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Management of *Sagittaria montevidensis* resistant to ALS and PSII mechanisms of action with saflufenacil associated with different adjuvants[1]

Manejo de ***Sagittaria montevidensis*** *resistente aos mecanismos de ação da ALS e do FSII com saflufenacil associado a diferentes adjuvantes*

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Abstract - This study aimed at evaluating the effective rates of saflufenacil and adjuvants for controlling *Sagittaria montevidensis* (sagitaria) populations resistant to ALS and photosystem II (PS II) inhibitor herbicides, and phytotoxicity to the Epagri 108 rice cultivar. Two biotypes of sagitaria (SAGMO) were tested: SAGMO 10 – with cross resistance to ALS inhibitor herbicides, and SAGMO 32 - with multiple resistance to ALS and PSII inhibitor herbicides. Treatments consisted of three different saflufenacil rates: 75; 112.5 and 150 g a.i. ha^{-1} with the addition of adjuvants Dash HC® (0.5% v/v); Assist® (1% v/v); Iharaguen-S® (0.5% v/v) or Veget'Oil® (1 L ha^{-1}). Evaluation focused on arrowhead control, phytotoxicity and shoot dry weight (DMAP) of the Epagri 108 rice cultivar. The saflufenacil rates of 75 to 150 g a.i. ha^{-1} did not provide efficient control for both sagitaria biotypes. Addition of the adjuvants Veget'Oil®, Assist® or Iharaguen-S® to saflufenacil did not alter the control efficiency of herbicide resistant sagitaria populations. The Epagri 108 cultivar presented selectivity to saflufenacil until 150 g a.i. ha^{-1}, without reducing DMAP. The phytotoxicity caused by saflufenacil, to the rice plants was minimal with the addition of Veget'Oil®. Rice plants shoot dry matter was not affected, regardless of the adjuvant used. Saflufenacil can be used until the rate of 150 g a.i. ha^{-1} associated with adjuvant Veget'Oil® for the management of sagitaria in rice fields, but it does not provide adequate control of biotypes SAGMO 10 and SAGMO 32.
Keywords: *Oryza sativa*; California arrowhead; multiple resistance

Resumo - Objetivou-se com esse trabalho avaliar o efeito de doses de saflufenacil e de adjuvantes no controle de sagitária resistente aos herbicidas inibidores de ALS e do Fotossistema II (FS II), e a fitotoxicidade a cultivar de arroz Epagri 108. Foram utilizados os biótipos de sagitária (*Sagittaria montevidensis*), SAGMO 10 - com resistência cruzada a herbicidas inibidores da ALS, e SAGMO

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32 - com resistência múltipla a herbicidas inibidores da ALS e do FS II. Os tratamentos constituíram-se da aplicação de doses de saflufenacil: 75; 112,5 e 150 g i.a. ha^{-1}; da adição dos adjuvantes Dash HC® (0,5% v/v); Assist® (1% v/v); Iharaguen-S® (0,5% v/v) e Veget'Oil® (1 L ha^{-1}) para cada dose do herbicida aplicado nos biótipos de sagitária. Avaliou-se o controle da sagitária, a fitotoxicidade e a massa seca da parte aérea (MSPA) do arroz. O saflufenacil, nas doses de 75 a 150 g i.a. ha^{-1} não proporcionou controle eficiente dos biótipos de sagitária. A adição dos adjuvantes Veget'Oil®, Assist® e Iharaguen-S® ao saflufenacil não alterou a eficiência de controle de sagitária resistente a herbicidas. A cultivar Epagri 108 apresenta tolerância ao saflufenacil até 150 g i.a. ha^{-1}, não ocorrendo redução da MSPA. A fitotoxicidade ocasionada às plantas de arroz pelo saflufenacil é menor ao se adicionar o Veget'Oil®. A massa seca das plantas do arrroz não foi afetada independente do adjuvante utilizado. O saflufenacil pode ser usado, até a dose de 150 g i.a. ha^{-1}, associado ao adjuvante Veget'Oil® para o manejo de sagitária infestante do arroz cultivar Epagri 108, porém não proporciona controle adequado dos biótipos SAGMO 10 e SAGMO 32.
Palavras-chaves: *Oryza sativa*; sagitária; resistência múltipla

Introduction

The majority of Brazil's rice production, approximately 77%, comes from the southern states of Rio Grande do Sul (RS) and Santa Catarina (SC), cultivated as irrigated rice. During the 2014/15 growing season in Santa Catarina 147.9 thousand hectares were devoted to rice production which yielded a total of 1,057.5 thousand tons. This was the second highest producing state in the country behind RS (CONAB, 2016).

