PLANTA DANINHA

SOCIEDADE BRASILEIRA DA CIÊNCIA DAS PLANTAS DANINHAS

SBCPD

<http://www.sbcpd.org>

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

Article

Is Increasing Doses of Imazapyr + Imazapic Detrimental to the Main Crop Rotation Alternatives to Flooded Rice?

O Aumento da Dose de Imazapyr+Imazapic é Prejudicial para as Principais Alternativas de Rotação de Culturas ao Arroz Irrigado?

ULGUIM, A.R.[1]*
CARLOS, F.S.[2]
ZANON, A.J.[1]
OGOSHI, C.[3]
BEXAIRA, K.P.[1]
SILVA, P.R.F.[4]

ABSTRACT - Imazapyr+imazapic ready mix is the main herbicide used to weed control in Clearfield® Rice System. The continuous use of high doses may increase their residual activity in the soil. The aim of this research was determine the sensitivity of cover crops and soybean in rotation with flooded rice to different levels of imazapyr+imazapic residues in the soil and its effect on soil microbiology. A field experiment was performed during the 2014/15 and 2015/16 seasons. In the first crop season, flooded rice was cultivated using herbicide rates in order to have levels of residue in the soil: 0, 140 (label dosage, equivalent to 73.5+24.5 g a.i. ha^{-1}), 280, 560, 840 and 1,120 g ha^{-1}. After crop harvest, during fall-winter, it was sown ryegrass, white clover and fallow (no soil cover); and in the followed season it was sown soybean in the same area (simulating rotation crop). At that occasion, soil samples were collected to perform soil microbiology analyses of acid phosphatase, β-glucosidase, fluorescein diacetate and urease activity. Growth characteristics on cover crops related to residue levels, and soybean development related to soil residue and cover crops used was evaluated. The results showed that the increase of imazapyr+imazapic doses caused a negative effect on the cover crops evaluated at 75 and 140 days after sowing, mainly at doses greater than twice the label dosage that also reduced the growth and yield of soybean. In general, the enzymes activity in soil decreased as the dose of the imazapyr+imazapic residue increased, showing lower microbiota activity to herbicides degradation.

Keywords: soil residue, ryegrass, white clover, soybean.

*** Corresponding author:**
<andre.ulguim@ufsm.br>

Received: December 9, 2018
Approved: April 16, 2019

Planta Daninha 2019; v37:e019217913

RESUMO - A mistura formulada de imazapyr + imazapic é o principal herbicida usado para o controle de plantas daninhas em arroz no sistema Clearfield®. O uso contínuo de altas doses pode aumentar sua atividade residual no solo. O objetivo deste trabalho foi determinar a sensibilidade de culturas de cobertura e soja em rotação com arroz irrigado a diferentes níveis de resíduo de imazapir + imazapic no solo, bem como seus efeitos na microbiologia do solo. Um experimento de campo foi realizado durante as temporadas 2014/15 e 2015/16. Na primeira safra, o arroz irrigado foi cultivado e utilizaram-se doses do herbicida para formar níveis de resíduo no solo: 0, 140 (dose de bula equivalente a 73,5 + 24,5 g i.a. ha^{-1}), 280, 560, 840 e 1.120 g ha^{-1}. Após a colheita, durante o outono-inverno, foram semeados azevém e trevo-branco e pousio (sem cobertura do solo); e na safra seguinte foi semeada soja na mesma área (simulando rotação de culturas). Na ocasião, amostras de solo foram coletadas para realizar análises microbiológicas da atividade de fosfatase ácida, β-glicosidase, fluoresceína-diacetato e urease. Avaliaram-se as características de crescimento nas plantas de cobertura relacionadas aos níveis

[1] Universidade Federal de Santa Maria (UFSM), Santa Maria-RS, Brasil; [2] Universidade Federal de Pelotas (UFPel), Pelotas-RS, Brasil; [3] Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (EPAGRI), Caçador-SC, Brasil; [4] Instituto Rio Grandense do Arroz (IRGA), Cachoeirinha-RS, Brasil.

Doi: 10.1590/S0100-83582019370100148

de resíduos no solo, assim como o desenvolvimento de soja relacionado aos resíduos e às plantas de cobertura utilizadas. Os resultados mostraram que o aumento da dose de imazapir + imazapic causou efeito negativo nas plantas de cobertura avaliadas aos 75 e 140 dias após a semeadura, principalmente em doses superiores ao dobro da dose de bula, o que também reduziu o crescimento e a produtividade da soja. Em geral, a atividade das enzimas no solo diminuiu com o aumento da dose do resíduo imazapyr + imazapic, mostrando menor atividade da microbiota à degradação dos herbicidas.

Palavras-chave: resíduo no solo, azevém, trevo-branco, soja.

INTRODUCTION

Weeds are one of the main limiting factors of the crop yield in flooded rice (*Oryza sativa* L.) and chemical control is the method most used by the farmers. The weedy rice (*Oryza sativa* L.) is characterized as the main weed that affects the flooded rice (Agostinetto et al., 2001), because it belongs to the same species of cultivated rice (*Oryza sativa*), which hinders the selective chemical control due to morphophysiological similarities. The introduction of rice cultivars using Clearfield® (CL) Technology allowed the control of harmful rice. The ready mixtures of imazethapyr + imazapic (75 + 25 g a.i. L^{-1}, respectively) and imazapyr + imazapic (525 + 125 g a.i. kg^{-1}, respectively) are compounds of the imidazolinone chemical group, and have high performance to control weeds in CL rice, mainly weedy rice (Bundt et al., 2015). However, from the appearance of resistant weedy rice biotypes, the farmers have been using high dosages of imidazolinone herbicides trying, without success, control these plants.

The persistence or residual effect of these herbicides in soil may vary from a few months to years, depending on the product, environment and soil characteristics (Senseman, 2007). The time of activity in soil can be positive when the period is long enough to maintain the crop free from the competition of weeds, but can be undesirable when it results in damage to the crops in a succession or rotation (Pinto et al., 2009a). The negative effect of the imidazolinone residue has been found in the development of maize plants, graniferous sorghum, tomatos and peppers sown in rotation compared to crops that use these herbicides (Alister and Kogan, 2005). There was some reports of fitotoxicity occurrence, reduction of the plant height and/or plant death because imidazolinone residues on flooded rice areas to ryegrass (Pinto et al., 2009a; Santos et al., 2014), non-CL rice (Marchesan et al., 2010; Souto et al., 2015), tomato (Kraemer et al., 2009) and corn (Pinto et al., 2009b).

