o estande de plantas, a altura e fitotoxicidade aos 14, 21 e 28 dias após a semeadura (DAS) e a massa da parte*

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Received: April 4, 2017
Approved: August 4, 2017

Planta Daninha 2019; v37:e019178636

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Doi: 10.1590/S0100-83582019370100052

aérea das plantas verdes (MPAV) e secas (MPAS) aos 28 DAS, em ambas as safras. De forma geral, a persistência do herbicida saflufenacil situou-se entre 25 e 35 dias. A beterraba foi mais sensível à presença de saflufenacil no solo do que o pepino. Os efeitos do herbicida foram superiores na safra de 2012/2013, em comparação à de 2011/2012.

Palavras-chave: inibidor da PROTOX, *Cucumis sativus* L., *Beta vulgaris* L., efeito residual.

INTRODUCTION

Currently, chemical control is the most commonly used method to control weeds in agricultural areas. The herbicide behavior in the environment, is essential to explain its efficiency on weeds, toxicity to cultivated plants, and impact on the environment, although it is a subject rarely explored in research conducted in Brazil. The application of herbicides with residual effect on the soil can cause damages to subsequent crops, being an important problem in intensive production systems, where two or more crops are harvested per year (Oliveira and Brighenti, 2011).

In soil, herbicides are subject to retention processes (sorption and adsorption), transformation (decomposition and degradation), transport (absorption, drift, volatilization, leaching, and runoff), and absorption by weeds and/or cultivated plants, determining their behavior and persistence (Spadotto, 2002). These processes are influenced by soil physical, chemical and biological characteristics (pH, organic matter, texture and mineralogy, temperature, and moisture) and the applied dose (Spadotto, 2002). Persistence can be defined as the herbicide ability in maintaining the molecule integrity, as well as its physical, chemical, and phytotoxic characteristics in the environment (Guimarães, 1987).

Testing with bioindicator plants is a technique used to identify and quantify the presence of an herbicide in the soil. Among the desirable characteristics of bioindicator species for herbicide bioassays are their ease of cultivation, accelerated development, and high sensitivity to the assessed herbicide (Nyffeler et al., 1982; Souza, 1999). Plant species differ among each other regarding their sensitivity to a particular herbicide, and the degree of sensitivity of a species varies among different herbicides. For this reason, assays are generally carried out to determine the most adequate species for bioassays. Diesel et al. (2012) determined the sensitivity of cucumber, watermelon, pumpkin, the hybrid zucchini Clarita, and beet to the herbicide saflufenacil and observed that cucumber and beet presented adequate characteristics for detecting saflufenacil since they showed a higher sensitivity to its presence in the soil.

Other bioassays have already used cucumber and beet species as bioindicators of the presence of herbicides in the soil (Blanco and Velini, 2005; Blanco et al., 2010; Guerra et al., 2011; Monquero et al., 2012; Diesel et al., 2012). For instance, sulfentrazone persistence (PROTOX inhibitor such as saflufenacil) in soils cultivated with soybean was assessed with sorghum (*Sorghum bicolor*) and beet (*Beta vulgaris*), showing that beet was more sensitive and hence preferential for bioassays of persistence in the soil (Blanco and Velini, 2005). The behavior of the herbicide sulfentrazone in a sandy clay loam soil cultivated with sugarcane was also assessed using beet as a bioindicator species (Blanco et al., 2010).

Saflufenacil is an herbicide developed for controlling dicotyledonous species in pre-emergence, incorporated pre-planting or post-emergence in crops such as sugarcane, corn, wheat, soybean, and cotton. This herbicide has a low volatilization (vapor pressure of 2.0×10^{-14} mmHg at 25 °C). It is a moderate acid, with a pKa of 4.3, water solubility of 30 mg L^{-1} at pH 5.0 and 2,100 mg L^{-1} at pH 7.0. The half-life ($t_{1/2}$) of saflufenacil is between 7 and 35 days (Basf Agricultural Products, 2008).

