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Effect of post-emergence applications of imazapyr and imazapic on the growth and grain yield of AHAS-transgene soybean plants

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Abstract

One of the factors that limit grain yield in soybean crops is weed competition. The objective of the present work was to evaluate the agronomic performance of an AHAS-transgene soybean cultivar (BRS 397; Cultivance®) subjected to different rates of Soyvance® (525 g kg^{-1} imazapyr, and 175 g kg^{-1} imazapic). Two field experiments were conducted in the 2015-2016 crop season in two sites with different soil and weather characteristics located in the state of Paraná, Brazil. A completely randomized block design was used, with 11 treatments consisted of different rates of Soyvance®. Phytotoxicity, chlorophyll index, first pod height, plant height, number of pods per plant, 100-seed weight, and grain yield were evaluated. The use of high rates of the herbicide decreased the final height and grain yield of soybean plants in approximately 25% in both locations, but did not affect their 100-seed weight and chlorophyll index. The use of rates of the commercial product higher than that recommended by the manufacturer (100 g ha^{-1}) may affect the agronomic performance of AHAS-transgene soybean plants.

Keywords: Imidazolinones, selectivity, ALS inhibitors.

Introduction

The world production of soybean (*Glycine max* L. Merril) is increasing due to the many crop protection technologies available for this species, which allow the application of herbicides in post-emergence by making them selective to the crop and enable a production that meets the global demand. A not appropriate weed management for soybean crops can result in significant losses. Thus, technologies that allow post-emergence application of selective herbicides is an important tool for farmers.

Brazilian soybean producers have shown good acceptance of transgenic technologies for this crop; soybeans crops covered an area of approximately 35 million hectares in the 2014/2015 crop season, and 93.5% of this total area was sown with genetically modified soybean seeds (Conab, 2016; Céleres, 2015). The main transgenic technology that has been adopted is Roundup Ready® (RR), which enables post-emergence applications of glyphosate. However, the intense use of this herbicide has significantly increased cases of resistance to this molecule. Currently, 307 cases of resistant species to 5-enolpyruvylshikimate-3-phosphate synthase (EPSPs) inhibitors have been reported all over the world, and eight of these species have been reported in Brazil (Heap, 2019).

The AHAS transgene (Cultivance® technology) was developed to provide weed control options and enable rotation of herbicides of different modes of action, thus mitigating the selection of resistant weed biotypes. Soybean plants with the Cultivance® technology tolerate post-emergence applications of imidazolinone-based herbicides, which are acetolactate synthase (ALS) inhibitors (Cib, 2016). ALS-inhibiting herbicides account for approximately 30% of all cases of weed resistance reported worldwide. There are already 19 confirmed cases of ALS resistance in Brazil (Heap, 2019); however, most reports of resistance refer to sulfonylureas. Thus, the use imidazolinone herbicides is an alternative for the prevention biotype resistance.

Soyvance® (BASF, Ludwigshafen, Germany) is an herbicide consisted of imazapyr (525 g kg^{-1}) and imazapic (175 g kg^{-1}) and is approved for post-emergence application on Cultivance® soybean crops in Brazil. In the state of Paraná, this product is approved for the control of weed species, including *Amaranthus viridis* L.*, Bidens pilosa* L.*, Brachiaria plantaginea* Hitchc.*, Commelina benghalensis* L.*, Digitaria horizontalis* Willd*,* and *Ipomoea grandifolia* (Dammer) O'Donell (Paraná, 2016). However, some cases of resistance to ALS inhibitors have already been reported, including resistance to the chemical group of imidazolinones, for example, *B. pilosa* resistance to imazaquin and imazethapyr (Heap, 2019).

The use of imidazolinone-resistant soybean cultivars is a new tool for weed control in soybean crops; it enables rotation of herbicides of different modes of action by rotating transgenic cultivars in the production system. Thereby, problems due to selection of herbicide-resistant weed biotypes (as is the case of glyphosate) will be avoided, especially in integrated weed management. The use of high rates of herbicide is not recommended because it can cause a great selection pressure on weeds for resistant biotypes, decreasing the longevity of the technology. Moreover, information on the tolerance level of the technology in the field are important.