The state of SC grows 90% of its rice in the pre-germinated seeding system, and the field can be flooded around 20 days before sowing. Approximately 20 days after flooding the field, pre-germinated rice seeds are planted into a water blade. This practice of soil preparation and sowing into a water blade creates an advantageous environment for aquatic weeds to develop. *Sagittaria montevidensis* (sagitaria) has adapted to this flooded environment, and is one of the most problematic weeds in flooded rice fields (Cassol et al., 2008), predominantly in crops under the pre-germinated system.

Sagitaria is widely spreaded throughout rice fields in southern Brazil, causing reduction in productivity. The lack of control causes the soil seed bank to increase, contributing to the increment of the population in the next crop (Cassol et al., 2008; Eberhardt et al., 2014).

Chemical control is widely used in rice crops, especially in SC, and at least one application of herbicides is administered during the growing season in virtually every rice field. However, the control of sagitaria has been problematic due to the appearance of biotypes with cross-resistance to enzyme acetolactate synthase - ALS - inhibitor herbicides, and with multiple resistance to ALS and Photosystem II - PSII - inhibitors (Eberhardt et al., 2014). This resistance is largely a result of annual herbicide applications with prolonged residuals, or those without any residual action in the same field and mode of action. Using highly efficient herbicides to control susceptible biotypes or even applying products in high or sub rates can intensify the selection pressure (Mariani et al., 2015).

A possible alternative for the control of resistant sagitaria populations is saflufenacil. Registered in Brazil in 2013, saflufenacil belongs to the pyrimidinedione family inhibiting the protoporphyrinogen oxidase enzyme - PROTOX (Soltani et al., 2009), and it should be applied in the post-emergence growth stage. However, there is still insufficient information on the use of saflufenacil in flooded rice, especially regarding the rate and the adjuvants to be used for the satisfactory control of sagitaria.

According to Mendonça et al. (2007), the use of adjuvants has shown the ability to improve the spray solution by enhancing the protection and absorption of herbicides. The use of adjuvants alters the properties of the spray solution, and it can influence the formation process, the spectrum of the drops, and their behavior in contact with the target (Maciel et al., 2013). Maciel et al. (2011) reported when working with adjuvants Agral®, Silwet®, Nimbus®, Naturl`Oleo® and Ag Bem® that there was significant increase in the control of *Brachiaria decumbens*, *B. humidicola*, *Setaria geniculata* and *Sorghum bicolor*, when associated to contact action herbicides paraquat and MSMA.

In addition, using adjuvants can also minimize the effects on the environment that may compromise the efficiency of a particular herbicide (Cunha & Silva, 2010). However, the primary difficulties of using them comes from the lack of knowledge on their action, and the implications of their control efficiency (Antuniassi, 2006). For example, little is known about adjuvants selectivity to the crops and if using them in conjunction with an herbicide can improve the efficacy of weed control. According to Cunha & Silva (2010), there is an insignificant amount of information on the topic, making it difficult to select or recommend adjuvants for the management of crop weeds.

With that in consideration, this paper focuses on evaluating the effect of saflufenacil and adjuvants rates for the control of sagitaria resistant to ALS and Photosystem II herbicides, and the phytotoxicity to Epagri 108 rice cultivar.

Material and Methods

The experiment was carried out in a greenhouse, in an entirely randomized experimental design, arranged in a 2x3x4 factorial design with four replications. In the first factor, two sagitaria biotypes were tested: SAGMO 10, with cross-resistance to ALS inhibitor herbicides, collected in Itajaí, SC, and SAGMO 32, with multiple resistance to ALS and PSII inhibitor herbicides, collected in Ilhota, SC. In the second factor, the rates of saflufenacil were allocated (75; 112.5 and 150 g a.i. ha^{-1}); and, in the third, adjuvants (Dash HC® (0.5% v/v); Assist® (1% v/v); Iharaguen-S® (0.5% v/v) and Veget'Oil® - 1 L ha^{-1}), applying the same rate of adjuvant in each rate of saflufenacil. A control treatment was also used without the application of herbicide and adjuvants. The application of herbicides was done using a CO_2 pressurized backpack sprayer equipped with a spray boom with four fan-like spray nozzles, series 110.02, spaced 50 cm, calibrated to apply a spray volume of 150 L ha^{-1}. The herbicides were applied when the sagitaria plants presented 10 to 15 cm of height, containing only lance-shaped leaves, and the rice plants were at 3-4 leaf stage (V_3 – V_4). It is important to highlight that, before this experiment, studies were done to confirm the resistance of sagitaria biotypes (SAGMO 10 and SAGMO 32) to ALS and PSII inhibitor herbicides (Moura et al., 2015). The experimental units were composed by plastic pots filled with 4 kg of soil, being identified as Eutrophic Albaqualf, Pelotas Mapping Unit (EMBRAPA, 2013), where 40 seeds of a sagitaria biotype were planted, or 10 seeds of Epagri 108 rice cultivar. It is also important to highlight that the plants of sagitaria and rice were grown in independent experimental units. Twelve hours before herbicide application, the water blade was removed from the experimental units and thinning was done to the plants, keeping eight sagitaria plants and five rice plants per experimental unit. The sagitaria biotypes were submerged right after sowing, keeping a water blade of approximately 5 cm for the whole experiment. For the rice plants, flooding was done whenever necessary.

