SBCPD
PLANTA DANINHA
SOCIEDADE BRASILEIRA DA CIÊNCIA DAS PLANTAS DANINHAS
<http://www.sbcpd.org>
ISSN 0100-8358 (print)
1806-9681 (online)

Article

Changes in the Metabolism of Soybean Plants Submitted to Herbicide Application in Different Weed Management Systems

Alterações no Metabolismo de Plantas de Soja Submetidas a Aplicação de Herbicidas em Diferentes Sistemas de Manejo de Plantas Daninhas

FRAGA, D.S.[1]*
AGOSTINETTO, D.[1]
RUCHEL, Q.[1]
LANGARO, A. C.[1]
OLIVEIRA, C.[1]

ABSTRACT - Technologies that advocate the use of herbicide-resistant crops are alternatives to weed control, but they may cause oxidative stress and change secondary metabolism of plants. Therefore, this study aimed to evaluate changes in the secondary metabolism of soybean plants which contained Cultivance® (CV), sulfonylurea-tolerant soybean (STS) and Roundup Ready® (RR) technologies submitted to the application of the mixture of herbicides imazapyr and imazapic in "plant-apply" and "apply-plant" management systems. Two field experiments, in which soybean cultivars were submitted to increasing doses of the mixture of herbicides imazapyr and imazapic, were performed. Aerial parts of plants were collected 10 days after crop emergence and stored at -83 °C, until quantification of variables. In general, the soybean cultivar BRS382CV exhibited lower contents of chlorophyll than cultivars CD249STS and NA5909RR. Besides, increasing doses of the mixture of herbicides imazapyr and imazapic decreased contents of chlorophyll in the cultivars. Stress caused by herbicides induced more generation of ROS and effective response of the antioxidant system through enzymes SOD, CAT and APX.

Keywords: *Glycine max*, oxidative stress, chemical control

RESUMO - A adoção de tecnologias que preconizam a utilização de culturas resistentes a herbicidas é alternativa para controle de plantas daninhas, porém pode causar estresse oxidativo e alterar o metabolismo secundário da cultura. Dessa forma, o objetivo deste trabalho foi avaliar as alterações no metabolismo secundário de plantas de soja contendo as tecnologias Cultivance® (CV), tolerância às sulfonilureias (STS) e Roundup Ready® (RR), submetidas à aplicação da mistura dos herbicidas imazapyr e imazapic nos sistemas de manejo em "plante/aplique e "aplique/plante". Foram realizados dois experimentos em campo, onde cultivares de soja foram submetidos a doses crescentes da mistura dos herbicidas imazapyr e imazapic. Aos dez dias após a emergência da cultura, foi realizada coleta da parte aérea e armazenada a -83 °C, até a quantificação das variáveis. O cultivar BRS382CV apresenta, em geral, menores teores de clorofila comparado aos cultivares CD249STS e NA5909RR, e o aumento da dose da mistura dos herbicidas imazapyr e imazapic diminui o teor de clorofila das cultivares. O estresse causado pelo herbicida induz maior formação de EROs, ocasionando elevada resposta do sistema antioxidante através das enzimas SOD, CAT e APX.

Palavras-chave: *Glycine max*, estresse oxidativo, controle químico.

*** Corresponding author:**
<fragadiegos@gmail.com>

Received: October 17, 2018
Approved: February 21, 2019

Planta Daninha 2020; v38:e020215525

[1] Universidade Federal de Pelotas, UFPel, Pelotas-RS, Brasil.

Doi: 10.1590/S0100-83582020380100043

INTRODUCTION

Weeds can cause significant losses in crop productivity if they are not adequately controlled. Thus, control strategies have been developed by biotechnology, such as herbicide-resistant crops and management systems. Cultivance® (CV), sulfonylurea-tolerant soybean (STS) and Roundup Ready® (RR) technologies were developed to enable efficient control of weed infestation from soybean sowing.

Regarding management systems, the so-called "plant-apply" one consists in applying a herbicide right after sowing, up to V_1 stage of the crop, thus, eliminating early competition (Basf, 2014). Another system that is used for managing weeds, which is called "apply/plant", uses pre-emergent herbicides and is based on track records of weeds in an area. This system is employed by many farmers in order to save time and maximize the use of their farm equipment (Oliveira Jr. et al., 2006).

The use of herbicides causes stress to plants and leads to the development of Reactive Oxygen Species (ROS), which damage plant metabolism. In normal conditions, plant production and removal are balanced, but, under stress, there may be imbalance between ROS and the antioxidant system, thus, ignoring removal mechanisms and triggering oxidative stress (Gill and Tuteja, 2010).

In general, ROS levels are controlled by the enzyme system which comprises antioxidant enzymes, such as superoxide dismutase (SOD) (converts the superoxide radical into hydrogen peroxide), ascorbate peroxidase (APX) (converts hydrogen peroxide (H_2O_2) into ascorbate) and catalase (CAT) (transforms hydrogen peroxide into water) (Karuppanapandian et al., 2011). These enzymes are important to mechanisms of recovery and protection of plants submitted to oxidative stress. In the non-enzymatic antioxidant system, several compounds, such as chlorophylls and carotenoids, can be found; they are responsible for the photoprotection of photosynthetic membranes, since they act as accessory pigments.

Therefore, this study aimed at evaluating changes in the secondary metabolism of soybean plants which contained Cultivance® (CV), sulfonylurea-tolerant soybean (STS) and Roundup Ready® (RR) technologies and were submitted to the application of the mixture of herbicides imazapyr and imazapic in "plant-apply" and "apply-plant" management systems.