The sensitivity of some crops supports the need for studies in order to identify the restrictions for the use of several crops (e.g. soybeans) that follow flooded rice in the CL System. For this, the evaluation of imidazolinone carryover to soybean is important because this is the main crop used in rotation with flooded rice in Brazil. The soybean cultivated in rotation with flooded rice has expanded in recent years and already occupies approximately 30% of the fields sown annually with flooded rice in Rio Grande do Sul State, Brazil (Zanon et al., 2016).

The degradation of imidazolinones in a field where flooded rice is produced can be influenced by agricultural practices such as: correction of acid soils, soil drainage and the use of cover plants (Santos et al., 2009). Soils cultivated with certain plant species have shown a faster reduction of contaminants compared to the soils that do not have crops (Santos et al., 2009), showing phytoremediation potential. This effect is due, partly, to the greater microbial activity characterized as the initial and main degradation mechanism of these herbicides in soil. Moreover, when the environmental conditions favor the development of microorganisms and the bioavailability of herbicides, the degradation of imidazolinones increases (Kraemer et al., 2009).

In order to evaluate and diagnose the microbiological quality of soil, a frequently used attribute is the activities of extracellular enzymes, because it is a sensitive indicator of environmental changes and can identify whether the management practices are appropriate (Matsuoka et al., 2003). Among the main determinations of enzymes that evaluate the biochemical condition of the soil is fluorescein diacetate (FDA) hydrolysis (Adam and Duncan, 2001). This is a non specific and sensitive method to evaluate microbial activity in the soil (Burns et al., 2013), since it determines a range of different hydrolases, such as proteases, esterases and lipases synthetized by the soil macrobiotic (Burns et al., 2013). On the other hand, β-glucosidase, acid phosphatase and urease are extracellular enzymes excreted by the soil microbiota which evaluate the transformation of organic compounds in the soil related to the biogeochemical cycles of carbon, phosphorus and nitrogen respectively (Burns et al., 2013).

Even with the growing interest in microbiological evaluations in agricultural and natural systems, the studies on the microbiological and biochemical dynamics in soils cultivated in a crop rotation that will include the cultivation of flooded rice are still rare. It is thus important to analyze the effect of the residue of different doses of the imazapyr + imazapic ready mix in succession and rotation crops of flooded rice, and also to diagnose the microbiological quality of soil. The objective of the study was to determine the sensitivity of cover crops and soybean in rotation with flooded rice to different levels of imazapyr + imazapic residues in the soil and the effect on soil microbiology.

MATERIAL AND METHODS

A field experiment was conducted during the crop seasons of 2014/15 and 2015/16. The soil of the experimental field is classified as Haplic Gleysol and represents the soils traditionally cultivated with flooded rice in Rio Grande do Sul State. Its chemical characterization sampled in 2014 is shown in Table 1.

Table 1 - Clay and chemical characterization of soil before beginning the experiment

Layer	Clay	OC[(1)]	pH	P	K	Ca	Mg
	(g kg^{-1})		(H_2O)	(mg dm^{-3})		(cmol$_c$ dm^{-3})	
0-20 cm	170	9.9	5.3	14.3	41.8	3.5	1.1

(1) Organic carbono.

In 2014/15 crop season, it was cultivated flooded rice and increasing rates of the ready mix imazapyr + imazapic herbicide were applied in an experimental design of Random Blocks, with four replicates. No adverse effects were observed on rice under these study conduction conditions (data not shown). The treatments were arranged in a Factorial Scheme where Factor A consisted of rates of ready mix imazapyr + imazapic (75 + 25 g a.i. L^{-1}, respectively) in order to simulate herbicide residue in the soil, which were: 0 (control), 140 (label dosage, equivalent to 73.5+24.5 g a.i. ha^{-1}), 280, 560, 840 and 1,120 g ha^{-1}, ie, 0, 1, 2, 4, 6 and 8 times the labeling dosage, respectively; and Factor B the main cover crops used by farmers during fall-winter in the areas planted with flooded rice, whose levels were ryegrass, white clover and fallow (no cover crop). In order to prevent plants from growing during the fallow treatment, glyphosate was applied more twice than the other treatments, when the growth of spontaneous plants occurred.

The experimental units consisted of plots, 1.7 x 2.5 m (4.25 m^2). The soil covers were planted by broadcast sowing in a no-till system, over rice straw, with sowing densities of 6 kg ha^{-1} of white clover and 25 kg ha^{-1} of ryegrass, according to farmers use in this conditions. Before sowing the cover crops, fertilization was performed with Di-ammonium Phosphate (DAP) (18-46-00), at a dose of 250 kg ha^{-1}, in order to correct soil fertility. White clover seeds were inoculated before sowing with a recommended inoculant for cultivation, while for ryegrass a nitrogen fertilization in the form of urea (100 kg ha^{-1}) was performed when the plants had between 6-8 expanded leaves. After cultivation for 145 days, glyphosate was applied over the whole area to control the cover crop and allow the soybeans sowing in the following crop season (2015/16).

The variables analyzed were reflectance of the canopy of the cover crops at 75 and 140 days after sowing (DAS), the crop height and phytotoxicity of the species at 140 DAS. The canopy reflectance was evaluated by Greenseeker radiometer that supplies the value of the “normalized difference vegetation index (NDVI). The equipment was positioned at a height between 0.8 and 1.0 m above the top of the canopy, and the readings are performed when there is linear displacement of the equipment over the experimental unit, with a working width captured by the sensor from 0.7 to 0.8 m. After the sensor is triggered, a reading is performed of a point over the canopy every 0.1 second, totalizing about 30 points measured in each experimental unit. Plant height was quantified using a millimetered ruler and phytotoxicity was done on a percentage scale where zero grade corresponds to absence of injuries and 100% to the death of the plants.

