https://doi.org/10.1590/1678-4499.20190074

BASIC AREAS - Article

Persistence of imazapyr+imazapic in irrigated rice area and effect on soybean due to soil moisture and phytoremediation in the off-season

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ABSTRACT: The residue of imidazolinone herbicides in soil can be detrimental to soybean cultivation in rotation with Clearfield® rice. Thus, this research aimed to evaluate the ryegrass phytoremediation capacity on the imazapyr+imazapic residue in the soil in two soil moisture conditions in the off-season, and the effect on soybean growth in the next growing season. Two experiments were conducted between 2016 and 2017 in Santa Maria, Rio Grande do Sul state, Brazil. It was used the randomized block design in a 3 × 2 factorial. The first factor being the residue in the soil of 0, 210 and 420 g c.p.$\cdot$ha^{-1} of the formulated mixture of the herbicides imazapyr+imazapic (525 + 175 g a.i.$\cdot$kg^{-1}) applied to rice in the 2015/2016 growing season, and the second factor being the presence or absence of ryegrass in the off-season. One experiment was conducted under soil moisture above 70% of field capacity in the off-season, and the other under ambient condition of soil moisture. The soil residue of the application of 420 g c.p.$\cdot$ha^{-1} of the imazapyr+imazapic herbicides performed 129 days before planting caused phytotoxicity and reduced dry matter of ryegrass under high soil moisture condition in the off-season in irrigated rice areas. Soybean, when planted 359 days after application, has its initial root and shoot growth affected by the residue of 210 and 420 g c.p.$\cdot$ha^{-1} rates in high soil moisture condition in the off-season, regardless of ryegrass cultivation during the same period. However, grain yield is not affected.

Key words: *Lolium multiflorum*, *Glycine max*, imidazolinones, residue.

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Received: Mar. 5, 2018 – Accepted: Nov. 14, 2018

INTRODUCTION

The Clearfield® system has been widely used in irrigated rice fields to control weeds such as weedy rice and barnyardgrass (Sudianto et al. 2013; Kraehmer et al. 2016). However, the continuous use of herbicides of the imidazolinones chemical group has led to the emergence of weed resistance cases, making it difficult to manage the crop.

The introduction of soybean into areas traditionally cultivated with irrigated rice from the Clearfield® system is an alternative to reduce damage from the occurrence of resistant weeds in these growing areas. However, imidazolinones have high persistence in the soil, which may compromise the success of crop introduction in rotation to the tolerant rice (Pinto et al. 2009a; Souza et al. 2016). The persistence of these herbicides is regulated by their sorption, which in turn is influenced by temperature and moisture, solution pH, organic matter content and soil texture (Kraemer et al. 2009b). Similarly, the intrinsic characteristics of imidazolinone molecules interfere with its persistence. While imazamox has a field half-life of 20-30 days, for imazapic this period is estimated at 120 days. For imazapyr, imazaquin and imazethapyr, the field half-life is 25-142, 60 and 60-90 days, respectively (Shaner 2014).

Soil moisture has fundamental importance on the persistence of imidazolinones. It influences the degradation of the herbicides, since aerobic microorganisms preferentially degrade the majority of the molecules of this group (Kraemer et al. 2009b; Martini et al. 2011). In ryegrass, the highest moisture degree promoted greater phytotoxicity to the plants in soil with imazethapyr+imazapic residue (Avila et al. 2010), proving the effect of this condition on the lower adsorption and degradation of the herbicides in the soil. The importance of soil moisture in the context of irrigated rice production areas is related to deficient drainage and low oxygenation of the soil in the autumn-winter period, which favors the longer persistence of imidazolinones. This condition may compromise the introduction of crops in succession and rotation in these areas.

Phytoremediation techniques, which use species capable of removing, immobilizing or transforming specific contaminants, are important tools for reducing the persistence of herbicides in the soil. Some studies point to the use of species as *Brachiaria brizantha* for phytoremediation of picloram (Braga et al. 2016) and *Dolichos lablab*, *Canavalia ensiformis* and *Crotalaria juncea* for sulfentrazone (Madalão et al. 2013). In areas of irrigated rice with imidazolinones residue, ryegrass stands out as a potential off-season crop for phytoremediation. It is adaptable to hydromorphic soils and can reduce the phytotoxicity of imazethapyr+imazapic in non-tolerant rice (Souto et al. 2015).

In this context, the use of phytoremediation during off-season, as well as the study of the influence of soil moisture on the dynamics of imidazolinone herbicide residue, is necessary when introducing crops in rotation with Clearfield® rice, such as soybean. Therefore, the objective of this research was to evaluate the ryegrass phytoremediation capacity on the imazapyr+imazapic residue in the soil in two conditions of soil moisture in the off-season, and the effect on soybean growth in the next growing season.