MATERIAL AND METHODS

Two field experiments were carried out from December 2014 to January 2015 in a completely randomized design, with four replicates. They were conducted in the following 3x5 factorial scheme: factor A corresponded to soybean cultivars BRS382CV, CD249STS and NA5909RR, which contained Cultivance® (CV), sulfonylurea-tolerant soybean (STS) and Roundup Ready® (RR) technologies, respectively, while factor B corresponded to increasing doses of the mixture of herbicides imazapyr and imazapic (0; 24.5; 49; 73.5 and 98 g i.a. ha^{-1}), up to the recommended dose of 98 g i.a. ha^{-1} (AGROFIT, 2014). The first experiment was carried out in the "plant/apply" system, in which treatments were applied one day after sowing. The second experiment was conducted in the "apply/plant" system, in which soybean was sowed one day after herbicides were applied.

In both experiments, treatments were applied by a CO_2-pressurized backpack sprayer equipped with a 110.015 flat fan spray nozzle. It was calibrated to apply 120 L ha^{-1} syrup, to which 0.5% v/v of adjuvant Dash® was added. Soybean cultivars were manually sown; spacing was 0.5 m, 20 seeds were used per meter and every experimental unit stretched over 3 m^2. Ten days after crop emergence, leaf samples were collected in a 1-meter area – where a 0.5-meter border was left – and stored at -83 °C up to the analyses. The following variables were analyzed: contents of *a*, *b*, *a*/*b* and total chlorophylls, total carotenoids, hydrogen peroxide, lipid peroxidation, total proteins and activity of enzymes SOD, CAT and APX.

Contents of chlorophylls (*a*, *b*, total and *a*/*b* relation) and total carotenoids were determined by the methodology described by Hiscox and Israelstam (1979) and calculated by Lichtenthaler's equations (1987). Results were expressed as mg g^{-1} fresh mass (FM). Besides, the *a*/*b* relation was calculated by the arithmetic calculation of the ratio of both variables.

Cell damage in tissues was determined in terms of content of hydrogen peroxide (H_2O_2), as described by Sergier et al. (1997), and of thiobarbituric acid (TBARS) reactive substances, through accumulation of malonic aldehyde (MDA), as described by Heath and Packer (1968).

In order to determine the activity of antioxidant enzymes SOD, CAT and APX, protein contents of samples were quantified by the Bradford assay (1976). SOD activity was determined by the methodology adapted from Giannopolitis and Ries (1977), while CAT and APX activities were found by methodologies described by Azevedo et al. (1998) and by Nakano and Asada (1981), respectively.

Resulting data were analyzed regarding normality (Shapiro-Wilk test) and then submitted to the analysis of variance (pd”0.05). When statistical significance was found, means were compared by the Tukey's test (pd”0.05) to cultivars, while the regression analysis was carried out to doses. Data were adjusted to the equation of linear polynomial regression, as follows:

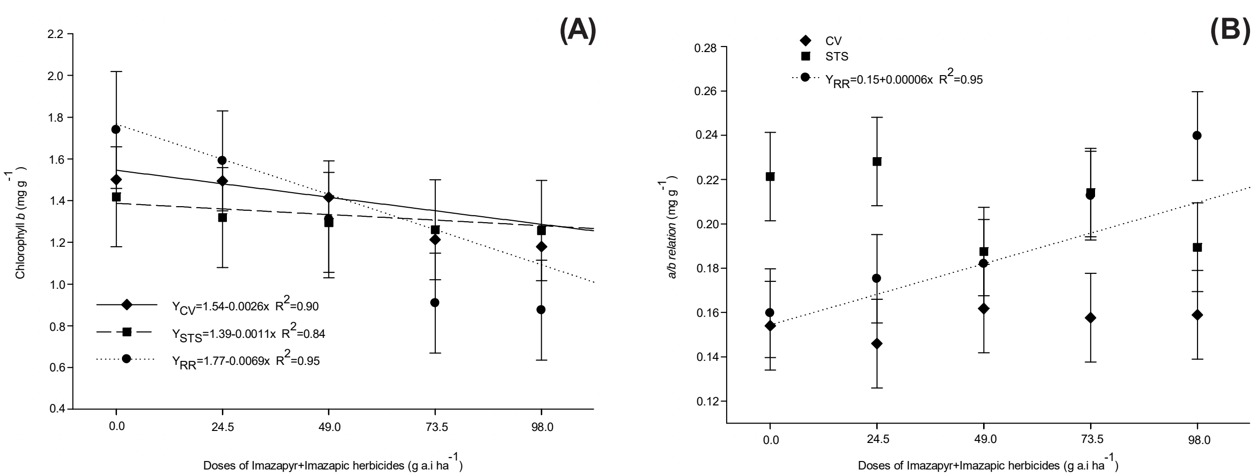
$$y = a + b.x$$

where y is the response-variable; x is the herbicide dose; and *a* and *b* are parameters of the equation (*a* is the intercept or linear coefficient and *b* is the slope of the straight).