In the following crop season (2015/16) the effect of the imazayr+imazapic residue on the soybean crop was evaluated after the use of the cover crops. The experimental design used was random blocks and the treatments were arranged according to a Factorial Scheme whose factor A consisted of the doses (levels of residue) of imazapyr + imazapic, as mentioned previously, and factor B consisted of the soil cover crops, whose levels were ryegrass, white clover and fallow (without soil cover). About 30 days before the soybean sown, glyphosate was applied for cover crops control and to allow the crop implementation.

It was sown fifteen seeds of soybean per meter, in lines with a 0.50 m space row. Seeds were treated with pyraclostrobin + methyl thiophanate + fipronil and, immediately before sowing, they were inoculated with *Bradyrhizobium japonicum*. The correction of soil fertility followed a soil analysis (Table 1) based on the technical recommendations for soybean, with fertilization for the expectation of a yield of 6.0 ton ha^{-1}. At the same time of soybeans sown, soil samples were collected to perform soil microbiology analyses where the rate of hydrolysis of flurescein diacetate (FDA) was estimated using the method proposed by Adam and Duncan (2001) and urease, acid phosphatase and β-glucosidase were determined using the methodology described by García-Orenes et al. (2010).

During the soybean crop, it was made assessments of rate of growth (days cm^{-1}) and of node emission (days $node^{-1}$), plastochron (°C day $node^{-1}$), percentage reduction of height, number of nodes and legumes per plant (%), number of branches per plants, grains per legume, weight of a thousand grains and grain yield of soybean (kg ha^{-1}). To calculate the growth and node emission rate every seven days, the height of the soybean plants was quantified from the soil to the last node, and the number of nodes of ten plants per plot, and the rate was calculated based on equation:

$$y = \Sigma\, N_{n+1} - N_n / D_{n+1} - D_n$$

in which N indicates the value of the height or number of nodes during the evaluation period; D is the day of evaluation; and (n+1) is the period immediately after n.

The plastochron was estimated by the inverse of the linear regression coefficient between number of nodes and the accumulated thermal sum (STa) (Xue et al., 2004). The accumulated thermal sum (STa, °C.day) was calculated by adding the sum of the daily thermal sum (STd, °C.day), assuming the lower base temperature (Tb) values of 7.6 °C, temperature (Tot) temperature of 31.0 °C and upper temperature (TB) of 40 °C, proposed by Setiyono et al. (2007), for the vegetative phase of the soybean crop, where: Sta = ΣSTd

$$STd = (Tmed - Tb)\ 1day, \text{ when } Tb < Tmed \leq Tot$$

$$STd = (Tot - Tb)\ (Tmax - Tmed) / (Tmax - Tot), \text{ when } Tot < Tmed \leq Tmax$$

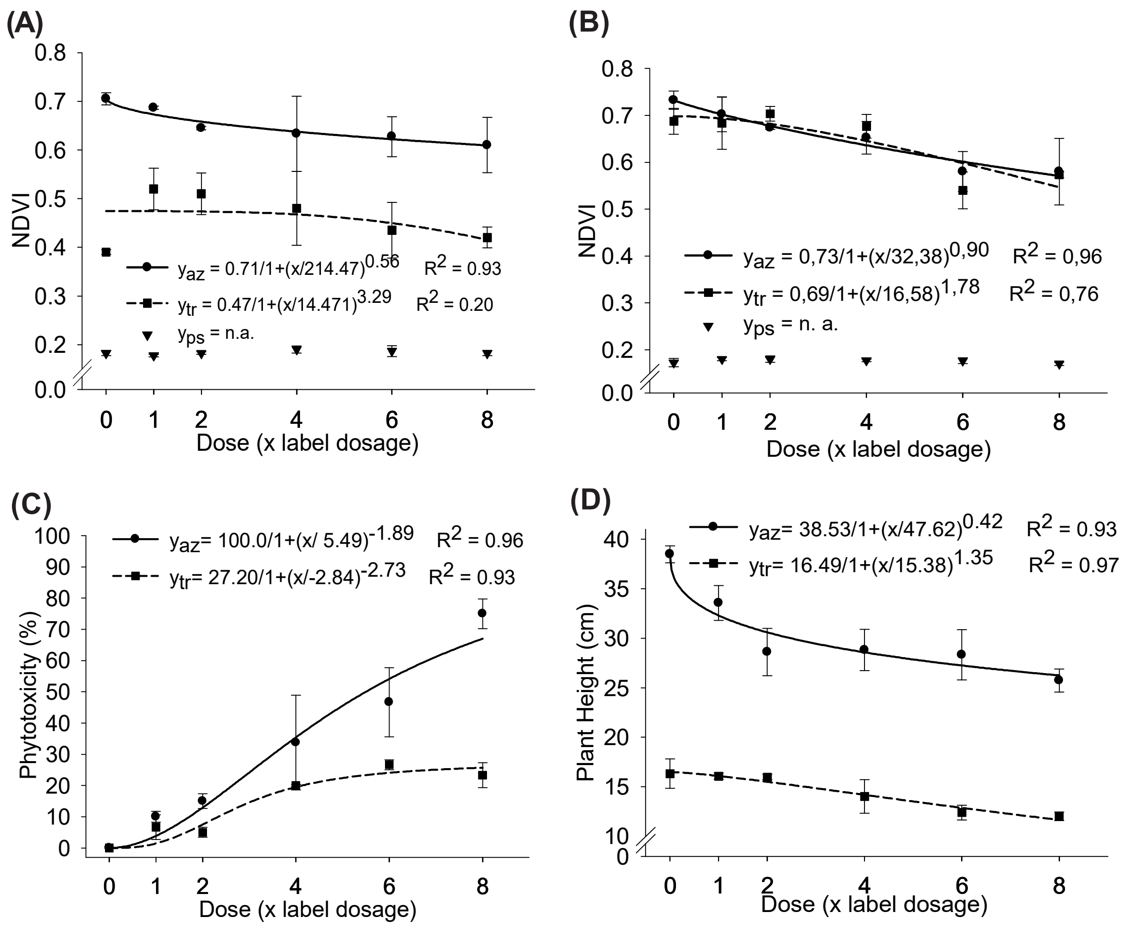
where: Tb is the lower basal temperature, Tot is the optimal temperature, TB is the upper basal temperature and the daily mean temperature (Tmed) is the arithmetic mean of the minimum and maximum temperatures.