A study developed by Rahman et al. (2014) aiming at quantifying the residual activity of saflufenacil at a dose of 17 g a.i. ha^{-1} in the soil found that the herbicide persisted in the soil for less than 14 days for the sensitive species clover, onion, carrot, and radish. In an experiment carried out in an Oxisol, 90 days after saflufenacil application in a soil under water restriction was necessary for cucumber toxicity levels to be reduced up to 8% (Monquero et al., 2012).

Saflufenacil application in the desiccation process of weeds in pre-sowing might present a risk for the implantation of sensitive crops. Many bean cultivars have shown sensitivity to this herbicide when applied in pre-emergence, even at a dose below that recommended (Diesel et al., 2014).

The behavior of saflufenacil in the environment is still little studied and there is a need for more information about the persistence of this herbicide in order to avoid damages in sensitive crops cultivated after its application. The aim of this study was to assess the persistence of the herbicide saflufenacil in an Oxisol (Latossolo Vermelho distroférrico, Brazilian Soil Classification System) by means of bioassays.

MATERIAL AND METHODS

Two experiments were installed, the first in the 2011/2012 season (January 20, 2012) and the second in the 2012/13 season (October 11, 2012), in a randomized block design with four replications. In both experiments, the herbicide saflufenacil (29.4 g a.i. ha^{-1}) was applied in an area cultivated with bean. Based on these field experiments, bioassays were performed in a greenhouse, where treatments consisted of eight periods of soil collection from the persistence studies developed in the field and from soil obtained in an area where the herbicide had not been applied (control). The soil in which the herbicide was applied is classified as an Oxisol (Latossolo Vermelho distroférrico, Brazilian Soil Classification System) (Embrapa, 2006), whose characteristics are presented in Table 1. The herbicide was applied with a CO_2 pressurized costal sprayer with nozzles 110.03 and a flow rate of 200 L ha^{-1}. The environmental conditions at application time season were air temperature of 25-28 and 26-29 °C, relative air humidity of 68-72 and 69-72%, and wind speed of 1.4-1.5 0.9-1.2 m s^{-1} in the 2011/2012 and 2012/2013 seasons, respectively.

Table 1 - Particle size distribution and soil chemical attributes of the Oxisol used in the experiments

Particle size distribution		Chemical attribute	
Component	%	Component	Value/Unit
Clay	55.7	OM[(1)]	49.50 g dm^{-3}
Sand	3.0	P_2O_5[(1)]	14.32 mg dm^{-3}
Silt	41.3	K_2O[(3)]	0.70 $cmol_c$ dm^{-3}
CEC[(4)]			17.63
pH[(5)]			5.6
H+Al[(6)]			5.35 $cmol_c$ dm^{-3}

[(1)] Organic matter; [(2)] Phosphorus; [(3)] Potassium; [(4)] Cation exchange capacity; [(5)] Soil pH; [(6)] Exchangeable acidity.

For each agricultural season, two bioassays were performed in a greenhouse, one of them with cucumber and the other with beet as bioindicator plants, using samples collected in the field experiment. Soil samples were collected at a depth of up to 10 cm, placed in pots with a volume of 0.3 dm^3, and stored in a freezer at -5 °C until the bioassays were performed.

The experimental design of the bioassays was a completely randomized design with four replications. Treatments consisted of periods of soil samples collected in both agricultural seasons on the day of saflufenacil application and at 5, 10, 15, 25, 35, 50, and 100 days after application (DAA), in addition to a control without herbicide application.

Soil samples were thawed 24 hours before starting the bioassays. A completely randomized design with four replications was used. Each replication was composed of four soil samples, totaling 16 samples for each treatment. These simple samples were homogenized and the soil was placed in eight pots with a capacity of 0.3 dm^3, with four replications for each studied species.

The bioindicator species cucumber and beet were previously selected among five species that produced the best results for detection of saflufenacil in contaminated soils (Diesel et al., 2012). For both species, three seeds were sown per pot at a depth of 1-2 cm. Pots were manually

watered daily in order to maintain the soil close to field capacity throughout the experimental period.