MATERIAL AND METHODS

Two experiments were conducted in the field in the 2016 off-season and 2016/2017 growing season in the municipality of Santa Maria (29°43' S, 53°43' W, 89 m altitude), state of Rio Grande do Sul, Brazil. The soil of the experimental area is classified as Eutrophic Arenic Hexic Planosol (Embrapa 2014b), with the following chemical characteristics in the layer of 0 to 0.1 m: pH water (1:1) = 5.8; effective CTC = 7.6 $cmol_c \cdot dm^{-3}$; base saturation = 63.7%; saturation by Al = 0; organic matter = 2%; clay = 25%; P-Mehlich = 12.8 $mg \cdot dm^{-3}$; K = 68 $mg \cdot dm^{-3}$; Ca = 5.3 $cmol_c \cdot dm^{-3}$; Mg = 2.2 $cmol_c \cdot dm^{-3}$. The climate of the area is characterized, according to the classification of Köppen, as subtropical humid (Cfa) (Alvares 2013).

The experimental design was a randomized block design in a 3 × 2 factorial with five replications. Factor A was composed of the residue of the formulated mixture of the imazapyr+imazapic herbicides (525+175 $g\ a.i. \cdot kg^{-1}$) in the soil at rates of 0, 210 and 420 $g\ p.c. \cdot ha^{-1}$. These rates were applied in irrigated rice in a previous experiment conducted in the 2015/16 growing season, with 50% of the rate applied in pre-emergence and the remaining in post-emergence of the crop. Before 2015/2016 growing season, the area had been cultivated with soybean (2012/2013), corn (2013/2014), and soybean (2014/2015). No imidazolinone herbicides were used in these growing seasons. Factor B was composed of the presence or absence of ryegrass in the off-season. The spontaneous vegetation in the experimental units without ryegrass was controlled during the off-season using the herbicide glyphosate at a rate of 1500 g a.e.·ha^{-1}. The dimensions of the experimental units were 4 × 4 m (16 m^2).

The experiments were conducted in two areas with distinct soil moisture conditions throughout the off-season, however the application of herbicides to rice in the previous growing season occurred in a similar way. From the emergence of ryegrass in one of the areas, an ambient soil moisture condition was recommended. In the other area soil moisture was maintained above 70% of the field capacity. To maintain this condition, four floods were required throughout the off-season. Soil moisture was monitored through sensors connected to an Onset HOBO U30 data logger positioned in the 0-0.05 m soil layer. The data obtained, together with the rainfall during this period, are shown in Fig. 1.

The rice harvest in the 2015/16 growing season was carried out in dry soil condition without further preparation. The ryegrass was planted at a density of 30 kg·ha^{-1} on rice straw on March 24, 2016 (129 days after herbicide application). Nitrogen fertilization of 30 kg·ha^{-1} was used at 30 and 60 days after emergence. The plants were desiccated 158 days after emergence (59 days before soybean planting) with the herbicide glyphosate at 1500 g a.e.·ha^{-1} rate. The soybean was planted on November 8, 2016 (359 days after herbicide application), using the cultivar BMX Valente RR, at the density of 28 seeds per m^{-2}. The base fertilization was of 21.5 kg.ha^{-1} of N, 86 kg.ha^{-1} of P_2O_5 and 86 kg.ha^{-1} of K_2O. Other cultural treatments were conducted according to technical recommendations for the crop (Embrapa 2014a).

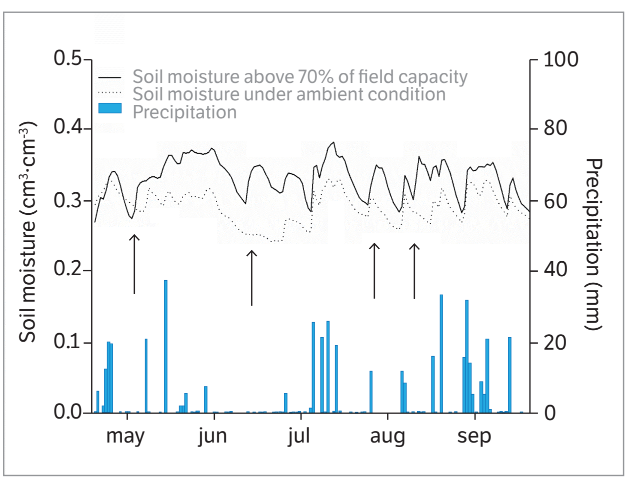

Figure 1. Mean daily soil moisture (cm^3·cm^{-3}) in experiments with soil moisture above 70% of field capacity and with soil moisture under ambient condition in the 0-0.05 m layer; and daily rainfall (mm) between April and September 2016. Arrows indicate the flood moments in the first experiment.

Phytotoxicity in ryegrass and soybean crops was evaluated at 20 days after plant emergence. Phytotoxicity percentages were attributed based on leaf color, width and growth, where zero represented no injury and 100 represented the death of plants (Frans et al. 1986). On the day of desiccation of ryegrass, two plant samples of 0.25 m^2 were collected in each experimental unit. The samples were oven dried at 65 °C for the determination of shoot dry matter.

