PLANTA DANINHA

SOCIEDADE BRASILEIRA DA CIÊNCIA DAS PLANTAS DANINHAS

<http://www.sbcpd.org>

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

Article

Morphological and Metabolic Changes in Soybean Plants Cultivated in Irrigated Rice Rotation and as Affected by Imazapyr and Imazapic Herbicides Carryover

Alterações Morfológicas e Metabólicas em Plantas de Soja Cultivada em Rotação ao Arroz Irrigado e em Função do Resíduo dos Herbicidas Imazapyr e Imazapic

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ABSTRACT - The appearance of soybean cultivars that are tolerant to herbicides from the imidazolinone group enables irrigated rice rotation conducted on Clearfield® system. The objective of this work was to evaluate the morphological and metabolic changes of soybean cultivars containing Cultivance® (CV), which is tolerant to imidazolinone, sulphonylurea toletant (STS) and Roundup Ready® (RR), which is tolerant to glyphosate as affected by carryover of increasing doses of imazapyr and imazapic herbicides mixture, applied in the management of irrigated rice crop. Thus, experiments were conducted on the field and in greenhouse, evaluating phytotoxicity, height, leaf area, shoot dry matter, as well as variables related to the secondary metabolism of plants like chlorophyll, carotenoids, hydrogen peroxide, lipid peroxidation and electrolytes leakage. Soybean cultivars RR and STS are susceptibles to the carryover of imazapyr and imazapic mixture applied in the management of irrigated rice crop, whereas the CV cultivar is resistant. The rise in the herbicide carryover dose increases the reactive oxygen species (ROS) production in soybean plants. The RR cultivar, in general, shows higher ROS production compared to the other soybean cultivars.

Keywords: imidazolinones, *Glicine max*, reactive oxygen species.

RESUMO - O surgimento de cultivares de soja tolerante a herbicidas do grupo das imidazolinonas possibilita a rotação de culturas com arroz irrigado, cultivado em sistema Clearfield®. O objetivo deste trabalho foi avaliar as alterações morfológicas e metabólicas de cultivares de soja contendo as tecnologias Cultivance® (CV), que é tolerante às imidazolinonas, tolerância às sulfonilureias (STS), e Roundup Ready® (RR), que é tolerante ao glyphosate, frente ao resíduo de doses crescentes da mistura dos herbicidas imazapyr e imazapic, aplicados no manejo da cultura do arroz irrigado. Para isso, foram realizados experimentos em campo e em casa de vegetação, onde foram avaliadas a fitotoxicidade, estatura, área foliar, massa da matéria seca da parte aérea, além de variáveis relacionadas ao metabolismo secundário das plantas, como teor de clorofilas e carotenoides, teor de peróxido de hidrogênio, peroxidação lipídica e extravasamento de eletrólitos. Os cultivares de soja RR e STS são suscetíveis ao resíduo da mistura dos herbicidas imazapyr e imazapic aplicado no manejo da cultura do arroz irrigado, enquanto o cultivar CV é resistente. A elevação da dose do resíduo do herbicida aumenta a produção de espécies reativas de oxigênio (EROs) em plantas de soja. O cultivar RR, em geral, apresenta maior produção de EROs, comparado aos demais.

Palavras-chave: imidazolinonas, *Glycine max*, espécies reativas de oxigênio.

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Received: June 15, 2016
Approved: September 2, 2016

Planta Daninha 2019; v37:e019165375

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Doi: 10.1590/S0100-83582019370100023

INTRODUCTION

Herbicides from the imidazolinone group are effective to control a wide spectrum of weeds; they are used in commercial mixtures, such as imazapyr+imazapic to control red rice, cultivated with the Clearfield® system. However, the presence of these herbicides in the soil may cause problems to non-tolerant cultures cultivated in succession or rotation (Bundt et al., 2015).

The rice/soybean rotation provides different advantages, since as well as facilitating the control of red rice, it also allows a better use of soil resources. However, there is a certain resistance to the use of this system due to the unfavorable physical characteristics for the cultivation of soybean, with little effective depth, impermeable layer close to the surface, little aeration and high density, which make the soil weak in terms of drainage (Vernetti and Vernetti Jr., 2013).

The rise of new technologies, such as Cultivance®, is an alternative for the management of red rice in areas with carryovers of herbicides from the imidazolinone group. These herbicides act inhibiting the acetolactate synthase enzyme (ALS), which act on the first step of the synthesis of the branched chain amino acids valine, leucine and isoleucine. The incapacity of cells to produce these amino acids causes cell death, since they are fundamental for the synthesis of proteins and derivates, as well as being essential for other metabolic routes.