RESULTS AND DISCUSSION

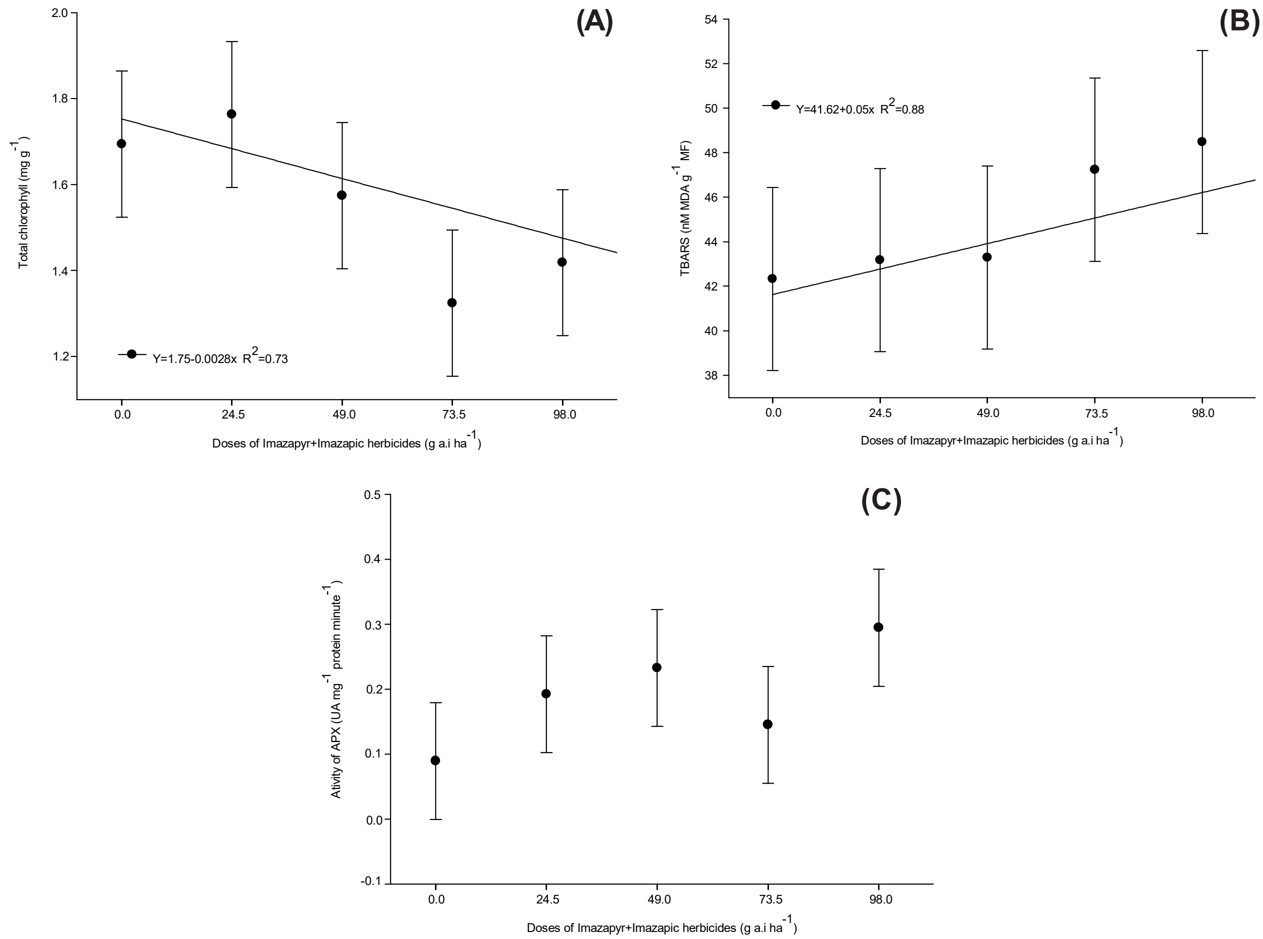
In the experiment that used the "plant/apply" system, interaction was found between both factors – cultivars and herbicide doses – in the cases of contents of *b* chlorophyll and *a*/*b* relation (Figures 1A and B). Concerning contents of total chlorophylls, lipid peroxidation and activity of enzyme APX, a simple effect of the factor dose was observed (Figures 2A, B and C). In the case of the content of hydrogen peroxide, interaction was found among factors under study (Figure 3). Some variables, i. e., contents of *a* chlorophyll, carotenoids, proteins, SOD activity and CAT activity did not exhibit any statistical significance (data not shown).

Regarding the content of *b* chlorophyll, there was no difference among cultivars under evaluation. In the case of herbicide doses, data adjusted to the linear polynomial equation; coefficients of determination (R^2) ranged from 0.84 to 0.95, a fact that showed satisfactory data adjustment. There was linear decrease of the variable as imazapyr+imazapic doses increased. Decreases were 0.03, 0.06 and 0.17 mg g^{-1} in every unit to which herbicides were added, considering cultivars STS, CV and RR, respectively (Figure 1A).



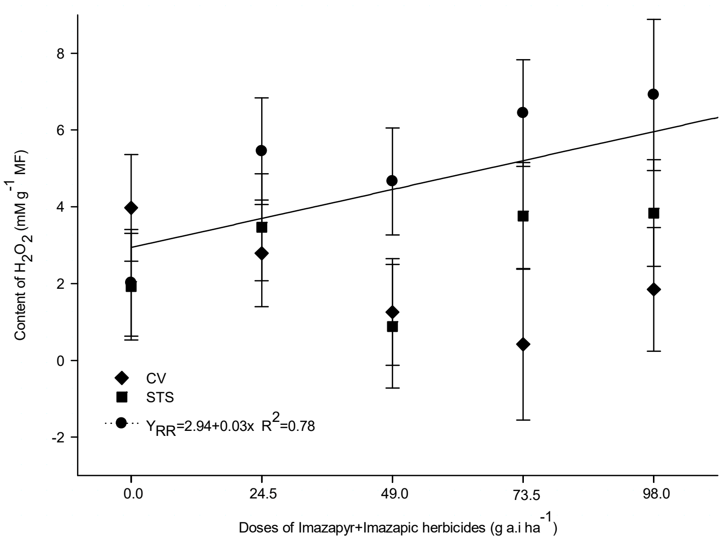
Points represent mean values of replicates among cultivars and bars represent confidence intervals of the mean.