The variables analyzed were the control of sagitaria and rice injury, at 14 and 28 days after herbicide application (DAH), attributing percentage scores that ranged from zero to 100%, where zero corresponded to no injury symptom and 100% to the death of the plants (SBCPD, 1995). The rice plants shoot dry matter was also assessed at 28 DAH. For that, the plant material collected was submitted to dry in a dryer at 60 ºC, until obtaining constant mass.

The data obtained was analyzed regarding its homoscedasticity and normality, being transformed into:

$$Yt = arcseno\sqrt{(y=0,5)/100}$$

When they did not present normal distribution and, later on, submitted to the variance analysis by the F test. When significant, the Fisher test was done to compare the sagitaria biotypes and the Duncan test to compare the means of herbicide rates and adjuvants, and the control means values were not considered because they were zero. All data was analyzed at a 5% probability, using the Winstat software (Machado et al., 2002).

Results and Discussion

The data referent to the control treatment without the application of herbicide and adjuvants were not considered in the analysis because they were only a reference for the evaluations. There was no interaction between the biotypes, herbicide rates and adjuvants for the variable sagitaria control at 14 and 28 days after herbicide application (DAH). There was statistical difference between biotypes for sagitaria control at 14 DAH (Table 1). Results showed that the maximum control was 70.1% for biotype SAGMO 32, although it was 11% higher than the control of SAGMO 10. The difference in the level of control observed among the biotypes may be attributed to the intrinsic capacity of biotypes in tolerating saflufenacil, an herbicide to which they are susceptible. The inefficient control of the sagitaria biotypes may have been caused by the development stage of the plants (10 to 15 cm) at herbicide application. The herbicide influences plant growth by inhibiting the action of enzymes or preventing the formation of some structure required for multiplication and growth of the cells (Concenço et al., 2007). For example, morphophysiological differences among susceptible and resistant ryegrass biotypes to glyphosate, resulted in a lower water transportation rate from the root to the aerial part of the plants (Concenço et al., 2007). Hamza et al. (2012) concluded that anatomical characteristics in biotypes of barnyardgrass (*E. crusgalli*) resistant to fenoxaprop-ethyl presented remarkable differences in cytology, compared to the susceptible one, in relation to the thickness of the leaf blade and to the diameter of the xylem vessels. This could contribute to the reduced translocation of the product, although this is not among the most probable causes of resistance in that biotype.

Table 1. Control (%) of sagitaria biotypes (*Sagittaria montevidensis*) resistant to herbicides at 14 days after herbicide application.

Biotype	Control (%)
SAGMO 10	58.85 b*
SAGMO 32	70.10 a
CV (%)	13.73

*Means followed by different lowercase letters in the column differ by the Fisher test at 5% probability.

In the literature, there is little information concerning the possible effects about the micromorphological characteristics of herbicide resistant weed biotypes, developed from the resistance mechanisms by the plant (Ferreira et al., 2012). Therefore, it becomes complex to predict how different resistant biotypes of the same species will behave when sprayed with herbicides that this populations may be susceptible.

The results show that the biotype with multiple resistance (SAGMO 32) tends to be less tolerant to saflufenacil than the one with cross-resistance. This can be explained by the fact that, in order to a biotype to develop resistance to an herbicide it must expend energy. Due to this, the biotype resistant a certain herbicide becomes more susceptible when applied with herbicides that control though a different mechanism of action. Thus, the biotype with multiple resistance to ALS and PSII inhibitors presents more susceptibility, when compared with cross-resistance to ALS inhibitors, from herbicides containing different action mechanisms, in this case saflufenacil, because multiple resistance requires greater energy expenditure.