At soybean V_3 and R_1 phenological stages (Fehr and Caviness 1977), a soil monolith was collected on the second planting line of each experimental unit containing five plants. Subsequently, the monoliths were washed in water for the removal of the plants without damage to the roots. Two plants from each extremity were neglected. The others were cut, separating the aerial part of the roots. Plant height was measured from the base of the stem to the insertion of the last trefoil with a ruler. Subsequently, the aerial part was dried in a forced circulation oven at 65 °C to estimate the dry matter. The roots were scanned in a high-resolution scanner (Epson Expression 11000 XL). The images obtained were processed in Winrhizo PRO software, obtaining the number of roots, length, and volume of roots per plant. In the R_1 stage, the nodules were separated from the roots and dried in an oven for the estimation of dry matter. At the end of the soybean crop cycle, an area of 6 m^2 per experimental unit was harvested. After cleaning and weighing the grains, the data were corrected to 13% of humidity and converted to kg·ha^{-1}.

The analyzed variables were submitted to the test of the presuppositions of the mathematical model (normality of the errors and homogeneity of the variances). The variance analysis of experiment data and significance of qualitative factor means (presence or absence of ryegrass) was performed using the F test ($p < 0.05$). The means of the quantitative factor (residue of herbicide rates), when significant, were submitted to polynomial regression analysis. For the data of phytotoxicity in the ryegrass, the transformation sine arc $\sqrt{[(y_0 + 0.5)/100]}$ was used.

RESULTS AND DISCUSSION

Experiment with soil moisture above 70% of field capacity in the off-season

There was an increasing linear behavior of the phytotoxicity in the ryegrass due to the increase of herbicide residue in the →

soil (Fig. 2a). The maximum percentage found was 17% in the highest rate (420 g c.p.·ha^{-1}). The effect of the herbicide residue was also observed in the shoot dry matter. There was a decrease of about 1400 kg·ha^{-1} at the highest rate, relative to the zero rate (Fig. 2c). However, in the 210 g c.p.ha^{-1} rate no significant difference was observed in relation to the same rate. These results corroborate with those of Avila et al. (2010). According to the authors, the formulated mixture of imazethapyr+imazapic herbicides caused greater phytotoxicity and lower plant height and shoot dry matter of ryegrass in higher soil moisture condition. Pinto et al. (2009b) also emphasize that there is a linear reduction in the dry matter of this crop due to the increase of the residue of these herbicides, applied to the rice in the previous growing season. According to Bundt et al. (2015), the seeding performed 128 days after application causes a reduction in the ryegrass dry matter. This finding corroborates with the present study, where the crop was planted 129 days after application.

Interaction between the factors was observed for the evaluation of phytotoxicity in soybean (Fig. 3a). The highest percentages, regardless of the herbicide residue, were observed where there was ryegrass in the off-season. The highest amount of residue yielded values of 32 and 40% of phytotoxicity in the area without and with ryegrass, respectively. For the shoot dry matter and plant height evaluations in the V_3 stage, there was a linear reduction as the herbicide residue increased (Figs. 3c and 3e). For the first evaluation, there was interaction between factors and the reduction was observed where there was no ryegrass in the off-season. For plant height, only rates residue were significant, which provided a reduction of about 12% over the zero rate. For corn, this reduction can be up to 62% when the application of imazethapyr+imazapic herbicides occurs 360 days before planting (Pinto et al. 2009a) and 35% in application 1100 days before planting (Sousa et al. 2012).

In R_1 stage, the behavior of shoot dry matter and plant height were similar to the V_3 evaluation, with reduction of these variables due to the increase of the herbicide residue (Figs. 4a and 4e). For the greater residue, a reduction was observed of approximately 38% and 16% for dry matter and