The objective of this work was to evaluate the tolerance of imidazolinone-resistant plants to post-emergence application of different rates of Soyvance® (525 g kg^{-1} imazapyr, and 175 g kg^{-1} imazapic) on the growth and grain yield of an AHAS-transgene soybean cultivar (BRS 397; Cultivance®).

Results and Discussion

Chlorophyll index and 100-seed weight of AHAS-transgene soybean plants after application of imazapyr + imazapic

No significant differences were found for chlorophyll index in the two areas evaluated in the present work (Table 1 and Table 2). It may be due to the evaluation time, which was at 54 days after application (DAA) of the herbicide, during the R1 reproductive stage. This shows that the plants had good recovery, overcoming the injuries caused by the herbicide product, whose symptoms start to decrease at 28 DAA. No significant effect was found for 100-seed weight in the two areas (Table 1), showing that this variable was not significantly affected by the increase in herbicide rates, despite presenting a slight decrease.

Phytotoxicity of imazapyr + imazapic in AHAS-transgene soybean plants

In Palotina, soybean injury was found at 7, 14, 21, and 28 DAA (Fig. 1) with the use of the lowest rates of the herbicide product—200 g ha^{-1} at 7 DAA, and 150 g ha^{-1} at the other evaluation times. The soybean injury increased as the herbicide product rate was increased up to 21 DAA. The symptoms of herbicide phytotoxicity decreased after 28 DAA, showing a possible recovery of plants from the injuries after application of high rates of the herbicide. In Brasilândia do Sul, injury symptoms were found at 21 DAA; however, all percentages remained lower than 11%, and the differences were not significant by the F test ($p \leq 0.05$) (Table 2).

Phytotoxicity symptoms in imidazolinone-resistant soybean plants increased up to 21 DAA, with a recovery of the plants after 28 DAA. Similar results were found for glyphosate-tolerant soybean plants that presented damages to the plant photosynthetic apparatus up to 34 DAA of a high rate of glyphosate (Zobiole et al., 2010a), showing the recover ability of plants from injuries caused by high rates of herbicides (Reddy et al., 2004). Moreover, Merotto et al. (2000) evaluated the tolerance of soybean cultivars to metsulfuron-methyl (sulfonylurea) and found phytotoxicity in cultivars caused by the application of high rates of the herbicide.

The phytotoxicity was lower at 28 DAA; the plots subjected to rates of up to 250 g ha^{-1} of the herbicide product showed less than 10% of visual injuries. However, at 28 DAA, the plants treated with herbicide rates higher than 450 g ha^{-1} showed more than 40% phytotoxicity symptoms.

First pod height and plant height after herbicide application

The first pod height decreased as the product rate was increased (Fig. 2); similar result was found for final plant height. The increase in imazapyr + imazapic rates resulted in a small decrease in first pod height, which directly affects the efficiency of mechanical harvesting operations in soybean crops (Nepomuceno, 2007). According to Queiroz et al. (1981), soybean plants must have first pod height of at least 13 cm to avoid losses during mechanical harvesting. However, the results of the present study showed that the herbicide rates that caused less or no injury to the plants caused a decrease in first pod height. Therefore, even with absence of chlorosis, the high rates of herbicide used affected the internodal distance at the first pod development.

In Palotina, even the highest rates of imazapyr + imazapic did not result in first pod height below 13 cm (Table 1). However, the data of Brasilândia do Sul showed that the application of herbicide product rates above 300 g ha^{-1} resulted in a first pod height of 13 cm or less, which may result in losses during mechanical harvesting. This effect was aggravated by the rainfall distribution in Brasilândia do Sul and by the soil field capacity, since medium-textured Ultisols are less favourable for crops than clayey Ultisols (IAC, 2016) because of their lower water retention and availability to plant roots, leading to a stress (Morando, 2014), which may aggravate injury symptoms caused by herbicides.

In both locations, the final plant height decreased as the herbicide rates was increased (Fig. 3). In Palotina, the plants showed greater tolerance, probably due the different soil type, mainly related to the water retention capacity. Albrecht et al. (2014) found the same effect for the application of glyphosate to Roundup Ready® soybeans (RR soybean), and a similar result when glyphosate was applied during the reproductive stage of the RR soybean plants. Krausz and Young (2001) found a decrease in the height of glyphosate-tolerant soybean plants subjected to application of imazethapyr.