The efficacy of post-emergent herbicides is closely related to the development stage of the weed, especially if these products act on metabolic processes. This makes them efficient on younger plants that produce meristematic tissues (Pinto et al., 2008). Therefore, the herbicides are expected to be more phytotoxic for younger plants that have a great amount of meristematic tissues, yet having little to no activity in older plants where tissues are differentiated. The application of saflufenacil in fleabane plants larger than 15 cm provided a low control level when compared to the application in smaller plants (Constantin et al., 2013).

Regarding the herbicide rates, there was statistical significance in sagitaria control at 14 and 28 DAH (Table 2). The rates of 112.5 and 150 g a.i. ha^{-1} of saflufenacil were more efficient in the control of sagitaria at 14 DAH, and the highest rate presented 5% more control when compared to the lower rate. At 28 DAH, saflufenacil (150 g a.i. ha^{-1}) presented a higher control level than the evaluation at 14 DAH, but it was still inefficient with only 21.88% of sagitaria biotypes controlled. It is worth to highligh that, to be considered efficient, an herbicide rate should present control level at least above 80% (Oliveira et al., 2009). However, none of the rates evaluated provided the desired baseline.

In studies by Eberhardt et al. (2014), for the rice cultivation season of 2009/10 and 2010/11, saflufenacil showed to be efficient, with control above 86% of the sagitaria plants with multiple resistance to ALS and PSII inhibitors, and the variation in rates from 70 to 147 g a.i. ha^{-1} did not interfere in relation to the control level.

Table 2. Control (%) of sagitaria with saflufenacil at 14 and 28 days after herbicide application.

Saflufenacil rates (g a.i. ha^{-1})	Control (%)	
	14 DAH	28 DAH
75	54.63 b*	11.41 b
112.5	67.66 a	13.13 b
150	71.25 a	21.88 a
CV (%)	13.29	25.70

*Means followed by different lowercase letters in the column differ by the Duncan test at 5% probability.

The application of saflufenacil with different adjuvants presented significant control of sagitaria at 14 and 28 DAH (Table 3). Saflufenacil, when applied with Veget'Oil®, presented higher control at 14 DAH in relation to Dash HC®, but it did not differ from Assist® and from Iharaguen-S®. At 28 DAH, saflufenacil, associated with Iharaguen-S®, exhibited a 27% increase of control when compared to the average of the other treatments associated to Veget'Oil® and to Assist®, but it did not differ statistically from them.

Table 3. Control (%) of sagitaria with adjuvants added to saflufenacil at 14 and 28 days after herbicide application.

Adjuvants	Control (%)			
	14 DAH		28 DAH	
Dash HC® (0.5% v/v)	59.38	b*	11.46	b
Assist® (1% v/v)	64.79	ab	14.38	ab
Iharaguen-S® (0.5% v/v)	63.75	ab	19.58	a
Veget'Oil® (1 L ha^{-1})	70.00	a	16.46	ab
CV (%)	16.62		25.98	

*Means followed by different lowercase letters in the column differ by the Duncan test at 5% probability.

The present study showed the importance of choosing the right adjuvant to be applied with saflufenacil so that the level of control of sagitaria is altered according to the product used. According to the technical recommendation for saflufenacil, Dash HC® must be added to the spray solution in post-emergence application. The addition of adjuvants may reduce the tension of the spray drops surface while increasing herbicide retention and distribution in the leaf surface

(Sanyal et al., 2006). The addition of adjuvants to saflufenacil ensured higher efficacy in the control of bindweed when comparing to saflufenacil applied in isolation (Knezevic et al., 2009).

Among the adjuvants, Veget'Oil® was the one that presented a better control (70%, at 14 DAH), corroborating the study done by Knezevic et al. (2009). This study showed that the control of several weed species, with saflufenacil, was greater with the addition of methylated seed oil and crop oil concentrate, in comparison with several adjuvants. The addition of mineral or vegetable oil to the herbicide reduces the solution's pH significantly, resulting in increased efficiency of weed control (Queiroz et al., 2008).

The highest level of sagitaria control by saflufenacil was with Veget'Oil®, and can be attributed to its capacity of dissolving the wax in the structure of the weeds leaves. When the waxes on the leaves become dissolved it eliminates barriers that reduce the absorption of the herbicides and induces leakage of the cell content, increasing absorption of herbicides, reducing drift, delaying the evaporation of the drops while acting as spreader and adhesive. Therefore, increasing the effect of the product on weeds (Maciel et al., 2011).