Crop yield was calculated by the plants harvested in the plot area and estimated as kg ha^{-1} with the humidity corrected to 13%. The quantification of the crop yield components, number of nodes, legumes per plant, grains per legume and weight of a thousand grains was performed based on all the plants collected to calculate yield, and then the direct quantification of variables was performed in all plants collected at $3m^2$ area.

The data were analyzed for normality using the Shapiro-Wilk test and the homocedasticity using the Hartley test and later submitted to analysis of variance considering the probability of 25% ($p \leq 0.25$) for the effect of interactions and 5% ($p \leq 0.05$) for the simple effects of each factor (Perecin and Cargnelutti Filho, 2008). When statistical significance was found, regression analysis and the Duncan test ($p \leq 0.05$) were performed.

RESULTS AND DISCUSSION

Interaction between the tested factors in cover crops (imazapyr + imazapic doses in irrigated rice from the 2014/15 crop versus winter cover crops) was observed for the analyzed variables, such as NDVI at 75 and 140 days after sowing of cover plants, phytotoxicity and plant height (Figure 1). However, in the growing season 2015/16, interaction between the tested Factors (doses of imazapyr + imazapic in irrigated rice from the 2014/15 crop versus soy from the 2015/16 crop) was observed for some analyzed variables on soybean, such as, growth rate, percentage reduction of height, number of nodes and legumes per plant (Figures 2A, 2C, 3B and 3C). For Factor A (simple dose effect), significance was found for node emission (Figure 2B), plastochron (Figure 2D), number of branches per plant (Figure 3A) and crop yield (Figure 3D). For the variable grain yield a simple effect was also found for the Factor B (soil cover) (Table 1), while for the variables number of grains per legume and weight of one thousand grains no statistical significance was observed.

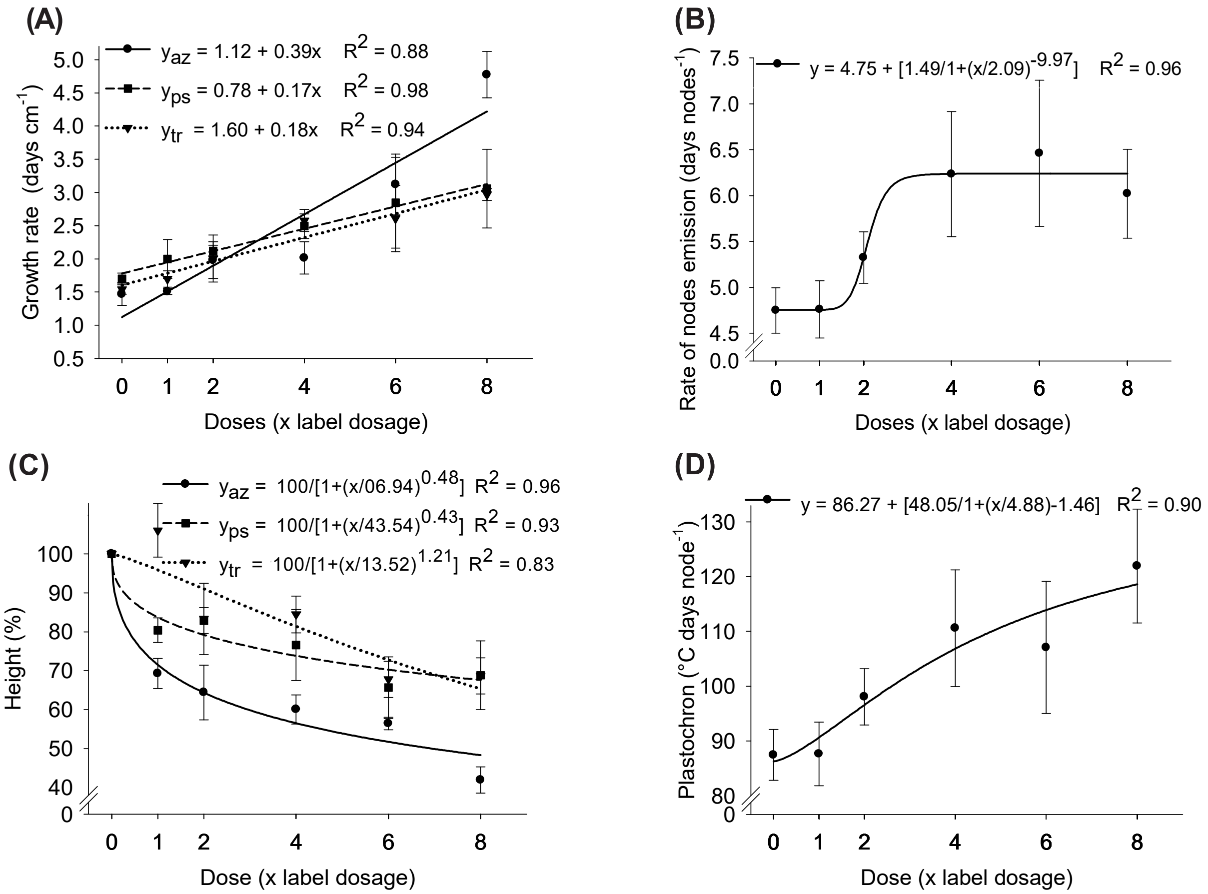


The dots represent the means and the bars the respective confidence intervals. az: ryegrass; ps: fallow; tr: white clover. Label dosage of imazapyr + imazapic = 73.5+24.5 g a.i. ha^{-1}.

Figure 1 - Normalized difference vegetation index (NDVI) of the canopy 75(A) and 140(B) days after sowing the covers, phytotoxicity (C) and plant height (D) of the soil covers in an area with different levels of imazapyr+imazapic residue applied in rice crop.

Cover plants

The NDVI difference between the covers was observed in the evaluation at 75 days after sowing (DAS), where the value of NDVI is higher in the ryegrass than in the white clover (Figure 1A). As for herbicide rates, NDVI decreased in the two assessment periods (75 and 140 DAS) and in both covers, with the largest difference between the highest and lowest residues of imazapyr + imazapic at 140 DAS (Figure 1B).



