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Response of Soybean Cultivars in Rotation with Irrigated Rice Crops Cultivated in Clearfield[®] System

Resposta de Cultivares de Soja em Rotação ao Arroz Irrigado Cultivado no Sistema Clearfield[®]

ABSTRACT - Soybean crop rotation with irrigated rice is an alternative for the management of weedy rice (*Oryza sativa*) resistant to herbicides from the imidazolinone chemical group. This work had the purpose to evaluate the phytotoxicity of soybean crops containing the technologies Cultivance® (CV) and tolerance to sulphonylurea and Roundup Ready[®] (RR), deriving from increasing doses of the imazapyr and imazapic herbicide mixture, applied in the irrigated rice crop management. Thus, the experiment was performed on field, where treatments consisted in three soybean cultivars (BRS382CV, CD249STS and NA5909RR) and five doses of the imazapyr and imazapic herbicides mixture (0, 49, 98, 196 and 392 g a.i. ha⁻¹). The evaluated variables were phytotoxicity, yield components and physiological quality of the seeds. Soybean cultivar BRS382CV is tolerant to herbicide carryover, applied in the management of weedy rice in irrigated rice crops, but the other cultivars are susceptible to it. Cultivar productivity, yield components and physiological quality of the seeds are affected by the carryover increase of the imazapyr and imazapic herbicide mixture.

Keywords: Oryza sativa, Glycine max, Cultivance®.

RESUMO - A rotação da cultura da soja ao arroz irrigado é alternativa para manejo de arroz-vermelho (Oryza sativa) resistente aos herbicidas do grupo químico das imidazolinonas. Objetivou-se avaliar a fitotoxicidade cultivares de soja contendo as tecnologias Cultivance[®] (CV) e tolerância às sulfonilureias (STS) e Roundup Ready[®] (RR), em decorrência do resíduo no solo de doses crescentes da mistura dos herbicidas imazapyr e imazapic, aplicados no manejo de plantas daninhas na cultura do arroz irrigado. O experimento foi realizado em campo, e os tratamentos consistiram de três cultivares de soja (BRS382CV, CD249STS e NA5909RR) e de cinco doses da mistura dos herbicidas imazapyr e imazapic (0, 49, 98, 196 e 392 g i.a. ha⁻¹). As variáveis avaliadas foram fitotoxicidade, componentes da produtividade e qualidade fisiológica das sementes. O cultivar de soja BRS382CV é tolerante ao resíduo da mistura desses herbicidas, aplicado no manejo de arroz-vermelho na cultura do arroz irrigado, sendo os demais cultivares suscetíveis. A produtividade dos cultivares, os componentes de produtividade e a qualidade fisiológica das sementes de soja são afetados pelo aumento do resíduo da mistura dos herbicidas imazapyr e imazapic.

Palavras-chave: Oryza sativa, Glycine max, Cultivance®.

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CAPEO Ministério Ministério da Correso da de Educação Ciência e Tecnologia

FAPEMIG

INTRODUÇÃO

Rice (*Oryza sativa*) stands out among the most important crops in the South of Brazil, especially due to the use of cultivars with high productive potential and the adoption of management practices to increase productivity. However, the average yields obtained in these crops are still below those achieved under experimental conditions, mainly due to the unsatisfactory control of weeds, especially weed rice (*Oryza sativa*) (Fleck et al., 2008).

Although the edaphoclimatic conditions in lowland areas do not ensure stable yields to soybean cultivation, it is an alternative for the rotation with irrigated rice in the southern region of Rio Grande do Sul (RS), mainly due to the ease in controlling weed rice and the consequent reduction of its seed bank. In the southern region of Rio Grande do Sul, the soybean area in rotation with irrigated rice, in the 2014/15 crop, was 284.1 thousand ha, with a yield of 2.1 ton ha⁻¹, becoming an option to diversify production and to avoid fluctuations in the price of rice, as well as being a good alternative, by improving fertility conditions and soil structure (IRGA, 2016).

Considering that weeds represent a major impact on agricultural production, due to the interference in crop productivity, new management strategies have been developed, such as herbicide-resistant crops. Among them, the Cultivance® technology (CV) allowed soybean to tolerate herbicides from the group of imidazolinones, which are not naturally selective to this crop, such as imazapyr and imazapic (BASF, 2014). The herbicides imazapyr and imazapic belong to the chemical group of imidazolinones and act on the inhibition of the enzyme acetolactate synthase (ALS).