The injuries in the soybean plants over the days after the herbicide application hindered the plant growth and decreased the internode length and the final plant height, differing from plants without herbicide application. Despite the tolerance to post-emergence application of imazapyr + imazapic, the development of the plants was affected by the highest herbicide rates used, which probably decreased grain yield.

Number of pods per plant and grain yield of AHAS-transgene soybean plants after imazapyr + imazapic application

The number of pods per plant found in Palotina presented no significant difference by the F test ($p \leq 0.05$) (Table 1). In Brasilândia do Sul, the pod number per plant was within the normal limits when applied the recommended rate of the herbicide product (100 g ha^{-1}) (Fig. 4). The final number of pods per plant in Brasilândia do Sul decreased as the herbicide rate was increased, presenting decreases of approximately 13% for the herbicide product rate of 275 g ha^{-1}, and 22% for the highest rate (500 g ha^{-1}). According to Oliveira Neto et al. (2009), high rates and mixtures of herbicides can cause injury to crops, reducing the number of pods per plant and, consequently, decreasing the grain yield.

Table 1. Analysis of variance and F test for number of pods per plant, first pod height, plant height, seed weight, chlorophyll index (a, b, and total), phytotoxicity, and grain yield of AHAS-transgene soybean plants grown in Palotina, PR, Brazil, after application of different rates of imazapyr + imazapic.

Mean Squares						
Factor	Number of pods per plant	First pod height	Plant height	Seed weight	Chlorophyll index *a*	*b*
Rate	13.82^{ns}	64.43^{ns}	364.48**	0.29^{ns}	0.55^{ns}	0.75^{ns}
Error	20.14	33.86	19.07	0.72	3.53	0.70
CV (%)	10.95	31.24	4.70	5.18	5.14	6.66
Mean	40.97	18.63	92.91	16.34	36.60	12.55
Mean Squares						
Factor	TC	Phytotoxicity 7 DAA	14 DAA	21 DAA	28 DAA	Grain yield
Rate	2.20^{ns}	338.30**	1530.60**	1842.06**	1439.09**	433342.06**
Error	6.33	4.11	15.77	30.73	10.43	115760.74
CV (%)	5.12	23.66	20.44	25.89	17.90	9.59
Mean	49.15	8.57	19.43	21.41	18.04	3548.05

CV = coefficient of variation; DAA = days after application of the herbicide; TC = total chlorophyll; ** significant at 1% probability, * significant at 5% probability, and ns = not significant by the F test.