The application of herbicides, even if a determined ingredient is selective for the culture, may cause alterations in the morphology of plants, such as phytotoxicity and consequent decrease in height, leaf area and dry matter mass of the aerial part (Song et al., 2007). The absorption of the herbicide by plants may cause alterations in the metabolism, bringing oxidative stress.

In plants, oxygen reactive species (ROS) may be produced in reactions occurred in mitochondria, chloroplast and peroxisomes. These molecules, such as hydroxyl radical (*OH), superoxide radical ($O_2{}^{*-}$) and hydrogen peroxide (H_2O_2), are extremely reactive and phytotoxic, causing lipid peroxidation and protein denaturing and are able to cause cell death (Gill and Tuteja, 2010).

In order to survive the action of ROS, plants have mechanisms that allow noticing and traducing external signals, in order to unleash adaptive responses. With this, plants developed two protection mechanisms: an enzymatic antioxidant system, where enzymes act as superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT); and another non-enzymatic one, where chlorophyll and carotenoids are included (Miller et al., 2011).

In the light of this, this work had the hypothesis that the carryover of the imazapyr and imazapic herbicide mixture causes phytotoxicity and reduces the height, leaf area and dry matter mass of the aerial part of susceptible soybean cultures, compared to resistant cultivars, containing the technology Cultivance®; and that the alteration on the secondary metabolism of soybean cultures that are susceptible to the herbicide is higher, compared to the resistant cultivar.

The objective of this study was to evaluate the morphological and metabolic changes of soybean cultivars containing the technologies Cultivance®, tolerance to sulfonylurea, and Roundup Ready® as affected by the carryover of increasing doses of the mixture of imazapyr and imazapic herbicides, applied in the management of irrigated rice crop.

MATERIAL AND METHODS

The study included experiments conducted on the field and in a greenhouse. On the field, the experiment was conducted in randomized block design, in the growing season 2014/15, where the management of weeds in the culture of irrigated rice was performed applying doses of imazapyr and imazapic mixture (0, 70, 140, 280 and 560 g ha^{-1}), where 140 g ha^{-1} was the suggested herbicide dose for control (AGROFIT, 2015). The other management practices were the recommended ones for the culture (SOSBAI, 2012).

In the greenhouse, the experiment was conducted in completely randomized experimental design, in factor scheme (3x5), with seven replications. The A factor was composed by the soybean cultivars BRS382CV (VC), CD249STS (STS) and NA5909RR (RR), containing the Cultivance®

technology, tolerance to sulfonylurea and Roundup Ready®, respectively); and the B factor, composed by carryovers of the imazapyr and imazapic herbicide mixture, applied in the management of the irrigated rice culture, according to what was described in the field experiment. The soil collected in the area up the 20 cm depth was placed in plastic pots with volumetric capacity of 3 L; 16 seeds were planted per pot and, afterwards, the thinning was performed to establish eight plants per experimental unit.

The evaluated variables were phytotoxicity for the culture, height, leaf area (LA), dry matter mass of the aerial part (APDMM) and secondary metabolites. Phytotoxicity was evaluated on day 20 and 30 after the emergence of soybean culture (DAE), corresponding to 375 and 385 days after the application of the herbicide in managing rice, on a percentage scale, where 0 (zero) represented the absence of damages and 100 (one hundred) the plant death (SBCPD, 1995). Height was evaluated on day 20 and 30 DAE with the help of a millimeter ruler, measuring the distance of the surface from the soil up to the insertion point of the last leaf. In order to evaluate the LA, soybean plants were sectioned at soil level, separating the leaves which were subsequently taken to a LI 3100C leaf area gauge. After quantifying this variable, the plant material was dried in an oven with forced air circulation, at 60 °C, for 72 hours and weighed to quantify APDMM.

On day 30 DAE leaf samples were collected, in three replications, and stored at -83 °C until secondary metabolism evaluations. The analyzed secondary metabolites were: chlorophyll and carotenoid content, hydrogen peroxide content, lipid peroxidation and electrolyte leakage.

The total contents of chlorophyll and carotenoids were determined with 0.1 g samples macerated in a mortar with 5 mL of acetone at 80% (v/v). The material was centrifuged at 12,000 rpm for 10 minutes, completing the volume for 20 mL with acetone at 80% (v/v). Chlorophyll a, b, total (a+b) and total carotenoid contents were calculated by the use of Lichtenthaler formulas (1987), starting from the absorbance of the solution, obtained by spectrophotometry at 647, 663 and 470 nm; the results were expressed in mg g^{-1} of fresh mass (FM).