Another technology developed to decrease the selection pressure of the intensive use of glyphosate on the Roundup Ready[®] (RR) soybean genotypes was sulfonylurea-tolerant soybean (STS), which was commercially released from the 2010/11 crop (COODETEC, 2016). These cultivars tolerate the chlorimuron-ethyl herbicide when applied in doses between 15 and 20 g a.i. ha⁻¹ to control glyphosate-resistant *Conyza* sp. (horseweed) (AGROFIT, 2016).

The application of herbicides for the management of weeds can generate stress for the crops, which is characterized as any negative effect on the growth and normal development of plant species. Stressful effects may lead to seed mass reduction, which would indicate a defective formation, leading to the insufficient physiological performance of the harvested material (Marcos-Filho, 2005).

The study hypothesized that the residue of the imazapyr and imazapic mixture used in rice cultivation does not cause phytotoxicity to the Cultivance soybean cultivar, and does not negatively affect the productivity and the physiological quality of seeds, but it causes phytotoxicity and reduces the productivity and seed quality of soybean cultivars STS and RR. Thus, the purposes of the study were to evaluate the phytotoxicity to soybean crop, productivity components and to the physiological quality of seeds containing the Cultivance® and tolerance to sulfonylureas and Roundup Ready® technologies, in relation to the residue of increasing doses of the imazapyr and imazapic mixture, applied in the irrigated rice crop management.

MATERIAL AND METHODS

The experiment was conducted on the field between October 2012 and March 2014, on a soil that was classified as solodic Eutrophic Hydromorphic Planosol (Embrapa, 2013). From the analysis of soil, the following values were obtained: pH (in H_20) = 5.4; pH (SMP) = 6.4; Al (cmol_c dm⁻³) = 0.2; Ca (cmol_c dm⁻³) = 2.1; Mg (cmol_c dm⁻³) = 1.0; P (mg dm⁻³) = 10.1; K (mg dm⁻³) = 20.0; CTCe = 3.4 (cmol_c dm⁻³); CTC (pH7) = 5.9 (cmol_c dm⁻³); 15% of clay; and 1.2% of organic matter.

In the agricultural year 2012/13, irrigated rice cultivar Puitá INTA CL was cultivated with the application of the herbicide imazapyr + imazapic [(525 g kg⁻¹ + 175 g kg⁻¹ (Kifix®)] at doses of 0, 49, 98, 196 and 392 g a.i. ha⁻¹ of the commercial product, with 98 g a.i. ha⁻¹ as the recommended dose for weed rice control (AGROFIT, 2016), in order to create the residue for the agricultural year 2013/14. Treatment application was performed during the post-emergence of the irrigated rice culture at the V3-V4 stage, with a CO_2 - pressurized backpack sprayer, equipped with a 110.015 fan-type nozzle, calibrated to apply 120 L ha⁻¹ of spraying mix added with 0.5% v/v of the



adjuvant Dash[®]. The other managements and cultural treatments were performed following the technical recommendations for irrigated rice (SOSBAI, 2012). After rice harvesting, at the end of March 2013, the area remained in fallow until soybean sowing, in November of the same year.

In the agricultural year 2013/14, soybean was sown perpendicularly to the herbicide application rows in the rice crop. The experimental design was in strip-plots, in a factorial arrangement (3x5), with four replications. Factor A was composed of soybean cultivars [BRS382CV (CV), CD249STS (STS) and NA5909RR (RR)], containing the Cultivance[®] and sulfonylurea-tolerance and Roundup Ready[®] technologies, respectively; and factor B, was composed by residues of the different doses of imazapyr + imazapic, as described. Each treatment was allocated in 3.06 x 3.6 m plots, with rows spaced 0.5 m apart, resulting in an area of 11.02 m².

The variable evaluated in the soybean crop was phytotoxicity, and, during the harvest, productivity, 1,000 seed weight (TSW), number of vegetables per plant, number of seeds per plant and physiological quality of the seeds were determined.

Phytotoxicity by the herbicide imazapyr + imazapic was evaluated at 10, 20, 30 and 67 (postflowering) days after the emergence of soybean (DAE), corresponding to 389, 399, 409 and 445 days after the application of the herbicide in the management of irrigated rice, using a percentage scale where 0 (zero) represented the absence of damages and 100 (one hundred) represented plant death (SBCPD, 1995).