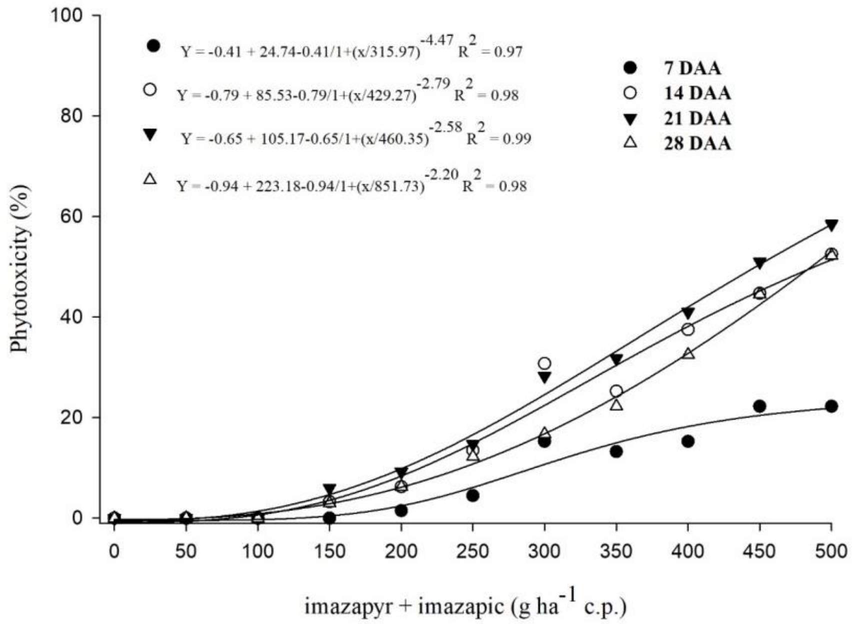

Fig 1. Phytotoxicity at 7, 14, 21, and 28 days after application (DAA) of herbicide (525 g kg^{-1} imazapyr and 175 g kg^{-1} imazapic rate) to AHAS-transgene soybean plants grown in Palotina, PR, Brazil, in the 2015-2016 crop season. Data fitted to a sigmoidal regression model.

Table 2. Analysis of variance and F test for number of pods per plant, first pod height, plant height, seed weight, chlorophyll index (a, b, and total), phytotoxicity, and grain yield of AHAS-transgene soybean plants grown in Brasilândia do Sul, PR, Brazil, after application of different rates of imazapyr + imazapic.

Medium Squares						
Factor	Number of pods per plant	First pod height	Plant height	Seed weight	Chlorophyll index *a*	*b*
Rate (R)	46.90**	19.90**	173.96**	0.93^{ns}	14.23^{ns}	1.39^{ns}
Error	11.73	3.25	26.01	1.26	5.95	1.82
CV (%)	9.40	12.05	6.10	6.97	7.44	12.33
Mean	36.44	14.97	83.67	16.10	32.81	10.93
Medium Squares						
Factor	Chl. Total	Phytotoxicity 7 DAA	14 DAA	21 DAA	28 DAA	Grain yield
Rate (R)	20.06^{ns}	315.22^{ns}	210.76^{ns}	21.02^{ns}	3.00^{ns}	779027.97**
Error	10.58	373.73	255.78	32.22	2.97	149607.68
CV (%)	7.44	81.01	96.38	96.44	161.29	12.21
Mean	43.74	23.86	15.59	5.89	1.07	3169.09

CV = coefficient of variation; DAA = days after application of the herbicide; TC = total chlorophyll; ** significant at 1% probability, * significant at 5% probability, and ns = not significant by the F test.

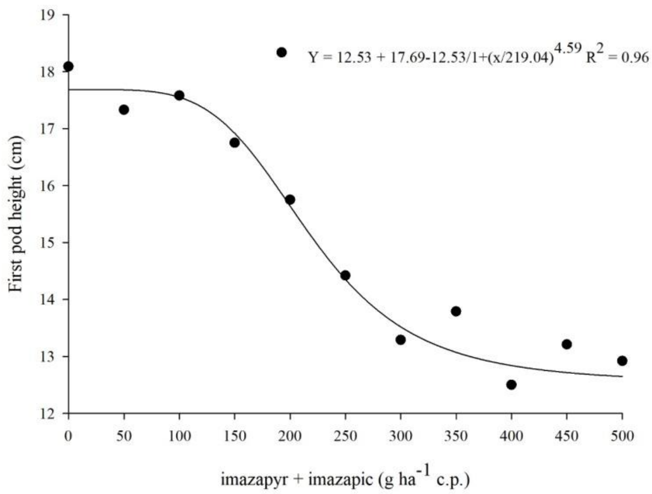

Fig 2. First pod height (cm) after application of herbicide (525 g kg^{-1} imazapyr and 175 g kg^{-1} imazapic rate) to AHAS-transgene soybean plants grown in Brasilândia do Sul, PR, Brazil, in the 2015-2016 crop season. Data fitted to a sigmoidal regression model.