Figure 1 - Contents of chlorophyll *b* (mg g^{-1}) (A) and relation between contents of chlorophylls *a*/*b* (mg g^{-1}) (B) in soybean cultivars BRS382CV, CD249STS and NA5909RR submitted to increasing doses of herbicides imazapyr and imazapic in a "plant/apply" system.



Points represent mean values of replicates among cultivars and bars represent confidence intervals of the mean.

Figure 2 - Contents of total chlorophyll (mg g^{-1}) (A), lipid peroxidation in terms of thiobarbituric acid (TBARS) reactive substances (nM MDA g^{-1} MF) (B) activity of enzyme APX (UA mg^{-1} protein minute^{-1}) (C) of soybean cultivars submitted to increasing doses of herbicides imazapyr and imazapic in a "plant/apply" system.



Points represent mean values of replicates among cultivars and bars represent confidence intervals of the mean.

Figure 3 - Contents of hydrogen peroxide (mM g^{-1} MF) in soybean cultivars BRS382CV, CD249STS and NA5909RR submitted to increasing doses of herbicides imazapyr and imazapic in a "plant/apply" system.

Concerning the *a/b* chlorophyll relation, there was difference among cultivar CV and the others when the imazapyr+imazapic dose was 73.5 g i.a. ha^{-1}. Likewise, difference was found among cultivar STS and the others when herbicide doses were 0.0 and 24.5 g i.a. ha^{-1}. Besides, at 98.0 g i.a. ha^{-1}, there was difference among cultivar RR and the others (Figure 1B). Concerning doses, data on cultivars CV and STS did not adjust to any model that could explain their behavior biologically. However, cultivar RR adjusted to the linear polynomial equation, whose R^2 was 0.95. Linear increase in the variable was observed in the case of cultivar RR, as herbicide doses increased; 0.00147 mg g^{-1} was the increase found in every unit to which herbicides were added (Figure 1B).

Data on the content of total chlorophyll adjusted to the linear polynomial equation, whose R^2 was 0.73. There was linear decrease in the variable as imazapyr+imazapic doses increased; 0.07 mg g^{-1} was the decrease found in every unit to which herbicides were added (Figure 2A). Decrease in the content of pigments due to the activity of herbicides is the consequence of oxidative stress, which leads to decrease in photosynthesis and shows that the content of chlorophylls may be a biomarker in plant growth. This consequence may be attributed to the fact that damage to the photosynthetic system result in decrease in chlorophyll levels (Gill and Tuteja, 2010).

Regarding lipid peroxidation, data adjusted to the linear polynomial equation, whose R^2 was 0.88. Linear increase in the variable was observed as imazapyr+imazapic doses increased; 1.225 nM MDA g^{-1} MF was the increase observed in every unit to which herbicides were added (Figure 2B).

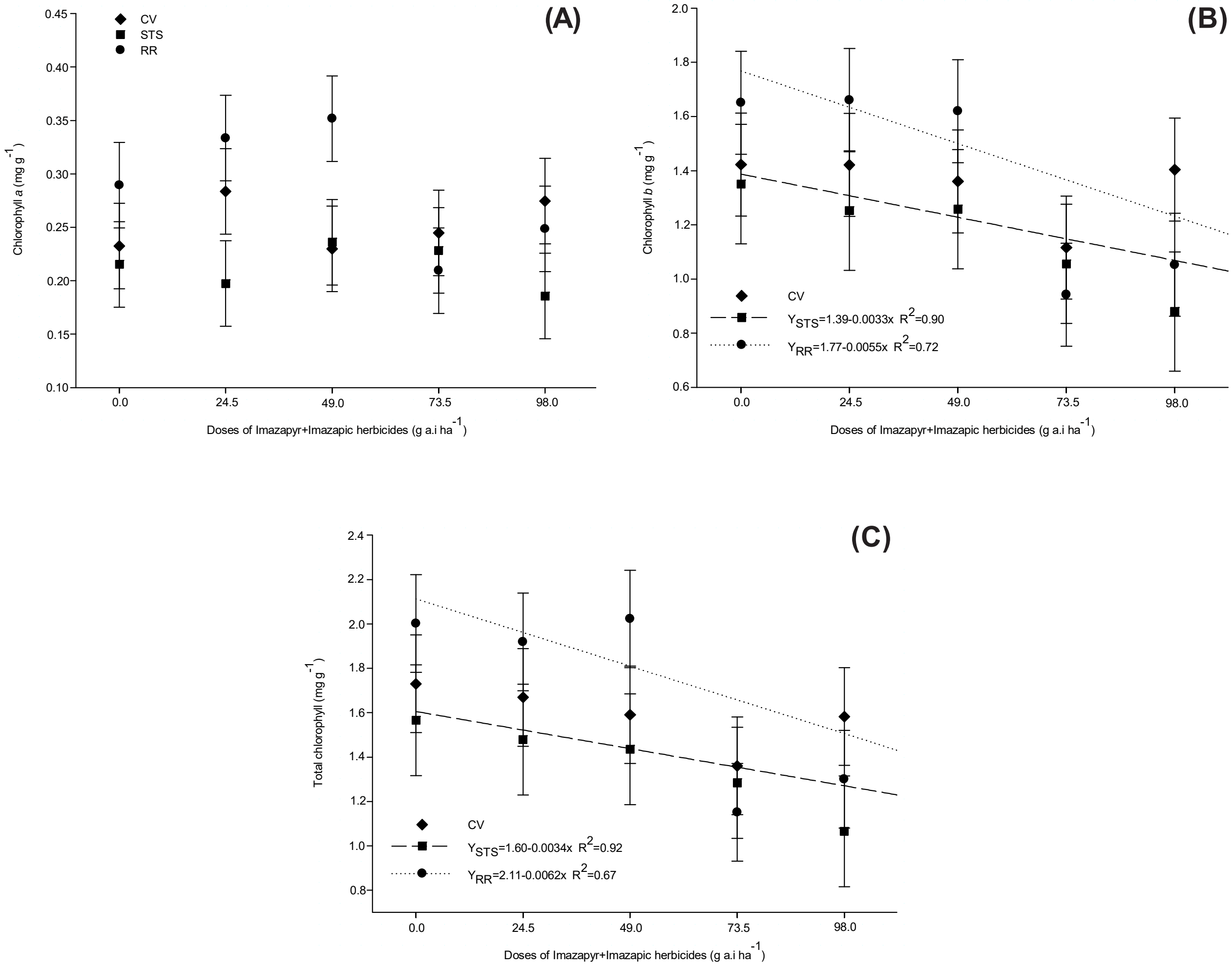
Activity of enzyme APX did not show any difference among doses. In addition, data did not adjust to any model that could explain its behavior biologically (Figure 2C). This result may be explained by the fact that the content of hydrogen peroxide increases as herbicide doses increase, since enzyme APX has high affinity with hydrogen peroxide. There was no significance in the case of activity of enzymes SOD and CAT, which shows that H_2O_2 accumulation was not enough to activate the plant defense mechanism.