Harvesting was performed in a usable area of 2.0 m², when the moisture content of seeds was around 18%. The variables number of vegetables per plant and number of seeds per plant were obtained from counting on ten random plants, collected in a usable area of each experimental unit. Productivity was obtained by weighing the seeds harvested in the 2.0 m² usable area of each plot; data were transformed into kg ha⁻¹ and corrected to 13% moisture. TSW was quantified in eight replications of 100 seeds, and the mean of the weighing in grams was calculated (Brasil, 2009).

The physiological quality of seeds was evaluated through tests on germination (G), first germination count (FGC), shoot length (SL), root length (RL), shoot fresh mass (SFM), root fresh mass (RFM), shoot dry mass (SDM), root dry mass (RDM), accelerated aging (AA), field emergence (FE) and emergence rate index (ERI).

Germination (G) was performed with four replications of 50 seeds per treatment, according to field plots. Sowing was done on a paper substrate, previously moistened in distilled water, at a proportion of 2.5 times the weight of the dry paper, and the rolls were kept in a germinator at 25 °C. The evaluation was performed eight days after sowing, according to the Rules for Seed Testing (Brasil, 2009), and the results were expressed as percentage of normal seedlings.

The first germination count (FGC) was evaluated five days after sowing, during the germination test.

The shoot length (SL), root length (RL), shoot fresh mass (SFM), root fresh mass (RFM), shoot dry mass (SDM) and root dry mass (RDM) were determined from four subsamples of 20 seeds per treatment, according to the methodology described by Nakagawa (1999).

Accelerated aging (EA) was performed by spreading seeds in a single layer on a metal mesh suspended in gerbox containing 40 mL of distilled water at the bottom. Subsequently, the boxes were capped and placed in a BOD chamber at 41 °C for 48 hours (Marcos-Filho, 2005). After this period, seeds were placed to germinate, according to the methodology described for the germination test, and they were evaluated on the fifth day, expressing results as percentage of normal seedlings.

For field emergence (FE), 200 seeds per treatment were sown in beds containing soil, distributed in four replications of 50 seeds. Evaluation was carried out in a single count of normal seedlings 14 days after sowing, and the results were expressed as percentage of normal seedlings (Nakagawa, 1999).

As for the emergence rate index (ERI), daily counts were performed together with the field emergence test, until the fourteenth day, considering only the seedlings with well developed shoots. The index was calculated according to Maguire (1962), using the formula:



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ERI = E1/N1+E2/N2 +...+En/Nn, where: E1, E2 and En = number of seedlings emerged in the first, second and up the last count; and N1, N2, Nn = number of days from the first, second and last count.

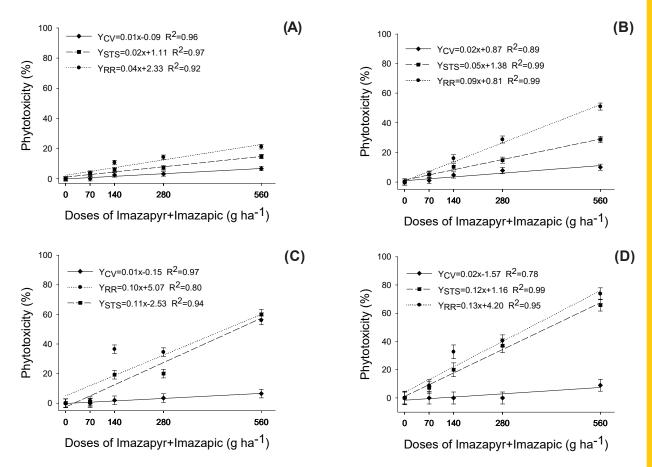
Data were analyzed as for normality (Shapiro-Wilk test) and, afterwards, they were submitted to analysis of variance ($p \le 0.05$). In case of statistical significance, a comparison was made between means, using Tukey's test ($p \le 0.05$) for the cultivar factor and regression analysis for the dose factor.

The regression analysis was performed with the help of the SigmaPlot 10.0 program, adjusting data to linear regression polynomial equations.