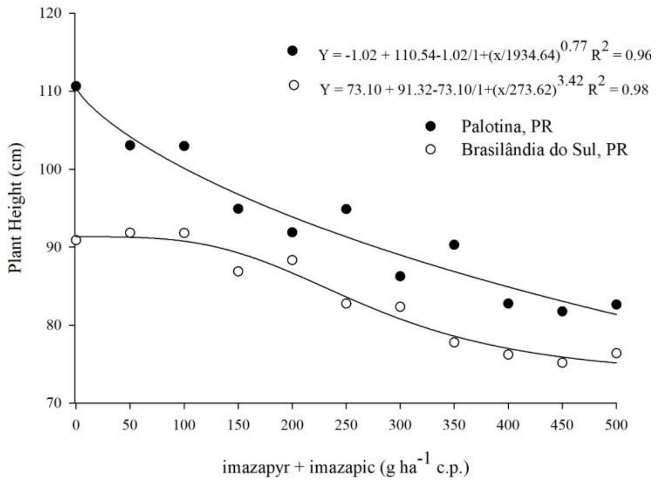

Fig 3. Plant height (cm) after application of herbicide (525 g kg^{-1} imazapyr and 175 g kg^{-1} imazapic rate) to AHAS-transgene soybean plants grown in Palotina and in Brasilândia do Sul, PR, Brazil, in the 2015-2016 crop season. Data fitted to a sigmoidal regression model.

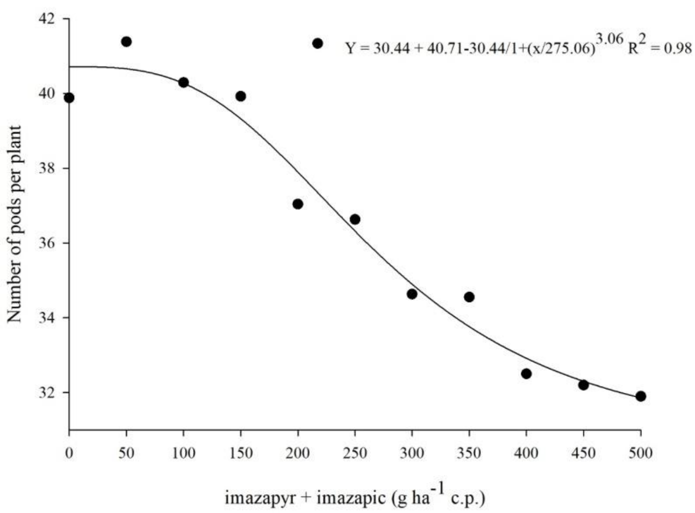

Fig 4. Number of pods per plant after application of herbicide (525 g kg^{-1} imazapyr and 175 g kg^{-1} imazapic rate) to AHAS-transgene soybean plants grown in Brasilândia do Sul, PR, Brazil, in the 2015-2016 crop season. Data fitted to a sigmoidal regression model.

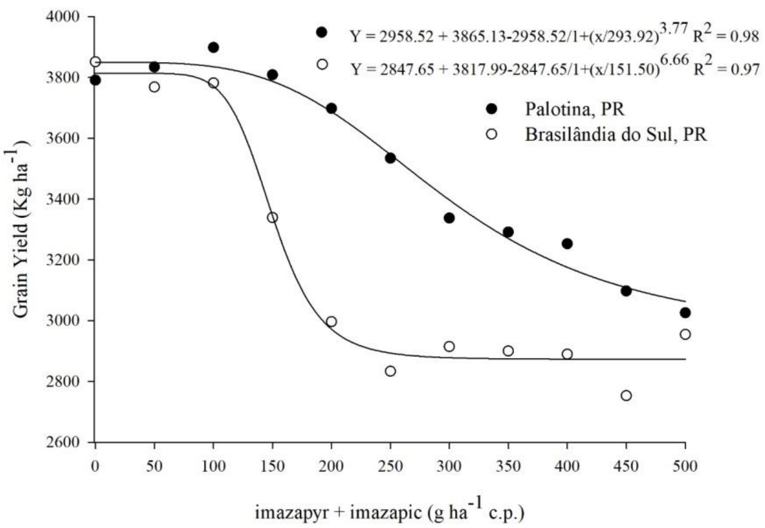

Fig 5. Grain yield (kg ha^{-1}) after application of herbicide (525 g kg^{-1} imazapyr and 175 g kg^{-1} imazapic rate) to AHAS-transgene soybean plants grown in Palotina and in Brasilândia do Sul, PR, Brazil, in the 2015-2016 crop season. Data fitted to a sigmoidal regression model.