Concerning the content of hydrogen peroxide, both cultivars CV and RR exhibited differences at the three highest doses under investigation. In terms of herbicide doses, data on cultivars STS and CV did not adjust to any model that could explain their behavior biologically. However, cultivar RR adjusted to the linear polynomial equation, whose R^2 was 0.78. In general, cultivar RR exhibited ascending linear behavior of the variable as imazapyr+imazapic doses increased; 0,735 mM g^{-1} MF was the increase found in the variable in every unit to which herbicides were added (Figure 3). Increase in the activity of enzyme SOD may be one of the reasons why there is high lipid peroxidation, since, as activity of SOD increases, there is also increase in contents of hydrogen peroxide (Gill and Tuteja, 2010).

In the experiment that used the “apply/plant” system, interaction was found between cultivars and doses concerning contents of *a*, *b* and total chlorophylls (Figures 4A, B and C). Regarding the relation between *a/b* chlorophyll and activity of enzyme SOD, simple effect of the factor dose was observed (Figures 5A and B). There was interaction among factors under study in the cases of the following variables: contents of protein, CAT activity and APX activity (Figures 6A, B and C). No statistical significance was found in the cases of contents of carotenoids, hydrogen peroxide and lipid peroxidation (data not shown).

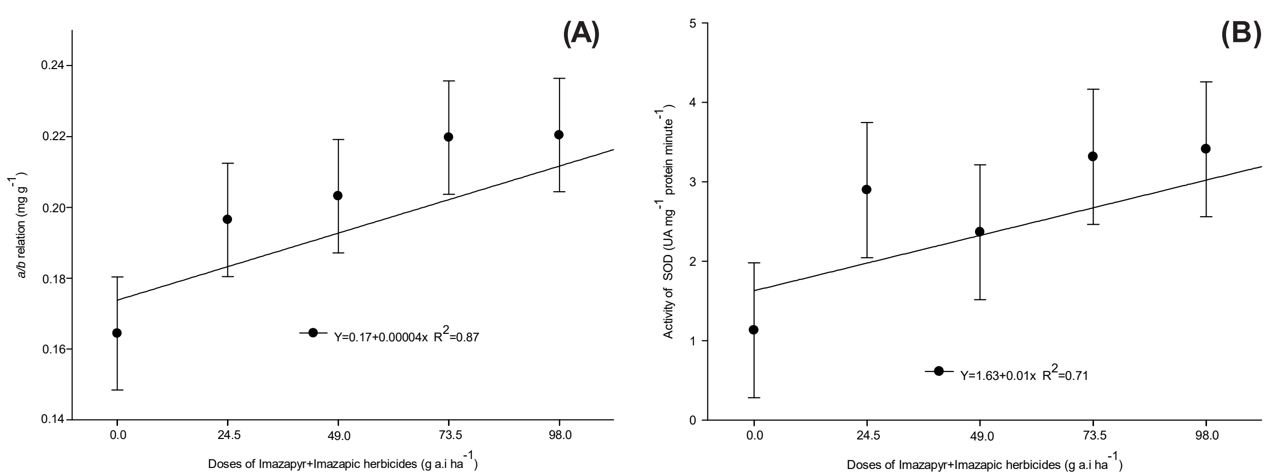
Concerning the content of *a* chlorophyll, its data did not adjust to the polynomial model (Figure 4A). Similar result was found with herbicides imazapyr+imazapic, which, in general, did not cause any effect on the content of *a* chlorophyll in irrigated rice plants (Langaro et al., 2016). On the other hand, herbicides bentazon, cyhalofop and penoxsulam led to effects on rice photosynthetic pigments (Nohatto et al., 2016).