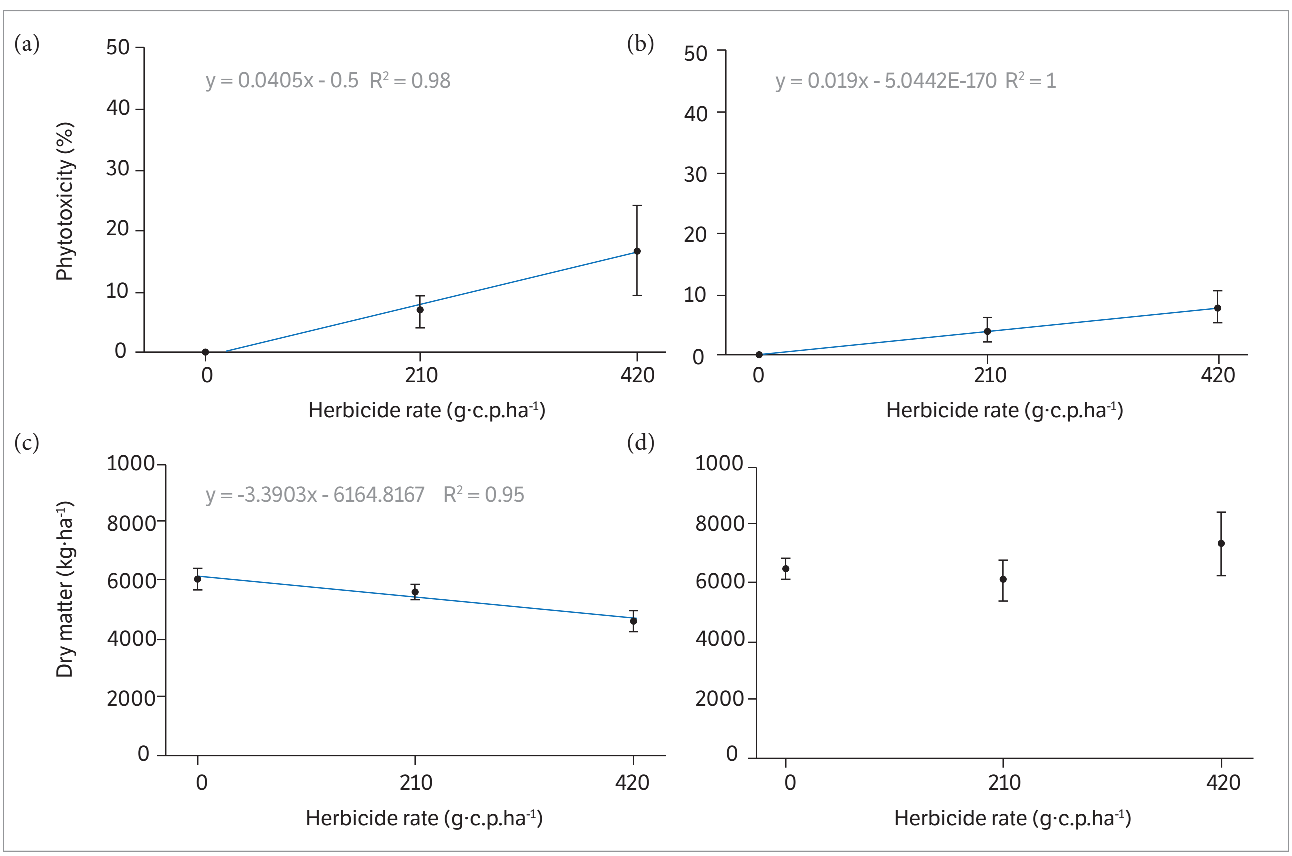

Figure 2. Phytotoxicity (%) and dry matter (kg·ha^{-1}) of ryegrass at 20 and 158 days after emergence, respectively, in relation to different rates of imazapyr+imazapic herbicides applied 129 days before seeding in an experiment with soil moisture above 70% of field capacity (a and c) and with soil moisture under ambient condition (b and d) in the off-season.