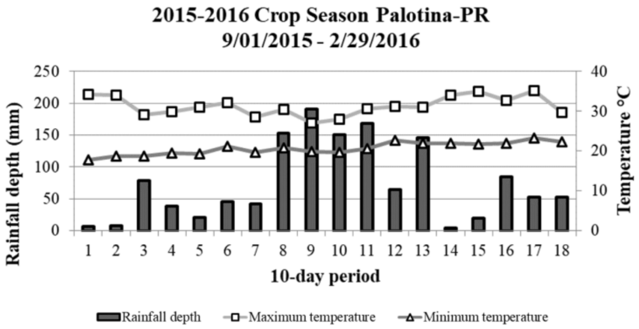

Fig 6. Average rainfall depth and air temperature in the 2015-2016 crop season, in Palotina, PR, Brazil.

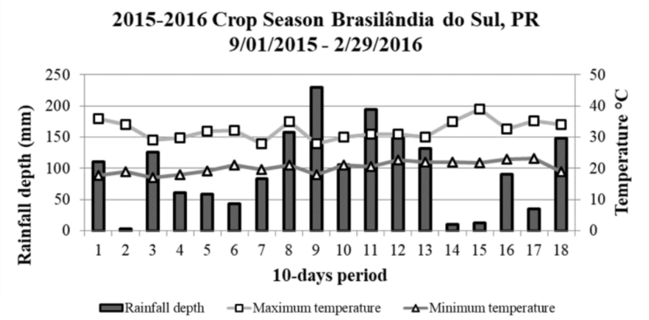

Fig 7. Average rainfall depth and air temperature in the 2015-2016 crop season, in Brasilândia do Sul, PR, Brazil.

Some studies have reported negative effects of increasing herbicide rates on number of pods per plant. Albrecht et al. (2011) found this result with application of increasing rates of glyphosate to RR soybean cultivars, regardless the stage of the plants at the herbicide application (vegetative and reproductive). Zadinello et al. (2012) found similar effect when applying glyphosate at the beginning of the reproductive stage.

Corrêa and Alves (2009) evaluated the application of a mixture of glyphosate and imazethapyr to transgenic soybean plants and found that the application of an imidazolinone had no effect on number of pods per plant. The decreases in number of pods per plant is connected to flower abortion during the reproductive period, which may be due to problems in the photosynthetic system or in the water use efficiency (Zobiole et al., 2010b). Martendal (2016) found a decrease in number of siliques in canola hybrids when using different rates of an herbicide consisted of imazethapyr (75 g L^{-1}) and imazapic (25 g L^{-1}) (Only®; BASF, Ludwigshafen, Germany). Variables that affect grain yield, such as number of pods per plant, plant height, and chlorophyll indexes, may be decreased as the rate of this herbicide is increased (Albrecht et al., 2014).

The grain yield of the imidazolinone-resistant soybean plants was affected by the increase of herbicide rates because of injuries in the plants during their vegetative stage, in both locations. This effect was more pronounced in Brasilândia do Sul, where the grain yield was, in general, lower than that found in Palotina (Fig. 5). The grain yield of the imidazolinone-resistant soybean plants decreased approximately 13% when the herbicide product was applied at a rate of 150 g ha^{-1}; however, there was a relative decrease of 25% when applying rates 4.5- to 5-fold higher than the recommended one. The use of high rates of herbicides is not recommended; it can result in greater selection pressure for resistant weed biotypes, thereby, decreasing the longevity of the technology.

Albrecht et al. (2014) found a decrease in grain yield of RR soybean plants grown in different sites and crop seasons when applying increasing rates of glyphosate. This effect was also found for application of glyphosate at the beginning of the reproductive stage, even to glyphosate-resistant soybean plants (Albrecht et al., 2011; Zadinello et al., 2012). According to Albrecht et al. (2012), combinations of herbicides may increase plant injury; they found that the use of a combination of glyphosate and chlorimuron-ethyl resulted in more significant decreases in grain yield than the use of glyphosate alone.