Contents of *b* chlorophyll exhibited differences between cultivars STS and CV when the herbicide dose was 98 g i.a. ha^{-1}. Low contents of *b* chlorophyll were only found in the case of cultivar RR when imazapyr+imazapic doses were 73.5 g i.a. ha^{-1} and 98 g i.a. ha^{-1}. Regarding herbicide doses, data on cultivar CV did not adjust to any model that could explain its behavior biologically, while both cultivars STS and RR adjusted to the linear polynomial equation, whose R^2 ranged from 0.72 to 0.90, thus, showing satisfactory adjustment. Linear decrease in the



Points represent mean values of replicates among cultivars and bars represent confidence intervals of the mean.

Figure 4 - Contents of chlorophyll *a* (mg g^{-1}) (A), chlorophyll *b* (mg g^{-1}) (B) and total chlorophyll (mg g^{-1}) (C) in soybean cultivars BRS382CV, CD249STS and NA5909RR submitted to increasing doses of herbicides imazapyr and imazapic in a "apply/plant" system.



Points represent mean values of replicates among cultivars and bars represent confidence intervals of the mean.

Figure 5 - Relation between contents of chlorophylls *a*/*b* (mg g^{-1}) (A) and activity of enzyme SOD (UA mg^{-1} protein $minute^{-1}$) (B) in soybean cultivars submitted to increasing doses of herbicides imazapyr and imazapic in a "apply/plant" system.

variable was observed as imazapyr+imazapic doses increased; decreases were 0.08 mg g^{-1} and 0.135 mg g^{-1} in every unit to which herbicides were added, considering cultivars STS and RR, respectively (Figure 4B).

The behavior of contents of total chlorophylls was similar to the one of *b* chlorophyll. Difference was found at the highest dose in the experiment with cultivars STS and CV. When herbicide doses were 73.5 g i.a. ha^{-1} and 98 g i.a. ha^{-1}, cultivar RR exhibited low contents of total chlorophylls. Concerning doses, there was no adjustment for data on cultivar CV, while the others adjusted to the linear polynomial equation, whose R^2 ranged from 0.67 to 0.92. There was linear decrease in the variable as herbicide doses increased; decreases were 0.08 mg g^{-1} and 0.15 mg g^{-1} in every unit to which herbicides were added, considering cultivars STS and RR, respectively (Figure 4C). The analysis of changes in the photosynthetic metabolism of sugar cane plants as the result of the herbicide paraquat showed decrease in contents of *a*, *b*, and total chlorophyll when herbicide doses increased (Chagas et al., 2008). Similarly, application of doses of herbicide imazaquin to different soybean cultivars led to decrease in contents of chlorophyll as the result of increase in its doses (Cayon et al., 1990).

Regarding the *a*/*b* relation and herbicide doses, data adjusted to the linear polynomial equation, whose R^2 was 0.87. There was linear increase as the result of increase in herbicide doses; increase was 0.00098 mg g^{-1} in every unit to which imazapyr and imazapic were added (Figure 5A).

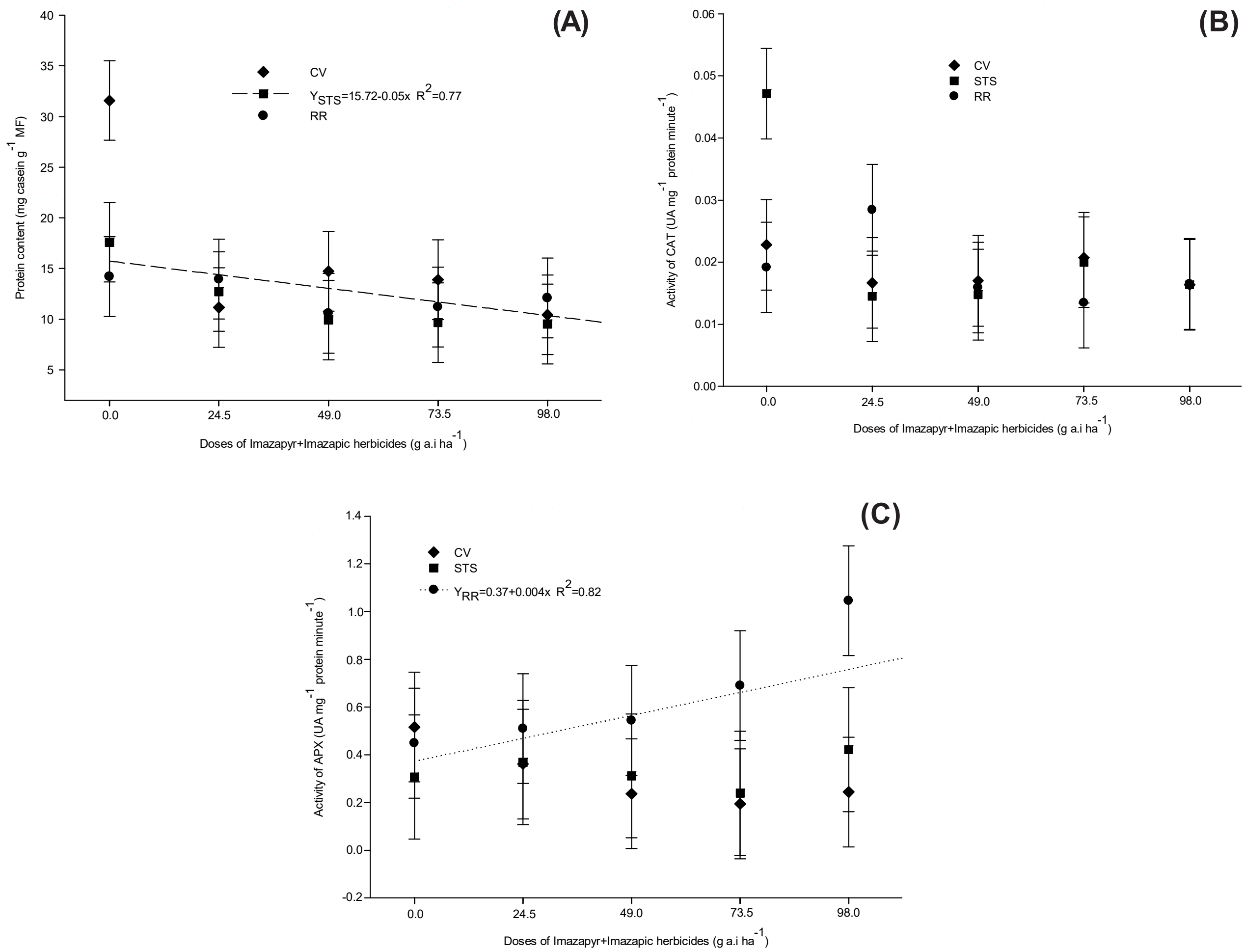
Data on SOD activity adjusted to the linear polynomial equation, whose R^2 was 0.71, which was considered satisfactory (Figure 5B). There was linear increase in the variable as doses of imazapyr+imazapic increased; 0.245 UA mg^{-1} protein $minute^{-1}$ was the increase found in every unit to which herbicides were added. In a similar study, increase in SOD activity was found as the result of herbicide lactofen, by comparison with the control treatment in a soybean crop (Ferreira et al., 2010). Besides, increase in the doses of herbicide oxyfluorfen was found to increase SOD activity in soybean plants (Cataneo et al., 2010).