Cell damages on tissues were determined in terms of hydrogen peroxide content (H_2O_2), as described by Sergier et al. (1997) and lipid peroxidation in terms of species reactive to thiobarbituric acid (TBARS), through the accumulation of malondialdehyde (MDA), as described by Heath and Packer (1968). In order to perform both analyses, 0.2 g of leaves were macerated with liquid nitrogen, homogenized in 2 mL of trichloroacetic acid (TCA) 0.1% (m/v) and centrifuged at 14,000 rpm for 20 minutes. In order to quantify H_2O_2, aliquots of 0.2 mL of supernatant were added to 0.8 mL of phosphate buffer 10 mM (pH 7.0) and 1 mL of potassium iodide 1 M, followed by agitation in vortex. The solution was kept static for 10 minutes at room temperature and, after that, the absorbance was read at 390nm. The H_2O_2 concentration was determined through the standard curve with known concentrations of H_2O_2 and expressed in mM g^{-1} FM.

In order to determine TBARS, aliquots of 0.5 mL of the aforementioned supernatant were added to 1.5 mL of acid (TBA) 0.5% (m/v) and trichloroacetic acid 10% (m/v) and incubated at 90 °C for 20 minutes. The reaction was paralyzed in ice bath for 10 minutes and, subsequently, the absorbance was determined at 532 nm, deducting the unspecific absorbance at 600 nm. The MDA concentration was calculated using the absorbance coefficient of 155 mM cm^{-1}, and the results were expressed in nM MDA g^{-1} of FM.

Cell damages were evaluated by the relative permeability of membranes, determined by electrolyte leakage, as described by Tarhanen et al. (1999). For this, 0.2 g of a sample were sectioned with scissors, placed in 25 mL of ultrapure water and left in water bath for four hours (25 °C). After this period, the initial conductance (iC) was verified, using a conductometer (Lutron, CD-4301). After this reading, the same samples were placed in water bath at 90 °C for two hours and the reading for the final conductivity (fC) was performed. The relative permeability was calculated by the relation iC/(iC+fC) x 100, and the result was expressed in percentage.

The obtained data were analyzed in terms of normality (Shapiro-Wilk test) and, subsequently, submitted o analysis of variance ($p \leq 0.05$). In case of statistical significance, the average comparison was performed, using the Tukey's test ($p \leq 0.05$) for the cultivar factor and the analysis of regression for the dose factor.

The analysis of regression was performed with the help of the SigmaPlot 10.0 software (Sigmaplot, 2007), adjusting data to the equations of polynomial regression, linear or quadratic type, depending on the variable.

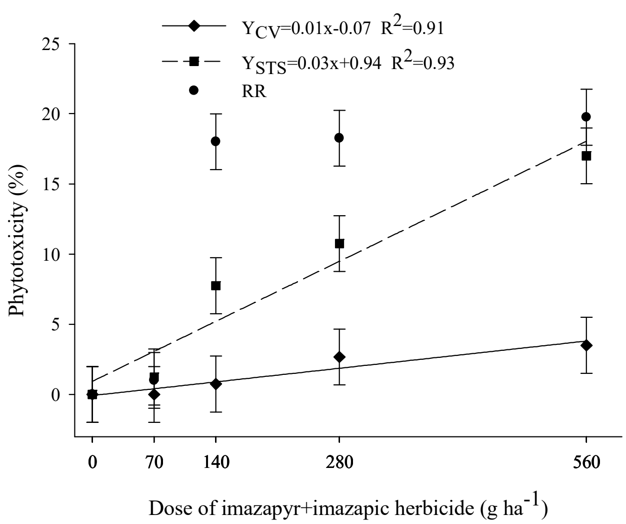
RESULTS AND DISCUSSION

There was an interaction between cultivar and dose factors for the phytotoxicity variables on day 20 and 30 after emergence (DAE), LA and APDMM (Figure 1, 2, 5 and 6). As for the height variable, on day 20 and 30 DAE, the simple effect of the cultivar (Table 1) and dose factors were observed (Figures 3 and 4).

On day 20 DAE, considering the carryover of the suggested herbicide dose (140 g ha^{-1}), cultivars STS and RR presented, approximately, 9 and 20 times more phytotoxicity respectively, compared to the cultivar CV (Figure 1). As for the herbicide doses, data referring to cultivar RR did not adjust to any model which biologically explained its behavior. As for cultivars STS and CV, it adjusted to the linear polynomial equation, with determination coefficient (R^2) varying from 0.91 to 0.93, demonstrating satisfactory adjustment. A linear increase in the phytotoxicity was observed as the dose increased; a double increase was verified in the variable for each added herbicide unit for the cultivar STS, compared to CV.