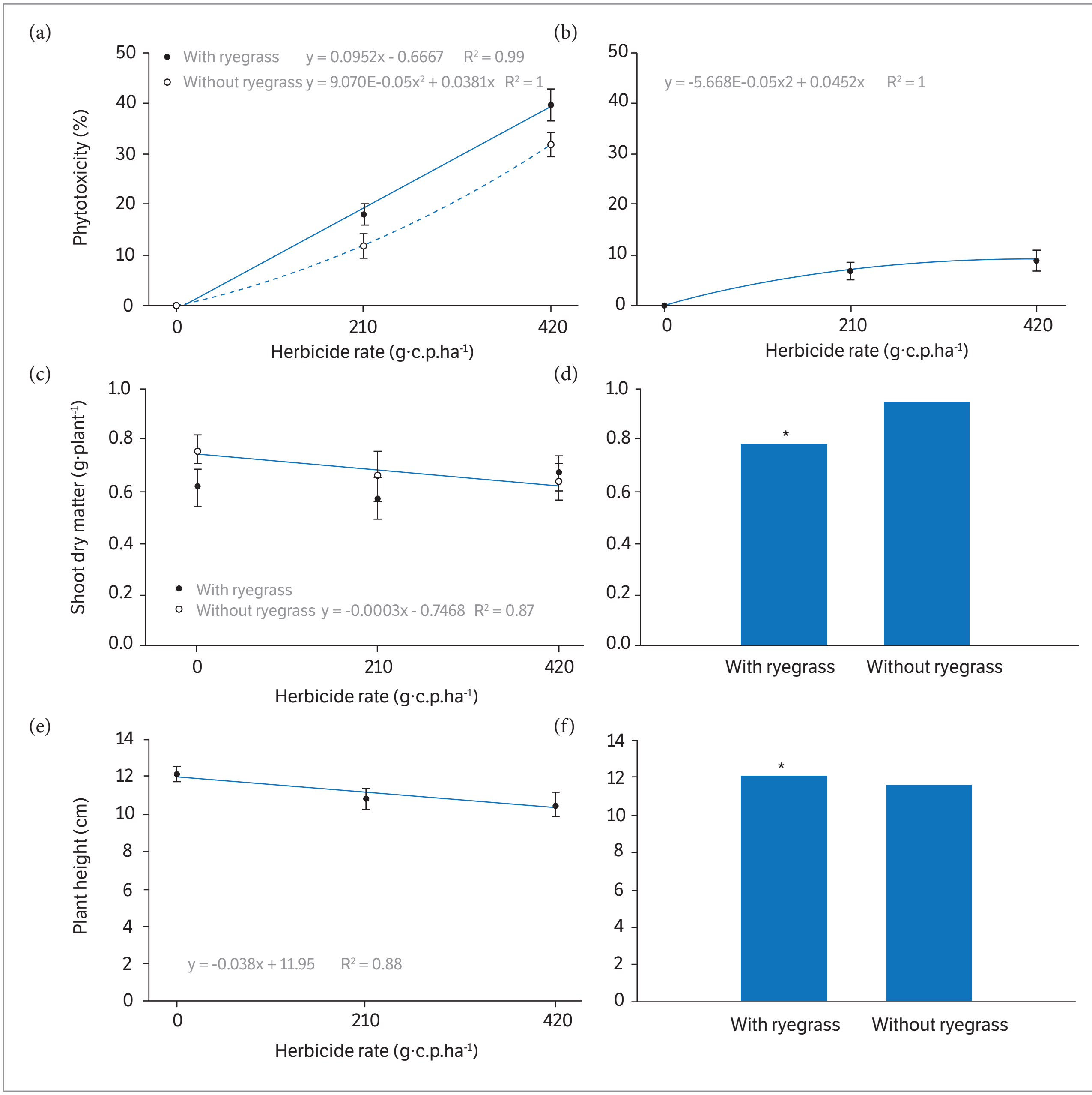

Figure 3. Phytotoxicity (%) at 20 days after emergence, shoot dry matter (g·plant^{-1}) and height (cm) of soybean plants evaluated in the V_3 stage,due to different rates of the herbicides imazapyr+imazapic applied 359 days before seeding, and presence or absence of ryegrass in the off-season, in an experiment with soil moisture above 70% of field capacity (a, c and e) and with soil moisture under ambient condition (b, d and f) in the off-season. *Means differ between the F test ($p < 0.05$).

height, respectively, in relation to the zero rate. The ryegrass factor was also significant, which reduced the dry matter and the height of the soybean plants (Table 1). However, there was no interaction between the factors. There was interaction between the factors for the dry matter of nodules in the R_1 stage (Fig. 4c). The area with ryegrass in the off-season showed a decrease in this variable in the greatest residue of the herbicides in relation to the residue of the rate of 210 g c.p.·ha^{-1}.

Ryegrass induced inferior results in interaction with the herbicide residue for the variables phytotoxicity, total root length at stage V_3, and dry matter of nodules at the R_1 stage. This suggests that the species is not indicated as phytoremediation of these herbicides for soybean cultivation in rotation with Clearfield® rice. Possibly the maintenance of a higher soil moisture throughout the growing season promoted by the ryegrass straw, with the high soil moisture