RESULTS AND DISCUSSION

There was an interaction between the factors cultivar and doses for the phytotoxicity variables at 10, 20, 30 and 67 DAE and grain yield. For these variables, data were adjusted to the linear polynomial equation, with the determination coefficient (R^2) ranging from 0.78 to 0.99 (Figures 1A, B, C, D and Figure 2). For the variables number of vegetables and number of seeds per plant, there was a simple effect for the dose factor, adjusting the linear polynomial equation with R^2 ranging from 0.95 to 0.96 (Figure 3A, B). There was no statistical significance for the TSW variable (data not shown).

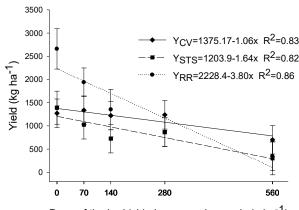
As for the variables about physiological quality of seeds FGC, G and AA, there was an interaction among the evaluated factors. Data about the variables were adjusted to the linear



Dots represent the mean values of the replications between cultivars and bars represent their respective mean confidence intervals.

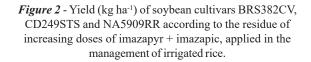
Figure 1 - Phytotoxicity (%) of soybean plants from the cultivars BRS382CV, CD249STS and NA5909RR according to the residue of increasing doses of imazapyr + imazapic, applied in the management of irrigated rice, evaluated 10 (A), 20 (B), 30 (C) and 67 (D) days after soybean emergence (389, 399, 409 and 445 ays after application, respectively).





Dose of the herbicide Imazapyr+Imazapic (g ha⁻¹)

Dots represent the mean values of the replications between cultivars and bars represent their respective mean confidence intervals.



polynomial equation, with R^2 from 0.78 to 0.93 (Figure 4A, B and C). For the variables SL and RL, there was a simple effect for the dose factor, and data adjusted to the linear polynomial equation, with R^2 from 0.75 to 0.79 (Figure 5A, B). For the ERI, FE, SFM, RFM, SDM and RDM variables, no statistical significance was observed (data not shown).

At 10 DAE, considering the residue of the recommended herbicide dose of 98 g a.i. ha⁻¹, cultivars RR and STS presented 3.3 and 1.4 times more phytotoxicity compared to the resistant cultivar CV (Figure 1A). In relation to herbicide doses, a linear growth in phytotoxicity was observed according to the dose increase. There was an increase from two and three times in the variable for each unit added with herbicide for cultivars STS and RR, respectively, compared to CV (Figure 1A).

At 20 DAE, it was observed that, at the 98 g a.i. ha^{-1} dose, cultivars STS and RR showed

1.2 and 2.4 times higher phytotoxicity, respectively, compared to the cultivar containing the Cultivance® technology (Figure 1B). For the herbicide doses, the linear growth of the variable was observed according to the dose increase, with an increase of 1.5 and 3.5 times the variable for each unit added with the herbicide, for cultivars STS and RR, respectively, compared to CV (Figure 1B).

At 30 DAE, it is possible to observe that, at the recommended dose of 98 g a.i. ha⁻¹, the susceptible cultivars STS and RR presented phytotoxicity 8.6 and 17 times higher, respectively, compared to the cultivar CV (Figure 1C). As for herbicide doses, phytotoxicity grew linearly due to the dose increase, causing a nine- and ten-fold increase in the variable for each unit added with herbicide for the RR and STS cultivars, respectively, in comparison to CV (Figure 1C).

At 67 DAE, during the post-flowering of the crop, a similar behavior of the variable was verified in comparison to the evaluation at 30 DAE (Figure 1D). As for herbicide doses, it was observed that phytotoxicity increased linearly due to the dose increase, with an increment of about five times the variable for each unit added with herbicide for cultivars STS and RR, compared to CV (Figure 1D).

A similar result was found in a work to evaluate the phytotoxicity caused by the mixture of the herbicides imazapyr and imazapic, applied during the post-emergence of soybean plants containing the Cultivance[®] technology, where phytotoxicity was close to 10% in the evaluations at 13, 20 and 27 days after application of the product (DAP), and at 41 DAP, phytotoxicity was close to 20% (Cavenaghi et al., 2014). Studies with cultures that were non-tolerant to herbicides from the imidazolinone group, such as ryegrass, sown in rotation to Clearfield[®] rice, proved that the residual effect of these herbicides causes high phytotoxicity at early stages, when the product dose is increased (Süzer and Büyük, 2010; Pinto et al., 2011).