Stand, height, and phytotoxicity of plants were assessed at 21 and 28 days after sowing (DAS), as well as shoot fresh (SFM) and dry matter (SDM) at 28 DAS. The stand was determined by counting the number of plants surviving in the pots. Height was determined with a ruler graduated in millimeters by measuring the distance between plant base and the end of the last leaf. Phytotoxicity to bioindicator species was determined based on a visual scale of toxicity caused by herbicides proposed by Frans et al. (1986), where 0 represents the absence of effects or no toxicity and 100 represents the complete effect, i.e. the death or total destruction of the crop. For SFM, plants were cut close to the soil and weighed. Subsequently, these plants were dried in an oven at 60 ºC until constant weight for SDM determination. The data of all the variables were relativized, except for phytotoxicity, and the control without herbicide was considered as 100%.

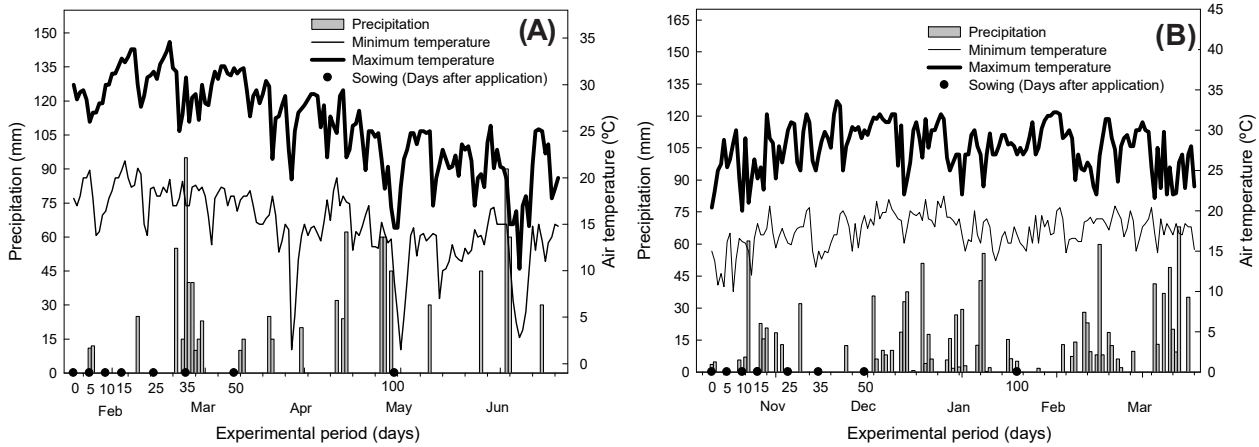
The dissipation constant (K) was obtained from the setting of a decreasing two-parameter exponential equation (f = a x exp(– x x)), being the parameter a the intercept on the ordinate axis and b, also referred to as k, the herbicide dissipation constant. The quotient between the decay constant (0.69315) and K generates the half-life values for each assessed response variable ($t_{1/2}$ = 0.693/k).

The results were submitted to the joint analysis of variance and when the interaction between factors was significant (agricultural season, bioindicator species, and period of soil sampling), the necessary slicing was performed conducted and the means were compared by Tukey's test ($p<0.05$) using the software Winstat (Machado and Conceição, 2005). The graphs were constructed using the software SigmaPlot 10.0. The data of response variables were adjusted through logistic equations.

RESULTS AND DISCUSSION

The analysis of the climatic data of both experimental periods showed higher temperatures in the 2011/2012 season when compared to the 2012/2013 season, mainly up to approximately 50 DAA of saflufenacil (Figure 1). In the 2012/2013 season, precipitations presented a lower intensity, but with a good distribution throughout the experimental period, up to 100 DAA of saflufenacil.

The analysis of variance for all studied response variables showed significant interactions at 5% level for the investigated factors. The data on the stand, height, phytotoxicity, SFM, and SDM were adjusted to the three- and four-parameter logistic equation and presented R^2 values between 0.66 and 0.99.



Sources: Precipitation (Experimental Area of the Universidade Tecnológica Federal of Paraná); Minimum and maximum temperatures (Meteorological Station of the Universidade Technológica do Paraná, Campus of Pato Branco).

Figure 1 - Daily precipitation and minimum and maximum air temperatures in the 2011/2012 (A) and 2012/2013 (B) seasons. Points marked on the abscissa axis indicate the periods of soil sample collection at each season.