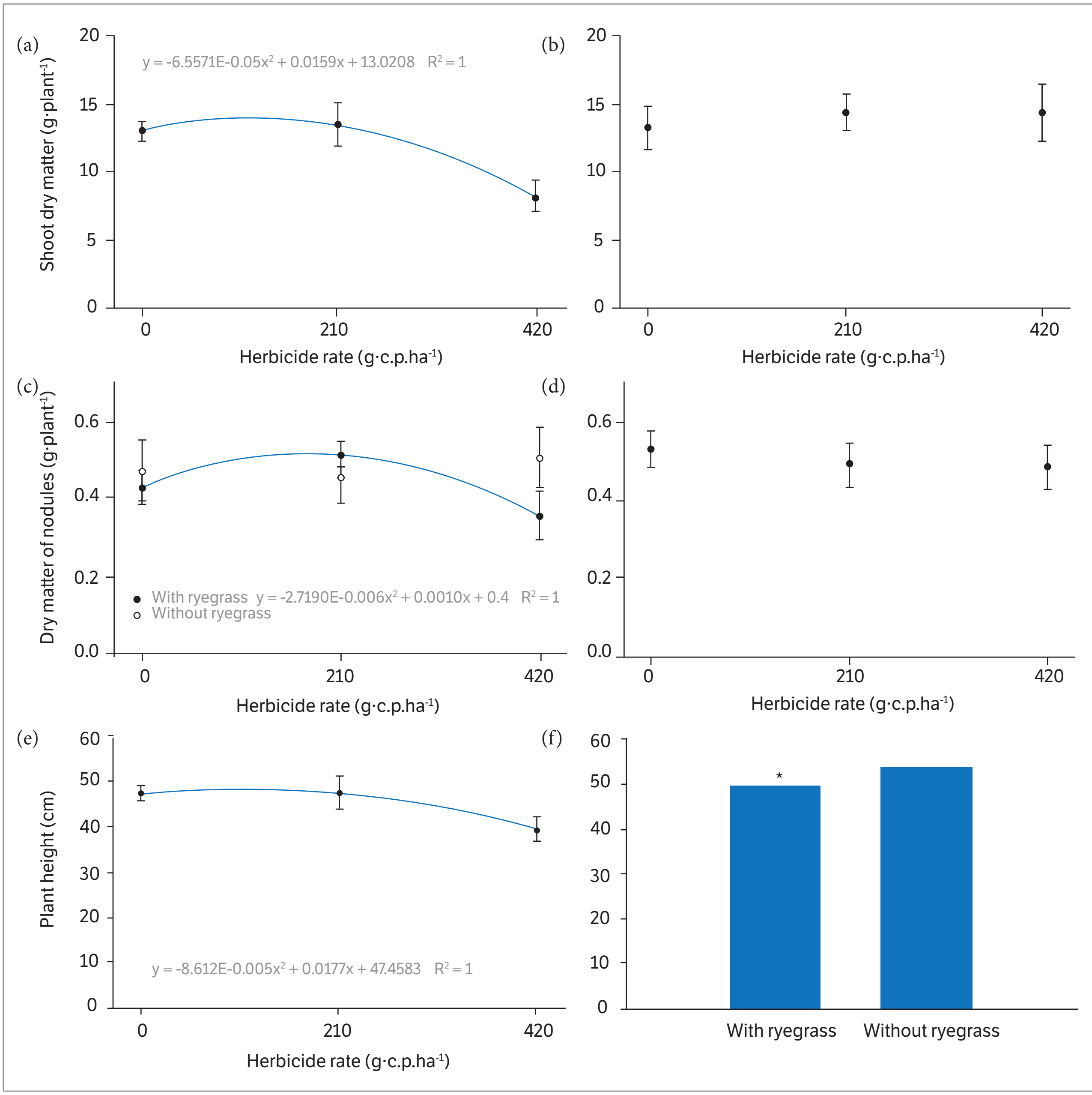

Figure 4. Shoot dry matter (SDM) (g·plant^{-1}), dry matter of nodules (g.plant^{-1}) and height (cm) of soybean plants evaluated at the R_1 stage, due to different rates of the herbicides imazapyr+imazapic applied 359 days before seeding, and presence or absence of ryegrass in the off-season, in an experiment with soil moisture above 70% of field capacity (a, c and e) and with soil moisture under ambient condition (b, d and f) in the off-season. *Means differ among the F test ($p < 0.05$).

during the off-season, has provided greater solubilization and less degradation of the residues of these herbicides, since they are preferentially degraded by aerobic microorganisms (Martini et al. 2011).

In the evaluations related to the root system of the soybean plants, the herbicide residue resulted in an increase in the number of root tips (Fig. 5a). This increase is possibly due to the symptom of the ALS-inhibitor herbicide in the roots known as "bottle brush". According to Clay (2013), plants express this symptom from 7 to 10 days after herbicide exposure. However the effect is temporary, not affecting crop grain yield. For the total root length there was interaction between the factors. The presence of ryegrass in the off-season resulted in a reduction of about 23.5% in this variable where there was herbicide residue (Fig. 5c). In this same environment, the

Table 1. Plant height (PH) (cm) and shoot dry matter (SDM) (g·plant^{-1}) at the R_1 stage and volume of soybean roots (cm^3·plant^{-1}) at the V_3 stage in an experiment with soil moisture above 70% of field capacity. Soybean grain yield (kg·ha^{-1}) in an experiment with soil moisture above 70% of field capacity and soil moisture under ambient condition in the off-season, in relation to the presence or absence of ryegrass during the same period.

Soil coverage in the off-season	PH (cm)	SDM (g.plant^{-1})	Roots volume (cm^3.plant^{-1})
	R_1	R_1	V_3
	Soil moisture above 70% of field capacity		
With ryegrass	43.5*	10.4*	0.66*
Without ryegrass	46.2	12.6	0.82
Mean	44.8	11.5	0.74
VC (%)	7.6	11.8	21

Soil coverage in the off-season	Grain yield (kg.ha^{-1})	
	Soil moisture above 70% of field capacity	Soil moisture under ambient condition
With ryegrass	3733^{ns}	3406*
Without ryegrass	3869	3765
Mean	3800	3585
VC (%)	6.2	9.3

*Means differ from each other in the column by the F test ($p < 0.05$). ^{ns}Not significant by F test.

root volume was reduced by about 28% in the residue in relation to the zero rate (Fig. 5e). Similarly, the presence of ryegrass in the off-season reduced root volume by 20% when compared to the area without the crop (Table 1), but there was no interaction between the two factors.

In the evaluations of roots performed in the R_1 stage, no significance was found for any of the factors in all variables analyzed for both experiments. This suggests that soybean plants were able to recover from the injuries caused by herbicide residue in the root system throughout their growth and development. Among crops such as beans, wheat and corn, soybean presents lower sensibility to the soil residue of the imazapir+imazapic herbicide (Ulbrich et al. 2005), which possibly explains this recovery.