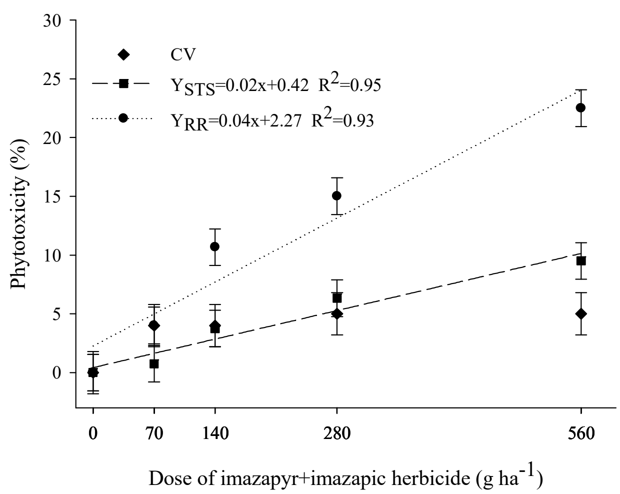
On day 30 DAE, it was observed that in the carryover of the suggested herbicide dose there was no difference between cultivars STS and CV, but cultivar RR presented twice more phytotoxicity compared to CV (Figure 2). As for the herbicide doses, data referring to cultivar CV did not adjust to the tested models. As for the other cultivars, data adjusted to the linear polynomial equation, with R^2 varying from 0.93 to 0.95, demonstrating adjustment. The linear increase in phytotoxicity was verified as the dose increased, observing that, as the carryover doubles, the phytotoxicity of cultivars RR and STS also doubles.

Similar results were found in the cultivation of irrigated rice containing the Clearfield® technology, where initial phytotoxicity was observed after the use of herbicides from the imidazolinone group, with later recovery of the plants (Villa et al., 2006). The application of herbicides from this group on sunflowers caused phytotoxicity to canola and beet cultures, planted



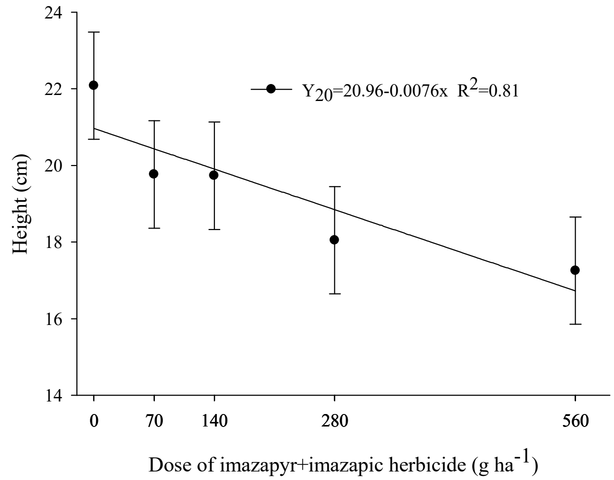
The points represent the average values of the replications among cultivars, and the bars, their respective confidence intervals of the average.

Figure 1 **-** Phytotoxicity (%) of plants from the soybean cultivars BRS382CV, CD249STS and NA5909RR, according to the carryover of increasing doses of imazapyr+imazapic, evaluated on day 20 after emergence (DAE) or 375 days after application.



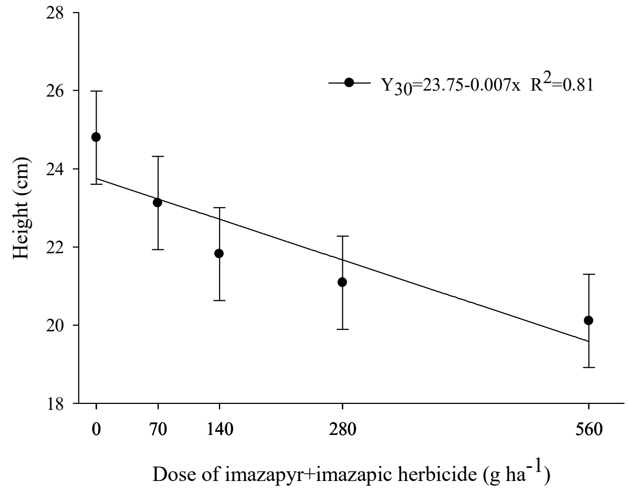
The points represent the average values of the replications among cultivars, and the bars, their respective confidence intervals of the average.

Figure 2 **-** Phytotoxicity (%) of plants from the soybean cultivars BRS382CV, CD249STS and NA5909RR, according to the carryover of increasing doses of imazapyr+imazapic, evaluated on day 30 after emergence (DAE) or 385 days after application.