As for the productivity variable, there was no difference between cultivars at doses equal to or greater than 98 g a.i. ha⁻¹. Generally speaking, it was possible to observe that, at the dose of 98 g a.i. ha⁻¹, cultivar STS was 59% less productive than cultivar CV. The cultivar RR showed an increase of 11% in productivity, at the same dose, compared to the cultivar CV (Figure 2). As for doses, there was a linear decrease of the variable as herbicide doses increased; it was possible to observe a decrease of 0.58 and 2.6 times the variable for each unit added with herbicide for cultivars STS and RR, respectively, compared to CV (Figure 2). The lower productivity loss of CV cultivar derives from its tolerance to imidazolinone herbicides, whereas its reduced productivity may be due to the lower adaptability of the cultivar to the characteristic water excess of lowland cultivations.



Research demonstrated that, in rice, phytotoxicity caused by herbicides, in many cases, does not affect productivity, since plants are able to detoxify/metabolize herbicides throughout their development (Villa et al., 2006). However, some rice cultivars cannot fully detoxify/metabolize the applied herbicide; therefore, decreases in the crop productivity occur (Petter et al., 2011). Examples of herbicides that are extremely selective because they undergo metabolic deactivation are sulfonylureas and imidazolinones (Oliveira Jr and Inoue, 2011), for which differences in selectivity between tolerant and susceptible species may be hundreds of times greater. In the case of sulfonylureas, tolerant species can rapidly transform herbicides into inactive products, while metabolism is much slower and less intense in susceptible species (Carvalho et al., 2009). As for imidazolinones, the main metabolites formed in plants are less toxic than the original compounds (Shaner and Mallipudi, 1991). It is worth highlighting that the metabolization rate of toxic compounds depends on the environment and may vary according to species and development stage of the plant (Kissmann, 1996). Thus, the same herbicide amount applied to one species

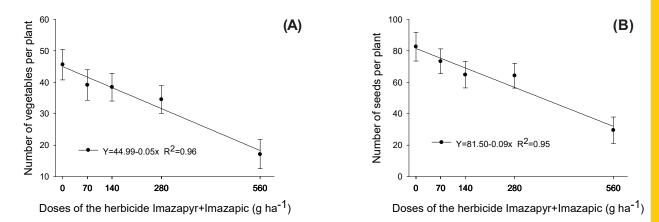
As for the variables number of vegetables and seeds per plant, the linear behavior of the curves showed that, as the herbicide dose doubled, the response variable was halved (Figure 3A, B). These results may be explained by the increase in the residue, supporting data about productivity.

may become phytotoxic under certain conditions and cause no damage on others.

As for the variables on physiological quality of seeds FGC and G, considering the residue of the recommended dose of 98 g a.i. ha⁻¹, no difference was observed among the evaluated cultivars; the mean values obtained were above 80%, complying with the established standards (Brasil, 2012) (Figure 4A, B). In a work to evaluate the influence of herbicides on the physiological quality of irrigated rice seeds, no differences were observed for the herbicide imazapyr + imazapic in the variables FGC, G and AA variables (Langaro, 2015).

Data about FGC and G for cultivars CV and STS did not fit any model that could biologically explain their behavior, but a linear decrease was observed for cultivar RR, both for FGC and G, as herbicide doses increased, with a 2.8% decrease of FGC and G for each unit added with herbicide (Figure 4A, B).