Increase in SOD activity as the result of stress caused by certain herbicides is due to ROS accumulation, mainly in conditions that may lead to cell death (Langaro et al., 2016). SOD, which is considered the first line defense against damage caused by ROS, catalyzes the conversion of superoxide anion into H_2O_2 and O_2 in chloroplasts, mitochondrias and peroxisomes, thus, interfering in concentrations of ROS involved in the Haber-Weiss reaction to produce the radical hydroxyl (Wang et al., 2012).

Contents of protein showed difference among cultivar CV and the others in the witness with no application, which exhibited the highest protein content for cultivar CV. Difference among the witness and the other doses was only found for cultivar CV (Figure 6A). Concerning herbicide doses, data on both cultivars CV and RR did not adjust to any model that could explain their behavior biologically. In the case of cultivar STS, data adjusted to the linear polynomial equation, whose R^2 was 0.77. In general, cultivar STS exhibited the descending linear behavior of the variable as doses of imazapyr+imazapic increased; decrease was 1.225 mg casein g^{-1} MF in every unit to which herbicides were added (Figure 6A). Proteins, which are found in all parts of cells, are fundamental to cell structures and functions. Changes in contents of protein may represent damage to plant growth and development. Some studies have shown that herbicide application may result in low protein synthesis (Sood et al., 2012).

Data on CAT activity did not adjust to the models under investigation (Figure 6B). Similar results were found when application of imazapyr+imazapic to irrigated rice plants resulted in lower activity of enzyme CAT than the one of the witness (Langaro et al., 2016).

Regarding activity of enzyme APX, difference was found among cultivar RR and the others when the herbicide dose was 98 g i.a. ha^{-1}. In terms of doses, data on both cultivars CV and STS did not adjust to any model that could explain their behavior biologically. However, in the case of cultivar RR, data adjusted to the linear polynomial equation, whose R^2 was 0.82. Decreasing linear behavior of the variable was observed as herbicide doses increased; decrease was 0.098 mg casein g^{-1} MF in every unit to which imazapyr+imazapic was added (Figure 6C). Application of pre-emergent herbicides oxadiazon and pendimethalin led to high activity of enzyme APX in irrigated rice plants (Langaro et al., 2017). A similar study showed that the activity of



Points represent mean values of replicates among cultivars and bars represent confidence intervals of the mean.

Figure 6 - Contents of protein (mg casein g^{-1} MF) (A), activity of CAT (UA mg^{-1} protein $minute^{-1}$) (B) and activity of APX (UA mg^{-1} protein $minute^{-1}$) (C) in soybean cultivars BRS382CV, CD249STS and NA5909RR submitted to increasing doses of herbicides imazapyr and imazapic in a "apply/plant" system.

enzyme APX increased in both wheat leaves and roots submitted to chlortoluron application (Song et al., 2007).

In order to keep ROS under control, plants exhibit balance between ROS and their antioxidant system. Increase in SOD activity usually leads to increase in contents of hydrogen peroxide. Since enzymes of the antioxidant system do not directly eliminate *OH, regulation of their precursors O_2^{-*} and H_2O_2 is a fundamental step to prevent risks caused by *OH, when enzymes SOD, APX and CAT work together (Bhattacharjee, 2010). Lipid peroxidation is one of the most investigated consequences of ROS activity in membrane structures, because it is one of the first responses given to damage caused by stress in plant tissues (Amri and Shahsavar, 2010).

Therefore, based on results of this study, it may be stated that further investigation is needed to help understand changes in secondary metabolism of soybean plants submitted to stress by herbicides. For instance, other molecules, systems and/or stages should be evaluated, not only because they are the most common tools in weed management, but also because damage to plant metabolism may affect crop productivity.