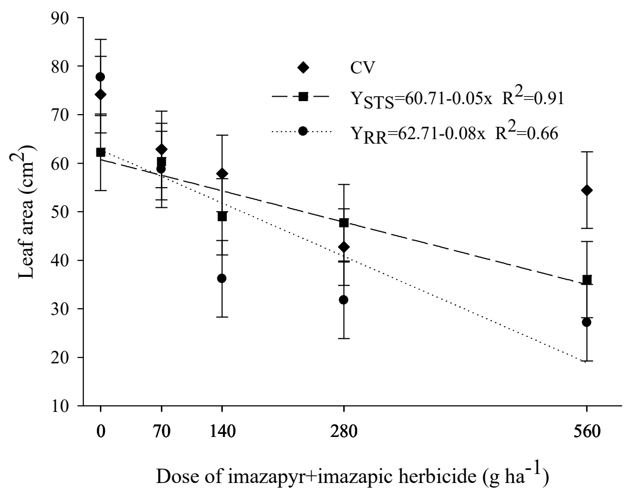
The points represent the average values of the replications among cultivars, and the bars, their respective confidence intervals of the average.

Figure 3 **-** Height (cm) of plants from the soybean cultivars submitted to the carryover of increasing doses of imazapyr+imazapic, evaluated on day 20 after emergence (DAE) or 375 days after application.



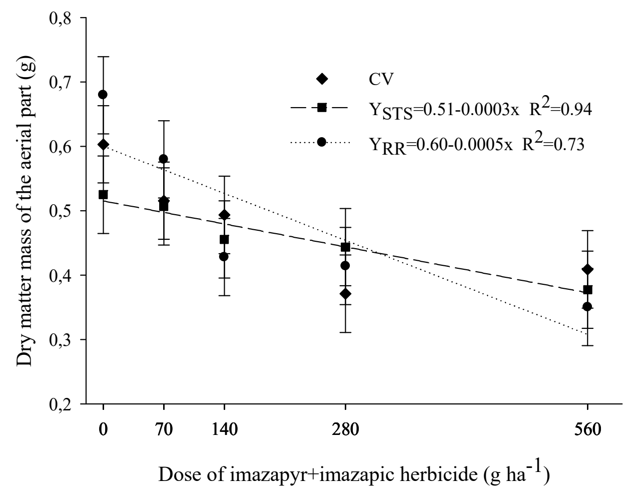
The points represent the average values of the replications among cultivars, and the bars, their respective confidence intervals of the average.

Figure 4 **-** Height (cm) of plants from the soybean cultivars submitted to the carryover of increasing doses of imazapyr+imazapic, evaluated on day 30 after emergence (DAE) or 385 days after application.



The points represent the average values of the replications among cultivars, and the bars, their respective confidence intervals of the average.

Figure 5 **-** Leaf area (cm^2) of plants from the soybean cultivars BRS382CV, CD249STS and NA5909RR, according to the carryover of increasing doses of imazapyr+imazapic, quantified on day 30 after emergence (DAE) or 385 days after application.



The points represent the average values of the replications among cultivars, and the bars, their respective confidence intervals of the average.

Figure 6 **-** Dry matter mass of the aerial part (APDMM) (g) of plants from the soybean cultivars BRS382CV, CD249STS and NA5909RR, according to the carryover of increasing doses of imazapyr+imazapic, quantified on day 30 after emergence (DAE) or 385 days after application.

in rotation (Süzer and Büyük, 2010). Moreover, the use of these herbicides in soybean culture caused phytotoxicity on maize plants planted in succession (Dan et al., 2012). Plants submitted to stress conditions, such as soybean in lowland environments, have more difficulties to metabolize herbicides that inhibit the ALS enzyme, keeping the product inside the plant for a longer time, which results into higher phytotoxicity (Roman et al., 2007).

Soybean CV and RR cultivars were higher on day 20 DAE, compared to the cultivar STS (Table 1). As for doses, data referring to the height adjusted to the linear polynomial equation,

Table 1 **-** Height (cm) of plants from the soybean cultivars BRS382CV, CD249STS and NA5909RR according to the carryover of imazapyr+imazapic, evaluated on day 20 and 30 after emergence (DAE) or 375 and 385 days after application

Cultivar	Height (cm)	
	20 DAE	30 DAE
BRS382CV	20.52 a	23.88 a
CD249STS	17.79 b	20.79 b
NA5909RR	19.77 a	22.07 b
VC (%)	10.61	7.65

Averages followed by the same letter in the column do not differ among themselves by Tukey's test at 5% probability.

with R^2 of 0.81, considered satisfactory. A linear decrease of the variable according to the increase in the herbicide dose was verified; it was observed that, as the carryover doubled, the variable was reduced in half (Figure 3). On day 30 DAE, the greatest height increase was observed on cultivar CV (Table 1). As for doses, data adjusted to the linear polynomial equation, with R^2 of 0.81, with a similar behavior to the one of the height on day 20 DAE (Figure 4).