In the assessment performed at 21 DAS of bioindicator species, no reduction was observed in plant stand in relation to the control in samplings performed from 25 DAA (Figure 2A and Table 2). In the assessment carried out at 28 DAS, a higher beet sensitivity to saflufenacil was observed when compared that of cucumber from the day of application to approximately 15 DAA (Figure 2B).

Plant height (Figure 3) was also negatively influenced by saflufenacil in the soil. In the 2012/2013 season, in the assessment performed at 21 DAS (Figure 3A), a higher reduction in

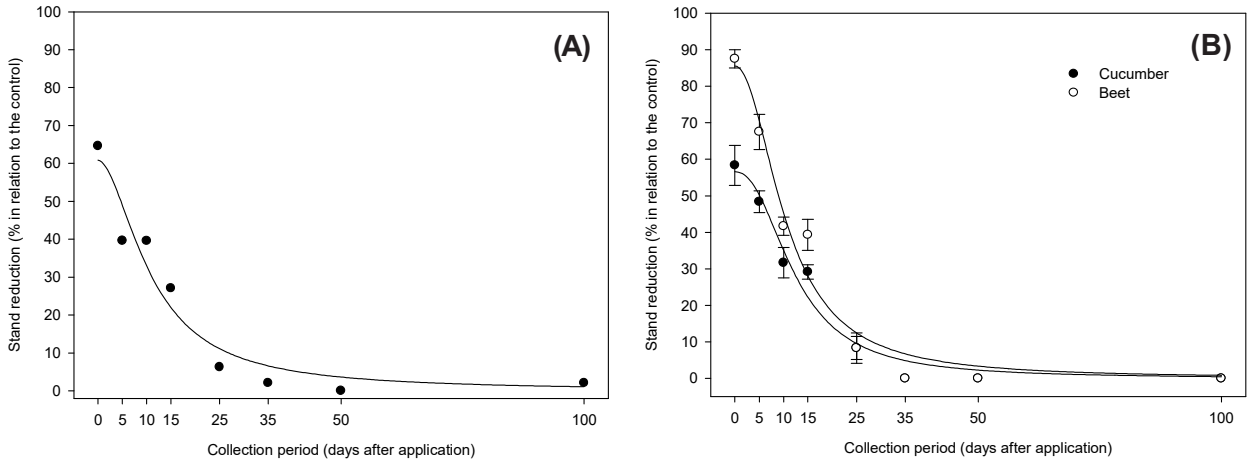


Figure 2 - Reduction of the plant stand for the average of agricultural seasons at 21 days after sowing of the bioindicator species (A) and for two bioindicator species at 28 DAA (B) in response to periods after saflufenacil application.

Table 2 - Summary of the values of persistence, dissipation constant, and estimated half-life as a function of the response variable, assessment period, and target species

Variable		Time required for absence of herbicide action (days)	K	Half-life (t½)
Stand 21 DAS		35	0.0654	10.6
Stand 28 DAS	Cucumber	35	0.0596	11.6
	Beet	35	0.0686	10.1
Height 21 DAS	Season 2011/12 Cucumber	25	0.0835	8.3
	Season 2011/12 Beet	50	0.0519	13.4
	Season 2012/13 Cucumber	35	0.0146	47.5
	Season 2012/13 Beet	50	0.0119	58.2
Height 28 DAS	Season 2011/12 Cucumber	35	0.0492	14.1
	Season 2011/12 Beet	35	0.0605	11.5
	Season 2012/13 Cucumber	25	0.0677	10.2
	Season 2012/13 Beet	50	0.0362	19.1
Phytotoxicity 21 DAS	Season 2011/12 Cucumber	25	0.0442	15.7
	Season 2011/12 Beet	25	0.0907	7.6
	Season 2012/13 Cucumber	25	0.0676	10.3
	Season 2012/13 Beet	25	0.0475	14.6
Phytotoxicity 28 DAS	Season 2011/12 Cucumber	25	0.0625	11.1
	Season 2011/12 Beet	25	0.1412	4.9
	Season 2012/13 Cucumber	25	0.0067	10.3
	Season 2012/13 Beet	25	0.0331	20.9
Fresh matter	Season 2011/12	25	0.0372	18.6
	Season 2012/13	25	0.0148	46.8
Fresh matter	Cucumber	25	0.0324	21.4
	Beet	25	0.0249	27.8
Dry matter	Season 2011/12	35	0.0183	37.9
	Season 2012/13	50	0.0180	38.5
Dry matter	Cucumber	25	0.0234	29.6
	Beet	35	0.0017	59.2