The soybean yield was not influenced by the residual herbicide rates (Table 1), which reinforces the plant resilience when exposed to this condition. Similar results are found in irrigated rice that is not tolerant to imidazolinones. Although the residues of imazetapir and imazapic have a negative influence on the plant stand, tillering and number of panicles, the grain yield was not impaired (Kraemer et al. 2009a). However, Marchesan et al. (2010) emphasize in their study that rice grain yield was not affected only after 705 days from herbicide application, and that at 371 days there was still an effect on this variable.

Experiment with soil moisture under ambient contidion in the off-season

For ryegrass, as in the previous experiment, it was observed a linear increase in the phytotoxicity as a function of the herbicide residue increase in the soil, being 8% the maximum percentage found in the greater residual rate (Fig. 2b). For shoot dry matter, no significant difference was observed (Fig. 2d).

In relation to soybean, only the residue of the rates was significant in the phytotoxicity evaluation, being that the maximum value found was 9% (Fig. 3b). Only the ryegrass factor presented significance for shoot dry matter and plant height in V_3 stage. In the crop area there was a reduction of approximately 18% in the shoot dry matter, in relation to the area without ryegrass (Fig. 3d). On plant height, the ryegrass area presented the greatest results (Fig. 3f). This is possibly due to the ripening occurred in the initial period of soybean growth due to the remaining straw of ryegrass, which did not undergo any type of management that placed it in greater contact with the soil before soybean planting. In R_1 stage, there was no significance for the factors in the shoot dry matter and dry matter of nodules (Figs. 4b and 4d). For plant height, the presence of ryegrass resulted in a reduction in this variable (Fig. 4f).

Number of root tips, total root length and root volume in V_3 stage, as in R_1 stage, were not affected →