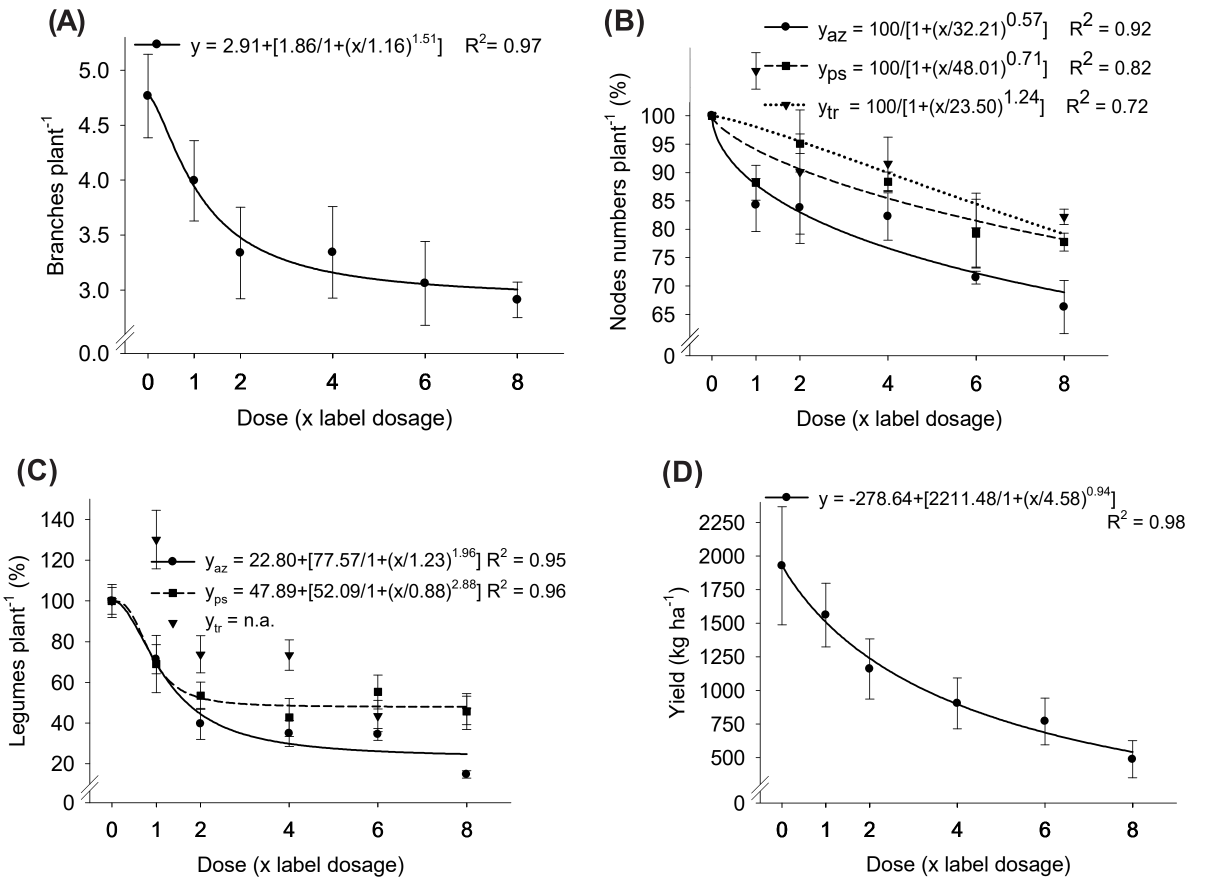
The dots represent the means and the bars the respective confidence intervals. az: ryegrass ps: fallow; tr: white clover. Label dosage of imazapyr + imazapic = 73.5+24.5 g a.i. ha^{-1}.

Figure 2 - Growth rate (days cm^{-1}) (A) and node emission (days $node^{-1}$) (B), height (%) (C) and plastochron (°C day $node^{-1}$) (D) of soybean plants cultivated in an area preceded by the application of doses of imazapyr + imazapic and different cover rice crops.

NDVI is the ratio between the difference of reflectivity in the near infrared and in the red bands, and by the sum of the same reflectivity (Zanzarini et al., 2013), and it can be used as an indicator of the quantity and condition of the vegetation. When the values are closer to one, indicate an area with more dense and vigorous vegetation, while the lower values and values close to zero indicate stress, less dense vegetation, or even denuded areas. Thus, the lower values of NDVI observed for the higher doses of the imazapyr + imazapic herbicide are due to the reduction of growth of the soil covers and to the symptoms of phytotoxicity caused by the herbicide that can be characterized as inhibition of growth and chlorosis of the points of growth and leaves evolving to necrosis (Senseman, 2007).

The phytotoxicity of ryegrass when exposed to the imazapyr + imazapic and imazethapyr + imazapic residue under controlled conditions was high beginning from twice the label dosages of these herbicides (Santos et al., 2014). For current study, divergent results were found, in which a phytotoxicity greater than 50% was observed beginning with six times the label dose of the first herbicide (Figure 1c), and this may be ascribed to the fact that this study was performed under field conditions due to meteorological characteristics during period. Furthermore, greater injury occurred in the ryegrass compared to the white clover, although the addition of phytotoxicity with the increase of the dose of herbicide occurred for both species (Figure 1C).

Plant height was impacted most by the increment of the dose of herbicide in the ryegrass plants, corroborating the results observed for phytotoxicity, with a reduction of 31% and 19% in the height of ryegrass and white clover, respectively at the highest dose of herbicide, correspondent to 8x the recommended rate, and compared to control dose (Figure 1D). These results converge



The dots represent the means and the bars the respective confidence intervals. Label dosage of imazapyr + imazapic = 73.5+24.5 g a.i. ha^{-1}. n.a.: without adjusting the data to the model.

Figure 3 **-** Number of branches (A), nodes (%) (B) and legumes (%) (C) per plant and yield (kg ha^{-1}) (D) of soybean cultivated in an area preceded by the application of doses of imazapyr + imazapic and different cover crops. az: ryegrass; ps: fallow; tr: white clover.

to that observed in previous studies, in which the impact of the imazethapyr + imazapic residue on ryegrass interfered negatively in the height and shoot dry matter (Pinto et al., 2009a). Thus, the differences observed can be due that fabaceae species presented higher phytoremediation factors for imidazolinones (Souto et al., 2015), and this may be due to the greater input of nitrogen in soil compared to the poaceae species.