Phytotoxicity showed no interaction between the rates of saflufenacil and adjuvants at 14 and 28 DAH. There was statistical significance between the herbicide rates in the phytotoxicity to rice plantas at 14 and 28 DAH (Table 4).

The rates used in this study, 75, 112.5 and 150 g a.i. ha^{-1} of saflufenacil, are very close to the recommendation rate for the application in flooded rice, which is 70 to 147 g a.i. ha^{-1}, so they presented low phytotoxicity to cultivar Epagri 108, both at 14 and at 28 DAH. Phytotoxicity was characterized by necrotic spots on sprayed leaves, especially in the first evaluation following the application; however, after this period, the phytotoxicity symptoms disappeared almost completely.

Table 4. Phytotoxicity (%) in Epagri 108 rice plants treated with saflufenacil at 14 and 28 days after herbicide application.

Saflufenacil rates (g a.i. ha^{-1})	Phytotoxicity (%)	
	14 DAH	28 DAH
75	10.93^{ns}	5.31^{ns}
112.5	9.68	4.37
150	9.68	4.00

^{ns} Did not present significant statistical difference.

The application of saflufenacil in rice presented initial phytotoxicity to the plants as informed by the product manufacturer. Later in the growth stage, phytotoxicity was reduced to the point where there was no deficiency in crop productivity. In the present study, the application of saflufenacil to rice plants caused a similar behavior to the one informed by the manufacturer, with high initial phytotoxicity present in the crop, which later was reduced, showing the ability of crop recovery to saflufenacil. In a study carried out by Camargo et al. (2011), the application of saflufenacil in the rate of 50 g a.i. ha^{-1} caused initial injury to rice of 15% at 8 DAH, but decreased to 3% at 24 DAH, resulting in no reduction of crop productivity.

The adjuvants tested in this study exhibited statistical significance in the phytotoxicity of rice at 14 and 28 DAH (Table 5). Saflufenacil, when applied with the adjuvants Veget'Oil® and Iharaguen-S®, displayed slower levels of phytotoxicity at 14 DAH. At 28 DAH, the application of saflufenacil + Veget'Oil® caused negligible injury between the adjuvants and the rice plants, where the symptoms were almost non-existent. It is clear that Veget'Oil® provided better control of sagitaria biotypes, and additionally caused lower phytotoxicity to the plants of cultivar Epagri 108 when applying saflufenacil, compared to the other adjuvants.

Veget'Oil® demonstrated superior characteristics in comparison to the other adjuvants due to its composition in breaking down the glaucous leaf barrier, reducing the size of the drops, providing better vegetable coverage when involving the pesticides molecules forming a thin coat on the leaves providing greater adherence to the sprayed surface and slower evaporation and photolysis rates (AGROFIT, 2016).

Table 5. Phytotoxicity (%) in Epagri 108 plants treated with adjuvants added to saflufenacil at 14 and 28 days after the herbicide application.

Adjuvants	Phytotoxicity (%)			
	14 DAH		28 DAH	
Dash HC® (0.5% v/v)	11.67	a*	5.83	a
Assist® (1% v/v)	10.41	ab	5.41	a
Iharaguen-S® (0.5% v/v)	9.16	b	4.58	a
Veget'Oil® (1 L ha^{-1})	9.16	b	2.92	b
CV (%)	12.71		27.79	

*Means followed by different lowercase letters in the column differ by the Duncan test at 5% probability.

The importance of choosing the right adjuvant to be used with saflufenacil consists of minimizing phytotoxicity to the crop, so that the injuries suffered by the plants do not reduce grain productivity. There was no interaction between saflufenacil rates and adjuvants for the variable dry shoot biomass of rice plants, both at 14 and 28 DAH, as it also did not have any statistical significance for factors rate and adjuvant (non-presented data).

Conclusions

Saflufenacil applied in the rates of 75 to 150 g a.i. ha^{-1}does not provide appropriate control for biotypes SAGMO 10 and SAGMO 32.

Rice cultivar Epagri 108 presents tolerance to saflufenacil until the rate of 150 g a.i. ha^{-1}, without effect on shoot dry biomass.

The addition of adjuvants Veget'Oil®, Assist® and Iharaguen-S® to saflufenacil, despite improving the effect of the herbicide, it may not provide the recommended the control of sagitaria resistant to herbicides.

The phytotoxicity to rice plants of Epagri 108 by saflufenacil is reduced when adding Veget'Oil®.

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