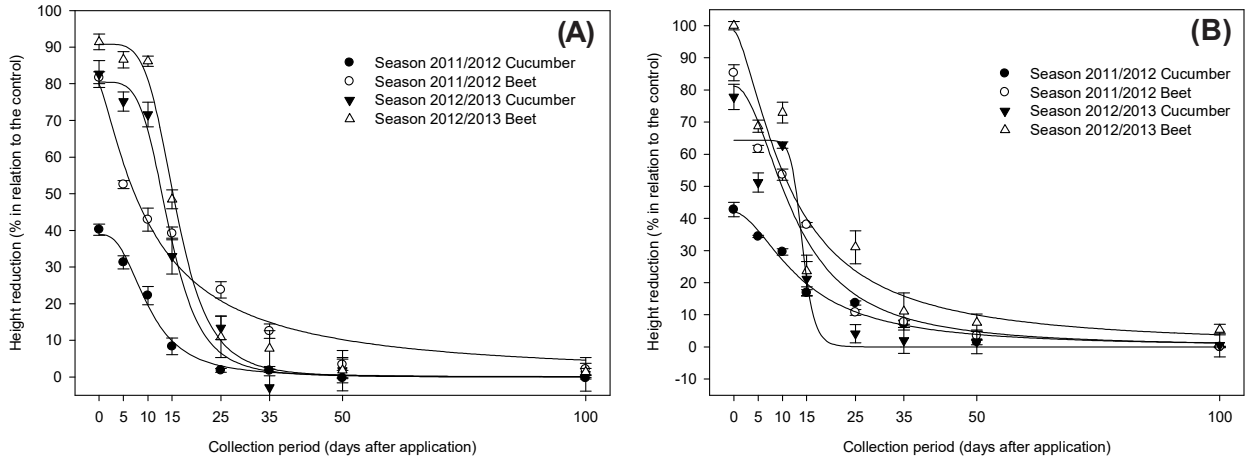


Figure 3 - Reduction of plant height for the 2011/2012 and 2012/2013 seasons at 21 (B) and 28 (C) days after sowing in response to periods after saflufenacil application.

height was observed for both species and a higher sensitivity of beet to saflufenacil when compared to the 2011/2012 season. At 21 and 28 DAS (Figures 3A and B), saflufenacil negatively influenced plant height up to 35 DAA in both seasons, period from which no difference was observed in relation to the control (Table 2).

In general, phytotoxicity assessments showed a higher sensitivity of beet to saflufenacil when compared to cucumber. These species differences were more pronounced in the 2011/2012 season than in 2012/2013 (Figure 4A and B). Beet and cucumber plants indicated toxicity by saflufenacil in the soil up to 25 DAA, with no differences in relation to the control from 35 DAA (Figure 4A, B and Table 2).

In the 2012/2013 season, the use of saflufenacil resulted in a higher reduction of SFM and SDM up to 10 DAA when compared to the 2011/2012 season (Figures 5A and 6A). The reductions of SFM in beet and cucumber in the soil samples collected on the day of saflufenacil application were 98 and 64%, respectively (Figure 5B), confirming a higher sensitivity of the beet to the herbicide, as previously mentioned for other variables. For both bioindicator species, periods of 25 and 35 DAA were required for SFM and SDM, respectively, to match the control (Figures 5B and 6B and Table 2).