Further, the highest lignin content observed in fabaceae, compared to the poaceae, may favor the sorption of imidazolinones in given cell organelles that are highly lignified, decreasing their availability in soil (Souto et al., 2015), consequently helping phytoremediation. For this purpose it is important that studies foresee a full cycle of crop rotation in the area previously treated with imidazolinones, to attenuate the residual effects of the herbicide in soil in the crops cultivated later. In this case, the soybean is an important alternative to crop rotation in flooded rice areas and the understanding the effect of soil residue due cover crop is needed during fall-winter. The results observed here showed that both ryegrass and clover cover crops had a negative impact due to an increased dose of imazapyr + imazapic, and the last one it seems more tolerant to herbicide residue on soil.

Soybean development

The data concerning soybean crop showed that according to the dose of herbicide, beginning with an average of four times the label dose, the growth rate (days cm^{-1}) (Figure 2A) and rate of node emission (days node^{-1}) (Figure 2B) of the soybean plants increases about one and two days for the growth of 1 cm and emission of 1 node, respectively, independent of the cover crop cultivated in fall-winter. The stress caused in the development of soybean plants can also be seen through the plastochron values which ranged from 90 to 120 °C day node^{-1} in the doses above twice the recommended, and which are superior to the values of 40 to 70 °C day node^{-1} found in basic studies of ecophysiology (Setiyono et al., 2007).

It was observed that the height (Figure 2C) and the nodes number in R8 (soybean maturity) (Figure 3B), in general decreased based on the label dose of imazapyr + imazapic. The reduction of the plant height after applying herbicides of the chemical group of imidazolinones is a frequent symptom observed in various crops, such as flooded rice (Marchesan et al., 2010), ryegrass (Pinto et al., 2009a) and corn (Pinto et al., 2009b). Besides, the greatest reduction occurred when soybean was preceded by ryegrass, the main cover crop used in a succession of crops in areas of flooded rice, compared to the other managements. In this context, it is believed that some species are able to metabolize the herbicides into compounds that are less toxic to the plant and to the environment, like some fabaceae species (Souto et al., 2013). Another possibility is phytostimulation, in which the microbial activity promoted by the release of root exudates which act to degrade the compound in soil is stimulated. These characteristics can explain the differences observed in cover crops growth and are related to soybean development, that in general, the combination of high imidazolinones residue in soil (upper than four-six times) and ryegrass cultivated during fall-winter promoted the most damages on soybean.

The herbicide residue in soil negatively affected the structures of the soybean plant which determine the yield potential, such as: number of branches, number of nodes and number of legumes per plant, and consequently promoted the reduction of soybean components yield after the application of twice the recommended dose of the herbicide (Figure 3). The number of branches found in soybean sown in herbicide residue control treatment ranges from four to six branches in modern varieties in Rio Grande do Sul State sown at the recommended time (Zanon et al., 2015), but as the herbicide is applied, the number of branches decrease (Figure 3A). It was also observed that the soybean yield was superior when preceded by ryegrass which provided a yield that was about 30% higher than the other cover crop managements (Table 2). This result can be explained by the high yield of soybean after ryegrass and without imidazolinone residue, where the soybean yield was around 2,500 kg ha^{-1} that promoted an elevating on average on that treatment, compared to fallow or white clover without residue that produced about 1,600 and 1,400 kg ha^{-1} soybean yield (data not shown), respectively. Thus, it can be due the agronomic benefits of a fabaceae crop after a poaceae like soybean and ryegrass, respectively.

Table 2 - Soybean yield (kg ha^{-1}) cultivated in an area preceded by different cover crops

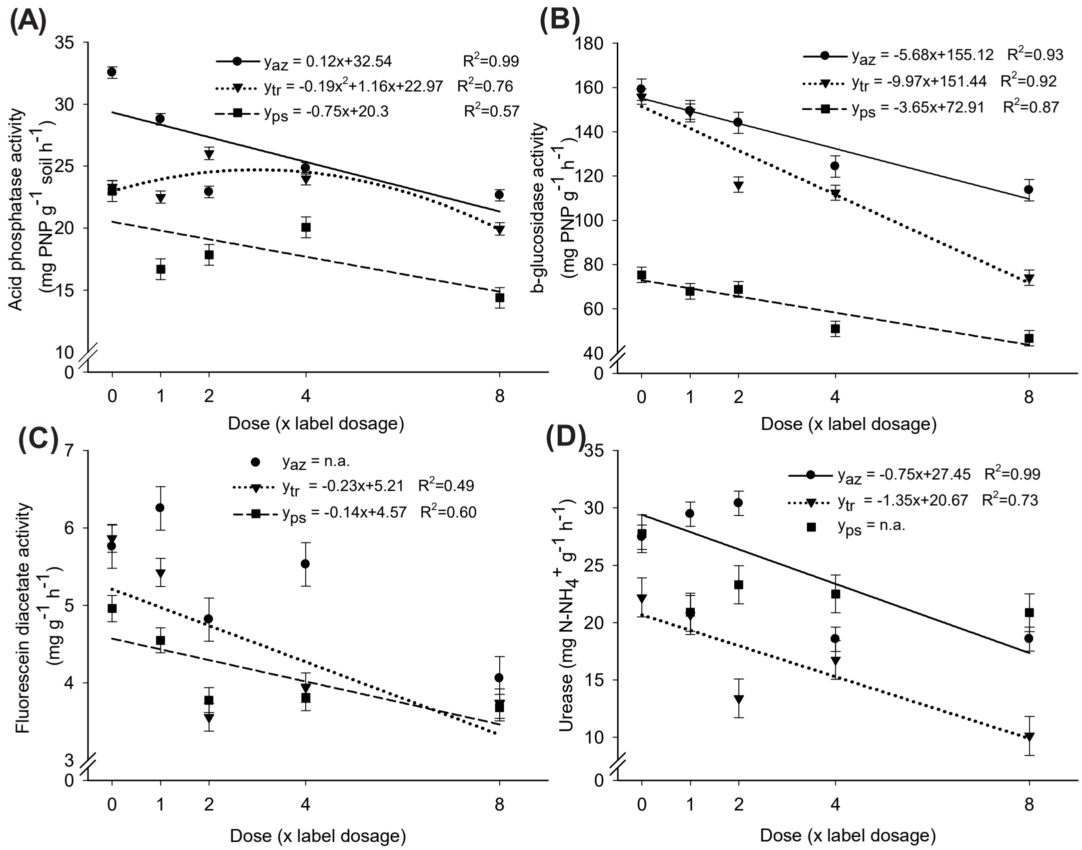
Soil cover	Yield (kg ha^{-1})
Ryegrass	1447 A
White clover	984 b
Fallow	1068 b
CV (%)	5.99