On maize plants submitted to herbicide carryover from the imidazolinone group, applied in the management of soybean culture, a reduction in the plants' height was observed (Dan et al., 2012). An increase in the carryover of imazapyr+imazapic caused a decrease in the height of ryegrass, white clover and bird's foot trefoil plants, planted in succession to irrigated rice (Martins, 2014). The reduction in the height of plants after the application of herbicides from the imidazolinone group was observed in other cultures, such as ryegrass, maize and sorghum (Pinto et al., 2011). Herbicides that inhibit the ALS enzyme are characterized by presenting, among other symptoms, inhibition of growth points and reduction or paralysis of the plant development (Roman et al., 2007).

For the LA variable, considering a carryover in the 140 g ha^{-1} dose, there was no difference between the cultivars STS and CV. Cultivar RR presented a LA approximately twice as low compared to CV (Figure 5). As for the herbicide doses, data referring to cultivar CV did not adjust to any model which biologically explained its behavior. As for cultivars STS and RR, data adjusted to the linear polynomial equation, with R^2 varying from 0.66 to 0.91, demonstrating satisfactory adjustment. There was a linear decrease on the LA according to the dose increase; as the carryover doubles, the variable becomes the half for cultivars RR and STS (Figure 5). The LA decrease in soybean cultivars may be explained by the increase in phytotoxicity, according to the elevation of the dose of imazapyr+imazapic herbicide carryover (Figure 2). This result was expected because the cultivar BRS382CV presents resistance to herbicides from the imidazolinone group.

As for APDMM, there was no difference among the evaluated cultivars, considering the carryover of the suggested dose of 140 g ha^{-1} (Figure 6). As for the herbicide doses, data referring to cultivar CV did not adjust to any tested model. The other cultivars adjusted to the linear polynomial equation, with R^2 varying from 0.73 to 0.94, demonstrating adjustment. A linear decrease of the variable was observed according to the dose increase; it was observed that, as the carryover doubled, the variable halved for cultivars NA5909RR and CD249STS (Figure 6). In a work to evaluate the carryover activity of imazapyr+imazapic in the soybean culture, there was no difference in APDMM for the carryover of the suggested dose of 140 g ha^{-1}. However, the increase of the carryover of these herbicides decreased the APDMM of white clover and ryegrass planted in succession to irrigated rice (Martins, 2014).

As for the variables chlorophyll a, b and total, on day 20 DAE there was no interaction between the cultivar and doses factors. There was simple cultivar effect for the variables chlorophyll b content and total (Table 2). There was no significance for carotenoid content, chlorophyll a content and the a/b relation (data not presented). On day 30 DAE, there was no significance for any of the variables related to chlorophyll (data not presented).

Contents of chlorophyll b and total presented similar behavior; a higher increased in the variables was observed for the cultivar RR, compared to CV and STS (Table 2). The content of chlorophyll in the leaves also indicates the level of damage that a determined stress, such as the use of herbicides, may be causing to the plant, since chlorosis is, normally, one of the first expressed symptoms (Catunda et al., 2005).

As for the variable hydrogen peroxide content, the behavior was similar in both plant material harvest periods. There was no interaction between the studied factors for the variable on day 20 and 30 DAE; in both evaluations, simple effect for the cultivar factor was observed. Moreover,

Table 2 **-** Content of chlorophyll b (mg g^{-1}) and total chlorophyll (mg g^{-1}) of plants from the soybean cultivars BRS382CV, CD249STS and NA5909RR submitted to the carryover of imazapyr+imazapic, evaluated on day 20 after emergence (DAE) or 375 days after application

Cultivar	Chlorophyll b (mg g^{-1})	Total chlorophyll (mg g^{-1})
BRS382CV	1.57 b	1.77 b
CD249STS	1.43 b	1.61 b
NA5909RR	1.90 a	2.14 a
VC (%)	22.86	23.32

Averages followed by the same letter in the column do not differ among themselves by Tukey's test at 5% probability.

both on day 20 and 30 DAE, the content of hydrogen peroxide was higher in the cultivars RR and CV, compared to STS (Table 3).

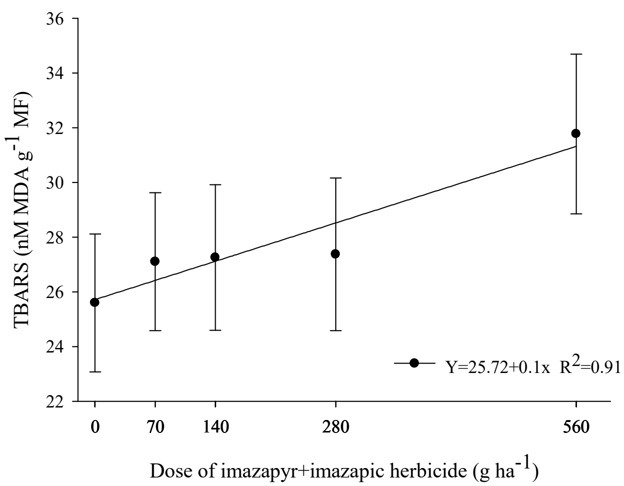
As for the lipid peroxidation variable, on day 20 DAE there was no interaction between the studied factors; only the simple effect was observed for the dose factor. Data adjusted in a satisfactory way to the linear polynomial equation, with R^2 of 0.91; a linear increase of the variable was observed according to the increase in the imazapyr+imazapic dose. As the carryover doubles, the value of the evaluated variable doubles (Figure 7).