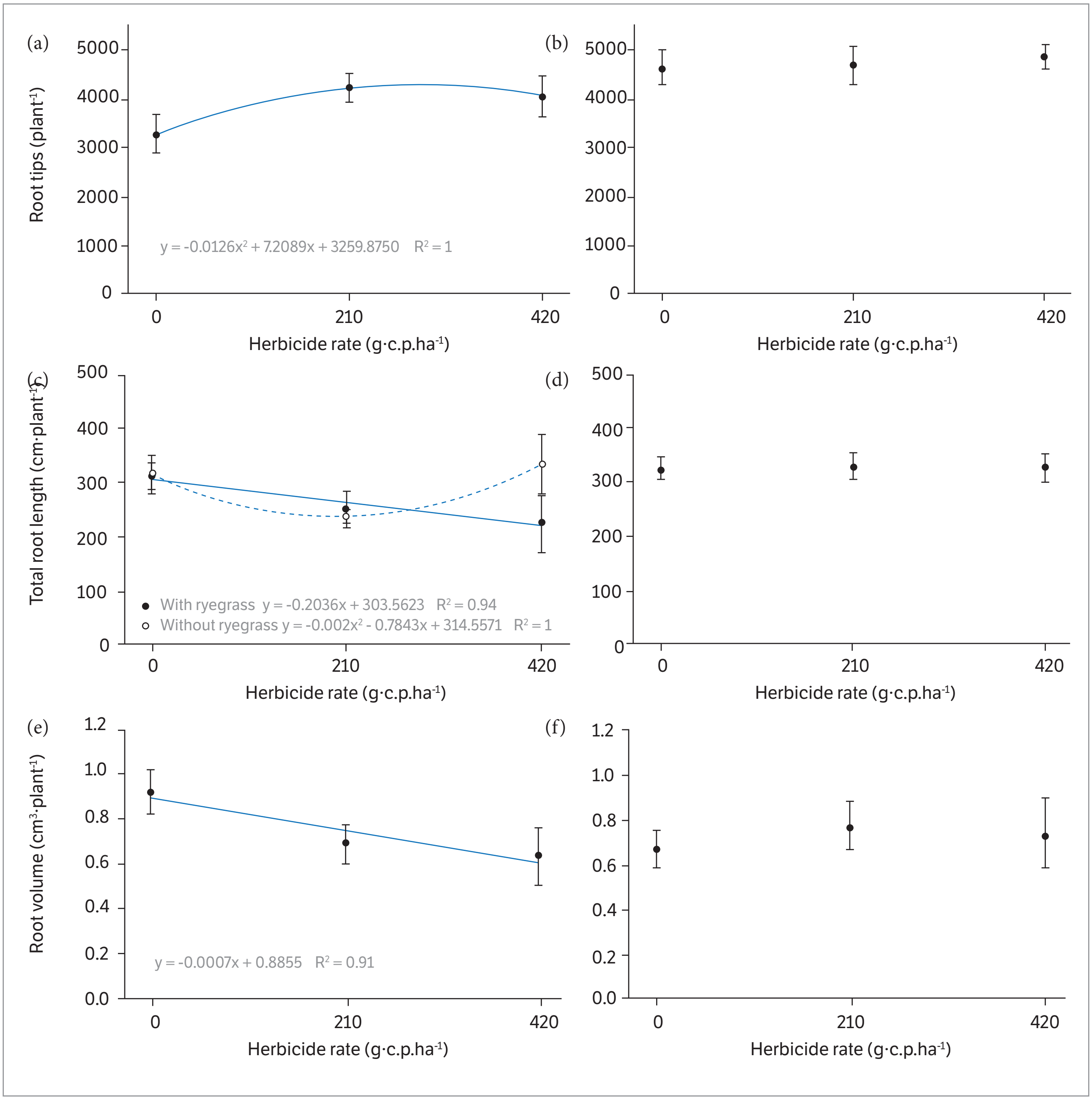

Figure 5. Number of tips, total length (cm·plant^{-1}) and root volume (cm^3·plant^{-1}) evaluated at V_3 stage due to different rates of imazapyr+imazapic herbicides applied 359 days before soybean seeding, and presence or absence of ryegrass in the off-season, in an experiment with soil moisture above 70% of field capacity (a, c and e) and with soil moisture under normal condition (b, d and f) in the off-season.

by herbicide residue and the presence of ryegrass in the off-season (Figs. 5b, 5d and 5f). However, ryegrass influenced grain yield. There was a reduction of approximately 360 kg·ha^{-1} when compared to the area with no ryegrass (Table 1). This crop had influence over more variables in this study in an isolated way, providing inferior results in relation to the permanence of uncovered area during the off-season. One of the explanations for this fact may be related to nitrogen immobilization. Straw decomposition and release of this nutrient is regulated by the C/N ratio. Therefore grasses, which have this high relation, tend to temporarily immobilize nitrogen (Acosta et al. 2014). In addition, there is the possibility of compounds releasing with allelopathic effect by ryegrass, which according to Nóbrega et al. (2009) reduces the emergence of seedlings and the fresh mass of soybean hypocotyls.

The difference in soil moisture between the two experiments during the off-season was determinant in the crops behavior (Fig. 1). The effect of the rates' residue of the herbicides was more emphasized, in the ryegrass as in the soybean, in the experiment with soil moisture above 70% field capacity in the off-season. There was probably less degradation of these herbicides in this condition due to the lower soil oxygenation and consequently lower activity of aerobic microorganisms. However, it is worth mentioning that for imazapyr the degradation in soil under anaerobic conditions is greater (Wang et al. 2006), suggesting that in the present study the herbicide with the greatest contribution to phytotoxicity in ryegrass and soybean was imazapic. In addition, the experiment with lower soil moisture may have provided greater herbicide rise in the soil along with water, from deeper layers to superficial layers, where there is more microbial activity, which may have resulted in greater degradation. Firmino et al. (2008a) identified upward behavior of the herbicide imazapyr in the soil, attributed to the evaporation that generated water movement by capillarity in the profile.

The high water solubility of imidazolinone herbicides may also have contributed to a greater availability of imazapyr and imazapic in the high soil moisture condition, with low clay content and soil organic matter, in addition to the pH of 5.8 in the 0 to 0.1 m layer, which provided low sorption to the soil matrix. The sorption of imazapyr is directly related to the organic matter, clay and the presence of iron oxides in the soil, while the pH is little related to this characteristic (Firmino et al. 2008b). According to a study by Aichele and Penner (2005), a higher percentage of imazamox, imazethapyr and imazaquin were found in soil solution at pH 7 in relation to pH 5. This reinforces the inverse relationship between pH and sorption of some herbicides imidazolinones.

In areas of irrigated rice where herbicides of the chemical group of imidazolinones are used, it is recommended to drain the soil throughout the off-season in order to stimulate the degradation of the residues and to minimize damage in successive crops to tolerant rice, such as ryegrass in the off-season and soybean in the next growing season. Moreover, the choice of genetic materials that have greater tolerance to deficit and to water excess also becomes important to reduce stresses to the plants that can potentiate the herbicide phytotoxicity.

CONCLUSION

The soil residue of the application of 420 g c.p.ha^{-1} of the formulated mixture of the imazapyr+imazapic herbicides performed 129 days before seeding causes phytotoxicity and reduction in the dry matter of ryegrass under high soil moisture conditon in the off-season in irrigated rice areas.

Soybean, when seeded 359 days after application, has its initial shoot and root growth affected by the residue of 210 and 420 g c.p.ha^{-1} rates in high soil moisture condition in the off-season, regardless of ryegrass cultivation during the same period. However, grain yield is not affected.

AUTHOR'S CONTRIBUTION

Conceptualization, Oliveira M. L., Marchesan E., Ulguim, A. R. and Coelho L. L.; Methodology, Oliveira M. L., Farias, J. G. and Coelho L. L.; Investigation, Oliveira, M. L., Soares, C. F., Farias, J. G., Fleck, A. G. and Coelho, L. L.; Writing – Original Draft, Oliveira M. L.; Writing – Review and Editing, Oliveira, M. L., Marchesan, E., Ulguim, A. R. and Coelho, L. L.; Funding Acquisition, Oliveira M. L. and Marchesan, E.; Resources, Marchesan, E.; Supervision, Marchesan E.

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