Means followed by the same letter do not differ significantly according to the Duncan test (p≤0.05).

It should be underscored that the negative impacts of the imazapyr + imazapic residue in the soil, in general, were more marked when overdoses of the herbicide were used, independent of the subsequent crop. Thus it should be highlighted that the farmers' practice of increasing the doses of herbicide aims to control weeds that are difficult to control. However it is known that this is not a sustainable practice, nor is it recommended, since it is not efficient for resistant weeds, increases the pressure of selection of resistance to herbicides, and it also provides greater persistence of herbicides in the soil, impacting the crops in succession and rotation as observed in this study. The soybean development and yield were negatively impacted when had high imidazolinone residue on soil, even after the soil covers have or not been cultivated in fall-winter.

Activity of extracellular enzymes from soil

The activity of soil microbiota, estimated from the activity of extracellular hydrolase enzymes, was affected by the increasing doses of herbicide (Figure 4). Acid phosphatase presented greater activity where ryegrass and clover were farmed during the fall-winter period possibly through



The dots represent the means and the bars the respective confidence intervals. Label dosage of imazapyr + imazapic = 73.5+24.5 g a.i. ha^{-1}. n.a.: = without adjusting the data to the model.

Figure 4 **-** The activity of the acid phosphatase (A), β-glucosidase (B), fluorescein diacetate (FDA) (C) and urease (D) after different cover crops were cultivated with increasing doses of imazapyr + imazapic. az: ryegrass; ps: fallow; tr: white clover.

the excretion of exudates by plants that stimulate the activity of acid phosphatase and by the excretion of the enzyme phosphatase in which the plants have the capacity of synthesis and exudation (Figure 4A). The activity of β-glucosidase also presented greater soil activity when ryegrass and clover crops were planted (Figure 4B). Similar behavior was seen in the FDA (Figure 4C) and urease (Figure 4D). The activity of the FDA under ryegrass crops and urease when fallow, did not influence the herbicide applications. The higher activity of β-glucosidase and urease in the presence of ryegrass and clover is associated with the carbon input to the soil, stimulated by exudation and the disposition to the soil of senescent roots, leaves and stems. The contribution of these organic compounds stimulates β-glucosidase activity, which is related to the breakdown of carbon-rich molecules such as cellulose and urease into nitrogen-rich molecules.

The composition of the mineral fraction of the soil affect the dynamics of the herbicides in the soil and consequently impact on the enzymatic activity of the soil that have a direct role in the metabolization of herbicidal molecules in the soil. The soil of the present study is a Gleysol that originates in sediments with low contents of iron oxides and organic carbon which are among the main soil compounds that perform sorption complexes with the molecules of herbicides and increase their persistence in the soil (Pateiro-Moure et al., 2009). Consequently, these characteristics contribute to a low persistence of xenobiotic molecules in soil, making them available to the microbial degradation. However, other aspects such as the period of flooding that occurs in the implementation of the rice crop, the low temperatures and the flooding during most of the winter in the South of Brazil are factors that tend to increase the persistence of imidazolinones in soils cultivated with flooded rice, especially when added at high doses.

In this scenario, even with the low presence of reactive groups, the soil organic matter (SOM), especially the humic fractions have a great capacity to adsorb herbicide molecules (Pateiro-Moure et al., 2009). Besides retaining the herbicide molecules in SOM, the physical constitution of the lowland soils, and the low total porosity of the soils diminish oxygen diffusion in this type of soil and, consequently, diminish the activity of the aerobic microbiota of the soil which are the most efficient to degrade organic compounds (Li et al., 2016). When available to the microbiota, the herbicides can be degraded by the microorganisms or can inhibit the activity of enzymes via the toxicity of the molecule and its metabolites, as observed in the current study (Figure 4) as by using imazethapyr on reduction the soil microbial biomass (Perucci and Scarponi, 1994).

The use of cover plants is an alternative to accelerate the degradation of agricultural pesticides in the soil due to the increase of the carbon sources, acceleration of nutrient cycling and maintenance of soil moisture level, which are important aspects for the increase of microbial activity in the soil (Balota et al., 2014). These factors contribute to elucidating the greater expression of the enzymes of soil where ryegrass and clover have been planted, compared to fallow treatment (Figure 4). In general, when the effect of ryegrass and clover were compared, there was a trend for greater enzyme activity where poaceae was cultivated, as compared to the fabaceae (Figure 4). Possibly this results from the greater development of ryegrass, evidenced by the greater height of the plants compared to clover (Figure 1D).

The increased dose of imazapyr + imazapic has a negative effect on the development of the ryegrass and clover cover crops, and it is most harmful at doses greater than twice the label dose, where the phytotoxicity and reduction plant height on ryegrass is more pronounced. The same, increasing doses reduce the growth and yield of soybean even after the soil covers have or not been cultivated in fall-winter. In this scenario, the negative impact on soil caused by overdoses of herbicides in an agricultural production system may cause irretrievable damage to the development and consequently grain yield and/or pasture crops. Besides, the adversities can go beyond the impacts on agricultural crops and affect soil health, significantly reducing the biochemical activity of soil which is the main natural resource used in farming.