As for the lipid peroxidation, on day 30 DAE there was interaction between the studied factors (Figure 8). Considering the carryover of the suggested dose of 140 g ha^{-1}, there was no difference among the cultivars. As for doses, data related to cultivars CV and STS did not adjust to the tested models; however, cultivar RR adjusted to the linear polynomial equation, with R^2 of 0.89, demonstrating satisfactory adjustment. The linear increase in the variable was verified as the dose increased, observing that, as the carryover doubles, the lipid peroxidation doubles for the cultivar RR.

Table 3 **-** Content of hydrogen peroxide (H_2O_2) (mM g^{-1} MF) of plants from the soybean cultivars BRS382CV, CD249STS and NA5909RR according to the carryover of imazapyr+imazapic, evaluated on day 20 and 30 after emergence (DAE) or 375 and 385 days after application

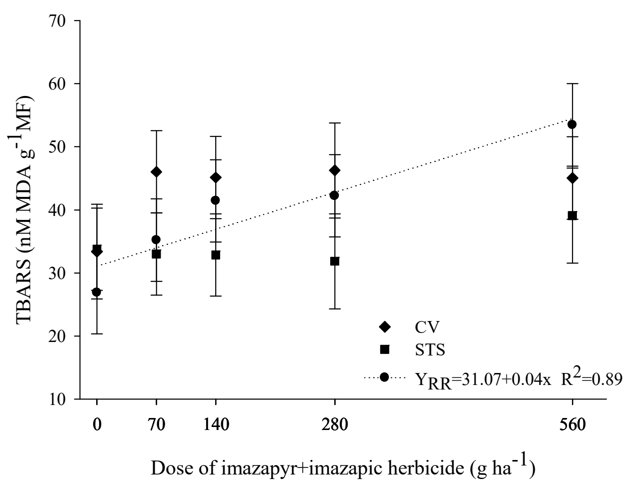
Cultivar	H_2O_2 content (mM g^{-1} MF)	
	20 DAE	30 DAE
BRS382CV	2.95 a	4.58 a
CD249STS	2.32 b	3.32 b
NA5909RR	3.18 a	4.21 a
VC (%)	20.08	22.80

Averages followed by the same letter in the column do not differ among themselves by Tukey's test at 5% probability.



The points represent the average values of the replications among cultivars, and the bars, their respective confidence intervals of the average.

Figure 7 **-** Lipid peroxidation in terms of species that are reactive to thiobarbituric acid (TBARS) (nM MDA g^{-1} MF) of plants from the soybean cultivars submitted to the carryover of imazapyr+imazapic, evaluated on day 20 after emergence (DAE) or 375 days after application.

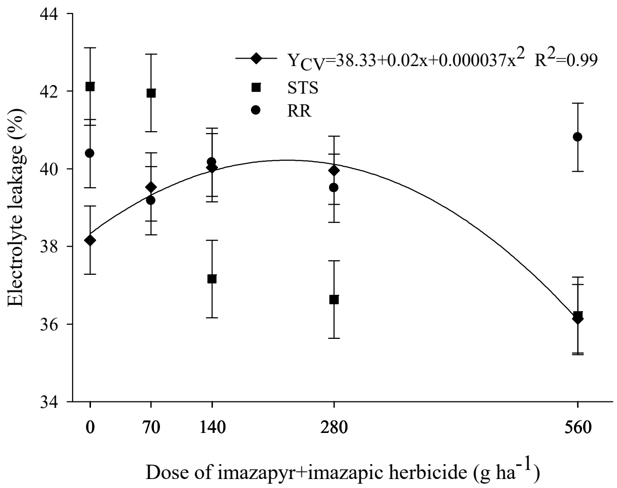


The points represent the average values of the replications among cultivars, and the bars, their respective confidence intervals of the average.

Figure 8 **-** Lipid peroxidation in terms of species that are reactive to thiobarbituric acid (TBARS) (nM MDA g^{-1} MF) of plants from the soybean cultivars submitted to the carryover of imazapyr+imazapic, evaluated on day 30 after emergence (DAE) or 385 days after application.