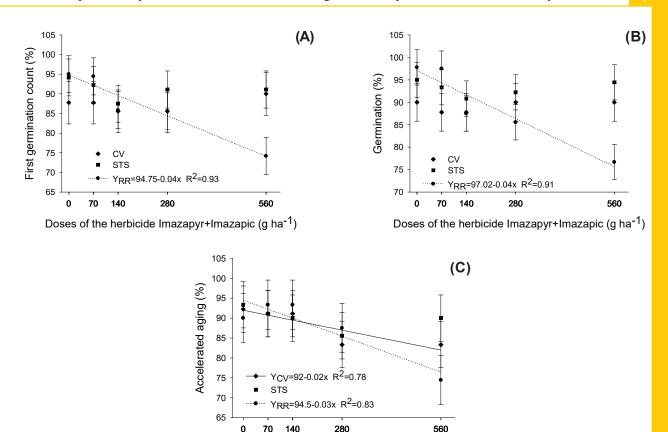
Based on the residue of the 98 g a.i. ha⁻¹ dose, no difference was observed among the cultivars evaluated for the variable AA; the mean values obtained in the germination test, even after accelerated aging stress, were above 90%, which is in accordance with the established standards (Brasil, 2012) (Figure 4C). Data on the variable for the STS cultivar did not fit any model that could biologically explain its behavior. On the other hand, for cultivar NA5909RR, a linear decrease of the variable was observed as herbicide doses were increased, with a reduction of 0.5 times in the variable for each unit added with herbicide, compared to BRS382CV (Figure 4C). Thus, the reduction of vigor by the accelerated aging test for cultivar RR caused by the herbicide residue may be related to the phytotoxicity observed for the cultivar or may also be related to the action



Dots represent the mean values of the replications between cultivars and bars represent their respective mean confidence intervals.

Figure 3 - Number of vegetables (A) and seeds (B) per plant of soybean cultivars according to the residue of increasing doses of imazapyr + imazapic, applied in the management of irrigated rice.





Doses of the herbicide Imazapyr+Imazapic (g ha⁻¹)

Dots represent the mean values of the replications between cultivars and bars represent their respective mean confidence intervals.

Figure 4 - First germination count (A) (FGC) (%), germination (B) (G) (%) and germination after accelerated aging test (C) (AA) (%) of the soybean cultivars BRS382CV, CD249STS and NA5909RR according to the residue of increasing doses of imazapyr + imazapic, applied in irrigated rice management.

mechanism of imazapyr + imazapic, which is more damaging in comparison to CV, since it is tolerant to this herbicide.

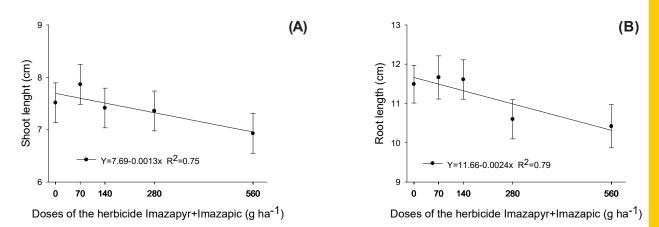
For the variables SL and RL of the seedlings, a linear decrease of the variables was observed according to the increase in the residue of imazapyr + imazapic, that is, as the dose doubled, the SL and RL of the seedlings halved (Figure 5A, B).

Soybean cultivars sown in different environments express their potentialities in relation to the occurred conditions, which change in space and time. As genotypes may respond differently to the environment, indications on the best season for each cultivar should be preceded by regionalized tests conducted in different environments (Peluzio et al., 2010).

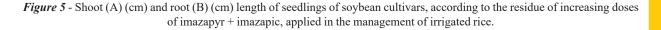
The maximum quality of soybean seeds is reached at physiological maturity, coinciding with the maximum accumulation of dry matter, vigor and germination (Popinigis, 1985). At the point of physiological maturity, the high moisture content of seeds hinders the mechanized harvesting of soybean and, therefore, seeds remain "stored in the field" until reaching the appropriate moisture content for the mechanical harvest. During this period, climatic conditions are rarely conducive to the maintenance of seed quality. Low temperatures favor seed quality; on the other hand, hot and humid conditions with excessive rainfall may compromise the germination and vigor of soybean seeds (Marcos-Filho, 2005). It is worth mentioning that there are differences regarding the tolerance of soybean seeds to unfavorable environmental conditions, depending on the cultivar.

Considering the aforementioned, there is a need for further research on the performance of soybean cultivars in rotation with Clearfield[®] rice. Knowing the management of residual





Dots represent the mean values of the replications between cultivars and bars represent their respective mean confidence intervals.



herbicides is a fundamental tool for producers to control weeds and for the consequent production of quality seeds.

Taking into account the results, it is possible to conclude that the BRS382CV soybean cultivar is tolerant to the residue of the herbicides imazapyr and imazapic, applied in the management of weed rice in the cultivation of irrigated rice, but cultivars CD249STS and NA5909RR are susceptible to them. The productivity of soybean cultivars, their productivity components and the physiological quality of seeds are negatively affected by the increase in the residue of the imazapyr and imazapic mixture.

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