Increased imazapyr + imazapic doses decrease enzymatic activity of the soil. However, the use of cover crops is an important alternative to mitigate the impact of the herbicide on the biochemical activity of soil. Thus, imidazolinones must be used rationally in rotation systems with flooded rice in order to avoid and/or minimize the negative effects on soil and its consequent impacts on the soil-plant system. Further studies involving the residual effect of herbicides on soils should be made, considering that many mechanisms that occur in the soil-plant system are not yet clearly known.

REFERENCES

Adam G, Duncan H. Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. Soil Biol Biochem. 2001;33:943-51.

Agostinetto D, Fleck NG, Rizzardi MA, Merotto Junior A, Vidal RA. Arroz vermelho: ecofisiologia e estratégias de controle. Cienc Rural. 2001;31:341-9.

Alister C, Kogan M. Efficacy of imidazolinone herbicides applied to imidazolinone-resistant maize and their carryover effect on rotational crops. Crop Prot. 2005;24:375-9.

Balota EL, Calegari A, Nakatani AS, Coyne MS. Benefits of winter cover crops and no-tillage for microbial parameters in a Brazilian Oxisol: A long-term study. Agric Ecosyst Environ. 2014;197:31-40.

Bundt AC, Avila LA, Pivetta A, Agostinetto D, Dick DP, Burauel P. Imidazolinone degradation in soil in response to application history. Planta Daninha. 2015;33(2):341-9.

Burns RG, DeForest, JL, Marxsen J, Sinsabaugh RL, Stromberger ME, Wallenstein MD, et al. Soil enzymes in a changing environment: Current knowledge and future directions. Soil Biol Biochem. 2013;58:216-34.

García-Orenes F, Guerrero C, Roldán A, Mataix-Solera J, Cerdà A, Campoy M, et al. Soil microbial biomass and activity under different agricultural management systems in a semiarid Mediterranean agroecosystem. Soil Till Res. 2010;109:110-5.

Kraemer AF, Marchesan E, Avila LA, Machado SLO, Grohs M, Massoni PFS, et al. Persistência dos herbicidas imazethapyr e imazapic em solo de várzea sob diferentes sistemas de manejo. Planta Daninha. 2009;27(3):581-8.

Li X, Zhang W, Liu T, Chen L, Chen P, Li F. Changes in the composition and diversity of microbial communities during anaerobic nitrate reduction and Fe(II) oxidation at circumneutral pH in paddy soil. Soil Biol Biochem. 2016;94(1):70-79.

Marchesan E, Bundt ADC, Avila LA, Agostinetto D, Nohatto MA, Vargas HC. Carryover of Imazethapyr and Imazapic to Nontolerant Rice. Weed Technol. 2010; 24:6-10.

Matsuoka M, Mendes LC, Loureiro MF. Biomassa microbiana e atividade enzimática em solos sob vegetação nativa e sistemas agrícolas anuais e perenes na região de Primavera do Leste (MT). Rev Bras Cienc Solo. 2003;27:425-33.

Pateiro-Moure M, Pérez-Novo C, Arias-Estévez M, Rial-Otero R, Simal-Gándara J, et al. Effect of organic matter and iron oxides on quaternary herbicide sorption–desorption in vineyard-devoted soils. J Colloid Interf Sci. 2009;333(2):431-8.

Perecin D, Cargnelutti Filho A. Efeitos por comparações e por experimento em interações de experimentos fatoriais. Cienc Agrotec. 2008;32:68-72.

Perucci P, Scarponi L. Effects of the herbicide imazethapyr on soil microbial biomass and various soil enzyme activities. Biol Fert Soils. 1994;17:237-40.

Pinto JJO, Noldin JA, Rosenthal MD, Pinho CF, Rossi F, Machado A, et al.Atividade residual de (imazethapyr+imazapic) sobre azevém anual (Lolium multiflorum), semeado em sucessão ao arroz irrigado, sistema clearfield®. Planta Daninha. 2009a;27(3):609-19.

Pinto JJO, Noldin JÁ, Machado A, Pinho CF, Rosenthal MD, Donida A, et al. Milho (Zea mays) como espécie bioindicadora da atividade residual de (imazethapyr+imazapic). Planta Daninha. 2009b;27:1005-14.

Santos JB, Ferreira EA, Fialho CMT, Santos EA, Galon L, Concenço G, et al. Biodegradation of glyphosate in rhizospheric soil cultivated with Glycine max, Canavalia ensiformis e Stizolobium aterrimum. Planta Daninha. 2009;27(4):781-7.

Santos LO, Pinto JJO, Piveta LB, Noldin AJ, Galon L, Concenço G. Carryover effect of imidazolinone herbicides for crops following rice. Am J Plant Sci. 2014;5:1049-58.

Senseman SA. Herbicide handbook. 9^{th}.ed. Lawrence: Weed Science Society of America; 2007.

Setiyono TD, Weiss A, Specht J, Bastidas AM, Cassman KG, Dobermann A. Understanding and modeling the effect of temperature and daylength on soybean phenology under high-yield conditions. Field Crops Res. 2007;100:257-71.

Souto KM, Jacques RJS, Avila LA, Machado SLO, Zanella R, Refatti JP. Biodegradação dos herbicidas imazetapir e imazapique em solo rizosférico de seis espécies vegetais. Cienc Rural. 2013;43:1790-6.

Souto KM, Avila LA, Cassol GV, Machado SLO, Marchesan E. Phytoremediation of lowland soil contaminated with a formulated mixture of Imazethapyr and Imazapic. Rev Cienc Agron. 2015;46:185-92.

Xue QW, Weiss A, Baenziger PS. Predicting leaf appearance in field-grown winter wheat: evaluating linear and non-linear models. Ecol Model. 2004;175:261-70.

Zanon AJ, Streck NA, Grassini P. Climate and management factors influence soybean yield potential in a subtropical environment. Agron J. 2016;108:1447-54.

Zanon AJ, Streck NA, Richter GL, Becker CC, Rocha TSM, Cera JC, et al. Contribuição das ramificações e a evolução do índice de área foliar em cultivares modernas de soja. Bragantia. 2015;74:279-90.

Zanzarini FV, Pissarra Teresa CT, Brandão FJC, Teixeira DDB. Correlação espacial do índice de vegetação (NDVI) de imagem Landsat/ETM+ com atributos do solo. Rev Bras Eng Agric Amb. 2013;17:608-14.