In general, the bioassays detected a residual effect of saflufenacil in the Oxisol between 25 and 35 DAA, although values up to 50 DAA were found for some response variables (Table 2). Several factors interfere with the persistence of an herbicide in the soil such as dose, and

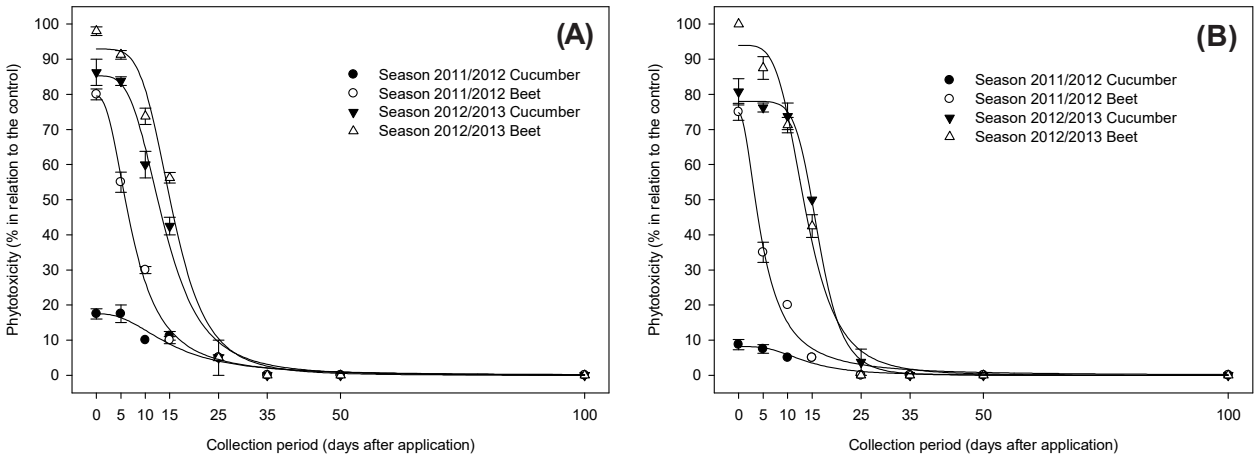


Figure 4 - Toxicity to beet and cucumber plants in the 2011/2012 and 2012/2013 seasons at 21 (B) and 28 (C) days after sowing in response to periods after saflufenacil application.

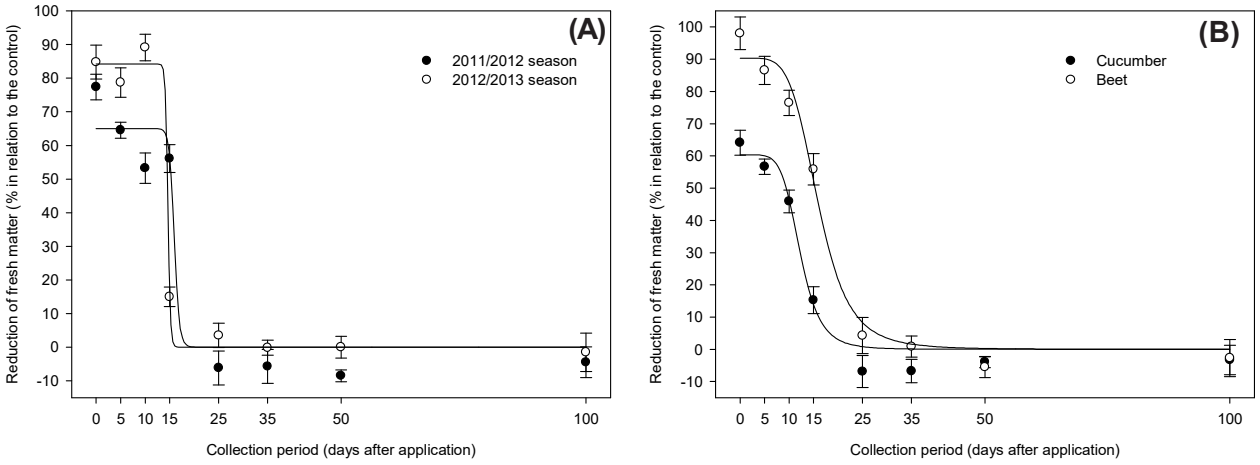


Figure 5 - Reduction of shoot fresh matter in two agricultural seasons (A) and two target-species (B) at 28 days after sowing in response to periods after saflufenacil application.