Results of this study show that, in general, cultivar BRS382CV has lower contents of chlorophyll than both cultivars CD249STS and NA5909RR and that increase in doses of the mixture of imazapyr and imazapic decreases contents of chlorophyll of cultivars. Increase in stress resulting from herbicide doses leads to more ROS and high response of the antioxidant system through enzymes SOD, CAT and APX.

REFERENCES

AGROFIT. Sistema de agrotóxicos fitossanitários. [accessed on Oct 10th, 2014]. Available at: http://agrof it.agricultura.gov.br/agrof it_cons/principal_agrofit_cons

Amri IE, Shahsavar A. Response of lime seedlings (*Citrus aurantifolia* L.) to exogenous spermidine treatments under drought stress. Aust J Basic Appl Sci. 2010;4:4483-9.

Azevedo RA, Smith RJ, Lea PJ. Response of antioxidant enzymes to transfer from elevated carbon dioxide to air and ozone fumigation, in the leaves and roots of wild-type and a catalase-deficient mutant of barley. Physiol Plant. 1998;104:280-92.

BASF – Sistema Cultivance. 2015. [accessed on Oct 10th, 2014]. Available at: http://www.agro.basf.com.b r/agr/ms/apbrazil/ptBR/function/conversions:/publish/content/APBrazil/solutions/cultivance/sistema.html.

Bhattacharjee S. Sites of generation and physicochemical basis of formation of reactive oxygen species in plant cell. In: Gupta SD. Reactive oxygen species and antioxidants in higher plants. Enfield: Science Publishers; 2010. p.1-30.

Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;7:248-54.

Cataneo AC, Chamma KL, Ferreira LC, Destro GFG. Atividade de superóxido dismutase em plantas de soja (*Glycine max* L.) cultivadas sob estresse oxidativo causado por herbicida. Rev Bras Herb. 2010;4:23-31.

Cayon DG, Lopes NL, Oliva MA, Silva JF. Teores de clorofilas e de proteína bruta em soja (*Glycine max* (L.) Merril) tratada com imazaquin. Rev Bras Fisiol Veg. 1990;2:33-40.

Chagas RM. Silveira JAG, Ribeiro RV, Vitorello VA, Carrer H. Photochemical damage and comparative performance of superoxide dismutase and ascorbate peroxidase in sugarcane leaves exposed to paraquat-induced oxidative stress. Pest Biochem Physiol. 2008;90:181-8.

Ferreira LC, Cataneo AC, Ramazzini Remaeh LM, Corniani N, Fumis TF, Souza YA, Scavroni J, Aparecido Soares BJ. A. Nitric oxide reduces oxidative stress generated by lactofen in soybean plants. Pest Biochem Physiol. 2010;97:47-54.

Giannopolitis CN, Ries SK. Superoxide dismutases: occurrence in higher plants. Plant Physiol. 1977;59:309-14.

Gill SS, Tuteja N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem. 2010;48:909-30.

Heath RL, Packer L. Photoperoxidation in isolated chloroplasts. I. kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys. 1968;125:189-98.

Hiscox JD, Israelstam GF. A method for the extraction of chlorophyll from leaf tissue without maceration. Can J Bot. 1979;57:1332-34.

Karuppanapandian T, Moon J-C, Kim C, Manoharan K. Reactive oxygen species in plants: their generation, signal transduction, and scavenging mechanisms. Aust J Crop Sci. 2011;5:709-25.

Langaro AC, Agostinetto D, Oliveira C, Silva JDG, Bruno MS. Biochemical and physiological changes in rice plants due to the application of herbicides. Planta Daninha. 2016;34:227-90.

Langaro AC, Agostinetto D, Ruchel Q, Garcia JR, Perboni LT. Oxidative stress caused by the use of preemergent herbicides in rice crops. Rev Cienc Agron. 2017;48:358-64.

Lichtenthaler HK. Chlorophylls and carotenoids: pigment photosynthetic biomembranes. Meth Enzymol. 1987;148:362-85.

Nakano Y, Asada K. Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 1981;22:867-80.

Nohatto MA, Agostinetto D, Langaro AC, Oliveira C, Ruchel Q. Antioxidant activity of rice plants sprayed with herbicides. Pesq Agropec Trop. 2016;46:28-34.

Oliveira Jr RS, Cavalieri SD, Arantes JGZ, Alonso DG, Roso AC, Costa JM. Interação entre sistemas de manejo e de controle de plantas daninhas em pós emergência afetando o desenvolvimento e a produtividade da soja. Planta Daninha. 2006;24:721-32.

Sergier I, Alexieva V, Karanov E. Effect of spermine, atrazine and comcination between them on some endogenous protective systems and stress markers in plant. Compt Rend l'Académ Bulgare Sci. 1997;51:121-4.

Song NH, Yin XL, Chen GF, Yang H. Biological responses of wheat (*Triticum aestivum*) plants to the herbicide chlorotoluron in soils. Chemosphere. 2007;68(9):1779-87.

Sood A. Kalra C, Pabbi S, Uniyal PL. Differential responses of hydrogen peroxide, lipid peroxidation and antioxidant enzymes in *Azolla microphylla* exposed to paraquat and nitric oxide. Biologia. 2012;6:1119-28.

Wang WN, Lu J, RenT, Li X, Su W, Lu M. Evaluating regional mean optimal nitrogen rates in combination with indigenous nitrogen supply for rice production. Field Crops Res. 2012;137:37-48.