The higher increase in the lipid peroxidation of sugarcane plants was caused by the application of increasing doses of paraquat herbicide (Chagas, 2008). ROS, which cause elevated content of hydrogen peroxide and lipid peroxidation, under normal conditions must be in balance with the anti-oxidant system of plants. The greater the stress caused, in this case by the increase in the herbicide dose, higher will be the formation of ROS. The process of oxidative stress is caused when there is an unbalance between ROS and the anti-oxidant system (Gill and Tuteja, 2010).

As for the electrolyte leakage variable, on day 20 DAE there was interaction among the studied factors (Figure 9). Considering the carryover of the suggested herbicide dose, there was no difference between the cultivars CV and RR; however, the cultivar STS presented 7% less leakage, compared to the resistant cultivar. As for herbicide doses, data referring to the cultivars STS and RR did not adjust to any of the tested models, whereas the cultivar CV adjusted to the quadratic polynomial equation, with R^2 of 0.99, demonstrating satisfactory adjustment. A quadratic behavior of the curve was observed; for the cultivar CV, the maximum point was 270.3 g ha^{-1}, which corresponds to 41.04 %.



The points represent the average values of the replications among cultivars, and the bars, their respective confidence intervals of the average.

Figure 9 - Electrolyte leakage (%) in plants from the soybean cultivars BRS382CV, CD249STS and NA5909RR, according to the carryover of increasing doses of imazapyr+imazapic, evaluated on day 20 after emergence (DAE) or 375 days after application.

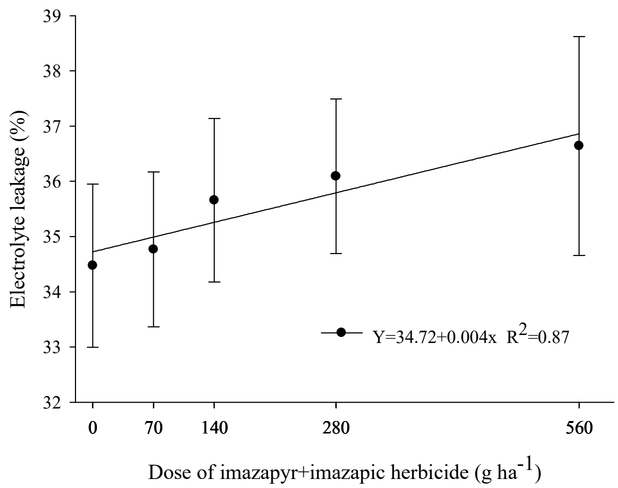
On day 30 DAE, there was no interaction between cultivar and doses for electrolyte leakage; there was simple effect for both studied factors. It was possible to observe higher electrolyte leakage in plants from the cultivar RR, compared to the others (Table 4). As for doses, data adjusted in a satisfactory way to the linear polynomial equation, with R^2 of 0.87. There was a linear increase in the variable according to the increase in the herbicide dose; it was verified that, as the dose value doubles, the value of the variable doubles (Figure 10).

The obtained result may be a consequence of the damages to the cell membranes coming from the lipid peroxidation, resulting into an leakage of the cell content to the medium that involves the damaged tissues. Thus, the determination of the electrolyte leakage in an incubation solution of plant tissues treated

Table 4 - Electrolyte leakage (%) in plants from the soybean cultivars BRS382CV, CD249STS and NA5909RR, submitted to the carryover of imazapyr+imazapic, evaluated on day 30 after emergence (DAE) or 385 days after application

Cultivar	Electrolyte leakage (%) on day 30 DAE
BRS382CV	33.19 b
CD249STS	34.98 b
NA5909RR	38.04 a
VC (%)	6.84

Averages followed by the same letter in the column do not differ among themselves by Tukey's test at 5% probability.



The points represent the average values of the replications among cultivars, and the bars, their respective confidence intervals of the average.

Figure 10 - Electrolyte leakage (%) in plants from soybean cultivars submitted to the carryover of increasing doses of imazapyr+imazapic, evaluated on day 30 after emergence (DAE) or 385 days after application.

with herbicides, through the electrical conductivity of the solution, constitutes one more variable for the evaluation of the herbicide effect at cell level (Li et al., 2000).

Results allow deducing that soybean cultivars NA5909RR and CD249STS are susceptible to the carryover of the imazapyr and imazapic herbicide mixture applied in the management of the irrigated rice culture, whereas cultivar BRS382CV is resistant. Moreover, the increase in the imazapyr+imazapic carryover dose increases the production of oxygen reactive species (ROS) in soybean plants; cultivar NA5909RR is, in general, the one presenting the highest production of ROS, compared to CD249STS and BRS382CV.

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