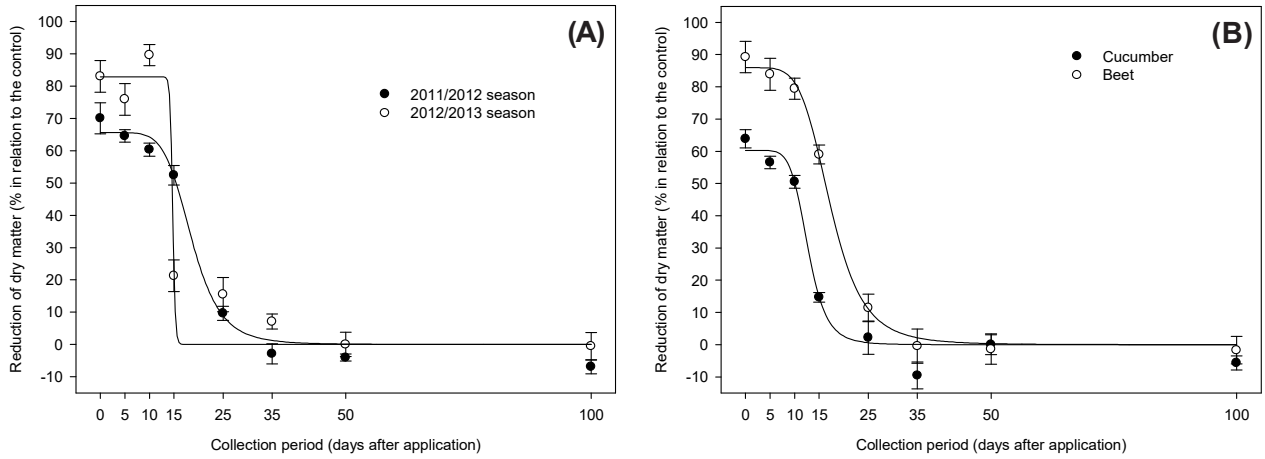


Figure 6 - Reduction of shoot dry matter in two agricultural seasons (A) and two target-species (B) at 28 days after sowing in response to periods after saflufenacil application.

climatic conditions, and soil physicochemical and biological characteristics (Camargo et al., 2013; Rahman et al., 2014).

The amplitude of half-life of saflufenacil in the Oxisol, estimated by the two-parameter exponential equations, ranged from 4.9 to 59.2 days (beet phytotoxicity at 28 DAA and shoot dry matter of beet, respectively) (Table 2). For shoot fresh and dry matter, the saflufenacil half-life was more pronounced for beet, demonstrating its higher sensitivity to this herbicide in the soil when compared to cucumber. Therefore, the time required for the absence of herbicide action is positively correlated with its half-life (Table 2).

Applications of 17 and 102 g a.i. ha^{-1} of saflufenacil in a silt-peaty soil with 9% of organic matter in New Zealand resulted in a residual effect of 14 and 28 days, respectively (Rahman et al., 2014), being in accordance with the results found in our study. In an experiment carried out in Brazil in an Oxisol, 90 days after saflufenacil application in a soil under water restriction was necessary for cucumber toxicity levels to be reduced up to 8% (Monquero et al., 2012), which is very higher than the persistence detected in our experiment. In this case, the absence of moisture reduces soil microbial activity and hence saflufenacil biodegradation (Oliveira Jr et al., 2006).

Soil organic matter contents are also related to herbicide persistence. The assessment of saflufenacil bioactivity in soils with different characteristics showed a higher herbicide bioavailability (indicated by the growth of the bioindicator species) in soil with low content (1.5%) and lower bioavailability in soil with high organic matter content (4%) (Gannon et al., 2014). The

organic matter in this study was 4.9% (Table 1), which may have influenced the lower availability of saflufenacil in the soil (maximum persistence of 50 days).

In general, saflufenacil efficacy on bioindicator species was higher in the 2012/2013 season when compared to the 2011/2012 season. This may be due to lower temperatures in the 2012/2013 season, mainly up to 50 days after herbicide application, when soil sample collections were concentrated. Low temperatures lead to a reduction in the biological degradation process of herbicides (Alletto, 2010). In addition, poorly distributed and more intense precipitation in the 2011/2012 season may have resulted in a higher leaching of saflufenacil when compared to the 2012/2013 season, removing the herbicide from the area where the emergence zone of weed seeds is concentrated.

Considering the assessed variables, saflufenacil persistence in the soil in the bioassay with beet varied from 25 to 50 DAA and from 25 to 35 DAA in the bioassay with cucumber (Table 2), which shows a higher sensitivity of the beet to saflufenacil in the soil. These results are in accordance with the studies carried out by Diesel et al. (2012), who assessed the sensitivity of five cultivated species to saflufenacil and detected a higher sensitivity of beet followed by cucumber among five tested species.

Species used in bioassays should be able to detect small amounts of herbicide in the soil. Considering the dataset, beet is more adequate to detect saflufenacil persistence in the soil when compared to cucumber since it is able to indicate with more precision a reduction in the development and detect saflufenacil presence for a longer period. Tolerance differences among cultivated species to herbicides are most often associated with the differential capacity of metabolizing the molecule, converting it into a less toxic metabolite for the plant (Merotto Jr and Vidal, 2001). However, other mechanisms may determine the differential tolerance among species, such as a reduced absorption or translocation, altered site of action, herbicide compartmentalization, among others.

PROTOX inhibitor herbicides present selectivity to cultivated species by means of minimal herbicide absorption and translocation, herbicide sequestration or even overexpression of the mitochondrial PROTOX enzyme, thereby reducing the excess protoporphyrinogen in the cytoplasm (Higgins et al., 1988; Matsumoto et al., 1999; Warabi et al., 2001). Soybean tolerance to the herbicides acifluorfen and flumiclorac is due to their lower absorption and translocation and high detoxification, whereas for corn a reduced leaf retention of flumiclorac and its high metabolization occurs (Ritter and Coble, 1981; Fausey and Renner, 2000).

There are indications that the mechanism of tolerance to saflufenacil is related to the presence of compounds that act in the inactivation of protoporphyrinogen IX, such as dithiothreitol, beta-mercaptoethanol, and ascorbic acid, i.e. selectivity occurs basically by the metabolization of herbicide molecule (Jacobs et al., 1996; Grossmann et al., 2010).

Thus, stand, height, and shoot dry matter of plants were the variables most sensitive to saflufenacil and phytotoxicity and shoot fresh matter were the variables with the lowest detection capacity of saflufenacil in the soil (Table 2). The sensitivity of the variable to detect changes in plant development in response to herbicide presence may be related to characteristics of the instrument used in the assessment, such as the level of detail of the scales used (phytotoxicity), or to the morphological characteristics of plants that allow characterizing with a higher or lower precision the reduction in development. For instance, the degree of detail of a phytotoxicity assessment scale may consider several characteristics, such as height reduction, plant growth atrophy, chlorosis and necrosis of tissues, among other symptoms. The higher the degree of detail is, the lower subjective the assessment becomes. Height determination may be more appropriate for some bioindicator species than for others. The use of species with a higher lateral development in detriment to those with a higher height development can make it difficult to characterize the herbicide effects if only plant height is measured.

In general, bioassays of saflufenacil persistence in an Oxisol present important information on the behavior of this herbicide in the soil and provide data for future researches. The results allowed concluding that saflufenacil application in desiccation would require a minimum period of 25 to 35 days before sowing sensitive crops such as potato, sunflower, cotton, bean, among others, although more detailed studies are needed for each of these species. These results

are supported by the need for a minimum interval of 25 days for the implantation of sensitive bean cultivar after weed desiccation with saflufenacil (BASF, 2016; Diesel et al., 2016).

In Brazil, saflufenacil is currently registered in the pre-emergence modality for use in rice, corn, and sugarcane (Agrofit, 2016) due to their tolerance. The use of crops with a higher tolerance to saflufenacil after desiccation or when it is necessary to introduce crops in a short time interval in an area where saflufenacil was applied brings a higher security, being a strategy recommended to farmers. In addition, it is necessary to investigate the behavior of other crops without register for saflufenacil, but with tolerance mechanisms, allowing their introduction in systems of rotation or succession.

Therefore, saflufenacil persistence in an Oxisol is between 25 and 35 days after its application. In addition, beet is more sensitive to saflufenacil in the soil when compared to cucumber and may be used in bioassays to detect this herbicide in the soil.

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