The imidazolinone-resistant soybean plants grown in Palotina showed higher tolerance to high rates of the herbicide product (Fig. 5). The technology proved to be efficient for tolerance to post-emergence applications of imazapyr + imazapic. The highest rates used decreased grain yields in up to 24%. However, under the climatic conditions in the 2015-2016 crop season and the soil conditions, the tolerance of the imidazolinone-resistant soybean plants to the herbicide product rates of up to 250g ha^{-1} applied in post-emergence was found in the AHAS-transgene production system. However, the use of the recommended rate is necessary to preserve the technology, reducing the selection pressure for resistant weeds.

The significant decrease in grain yield due to the use of the highest rates of the herbicide product is related to the resulting injuries in the plants at the vegetative stage. In Palotina, the phytotoxicity symptoms increased up to 21 DAA; however, the use of herbicide product rates greater than 450 g ha^{-1} resulted in more than 40% of injuries at 28 DAA. Therefore, there was a shorter time for planting, which affected the productive parameters and agronomic performance of the plants, since the grain yield is a result from other plant attributes (Carvalho et al., 2002).

Materials and Methods

Plant material

The BRS 397 soybean cultivar was chosen for the present study. It is in the maturity group 6.2, has indeterminate growth habit, purple flowers, gray pubescence, and light-brown hilum, and its grains present average protein and oil contents of 38.2% and 23.0%, respectively. These plants can reach heights of 80 to 100 cm, and 100-seed weight of 15 g (BASF, 2016).

The cultural practices used for this cultivar in the present study followed the guidelines of the Embrapa (2008). The seeds were sowed in both locations on 1 October 2015, at rate of 310,000 seeds ha^{-1}, with spacing of 0.45 m between rows. The plots consisted of six 5-meter rows, with an evaluation area of 3.6 m^2. The plots were kept free from weeds through manual weeding, as described by Embrapa (2008).

Field Experiments

Two field experiments with soybean crops under no-till system were conducted in different environments in western state of Paraná, Brazil, during the 2015-2016 crop season: one in Palotina (Area 1) (24°20′49′′S, 53°51′32′′W, and altitude of 346 m) and other in Brasilândia do Sul (Area 2) (24º05′′13′′S, 53º29′′32′′W, and altitude of 378 m).

The soil of the Area 1 was classified as Typic Hapludox (Latossolo Vermelho eutroferrico; Embrapa, 2006) of clayey texture (63.75% clay, 17.50% silt, and 18.75% sand), whose 0–20 cm layer presented pH ($CaCl_2$) of 4.5, 13.42 g dm^{-3} of organic matter, 59.09 mg dm^{-3} of P, and 0.27, 1.42, and 0.00 $cmol_c$ dm^{-3} of K^+, Ca^{2+}, and Al^{3+}, respectively. The soil of the Area 2 was classified as Typic Paleudalf (Argissolo Vermelho Amarelo distrofico; Embrapa, 2006) of medium texture (30% clay, 15% silt, and 55% sand), whose 0–20 cm layer presented pH ($CaCl_2$) of 5.3, 14.45 g dm^{-3} of organic matter, 30.44 mg dm^{-3} of P, and 0.30, 2.67, and 0.00 $cmol_c$ dm^{-3} of K^+, Ca^{2+}, and Al^{3+}, respectively.

The climate of the region encompassing both locations is Cfa (humid temperate with hot summers), according to the Köppen classification. The rainfall depth and air temperature data were monitored daily during the experiments in Palotina (Fig. 6) and Brasilândia do Sul (Fig. 6).

These sites were selected because they present different edaphoclimatic characteristics, thus enabling the evaluation of responses of these soybean plants under different conditions.

Experimental design and herbicide application

The experiments were conducted in a completely randomized block design with four replications. The treatments consisted of 11 rates (0, 50, 100, 150, 200, 250, 300, 350, 400, 450, and 500 g ha^{-1}) of the herbicide Soyvance® (BASF, Ludwigshafen, Germany), whose active ingredients are imazapyr (525 g kg^{-1}) and imazapic (175 g kg^{-1}). The product rate recommended by the manufacturer is 100 g ha^{-1}.

The treatments were applied when 50% of the plants had four expanded leaves (V_4 stage) using a CO_2-pressurized backpack sprayer under constant pressure (29 psi), with flow rate of 0.65 L min^{-1}. The sprayer had a spray boom containing six extended range flat spray nozzles (XR 11002; Teejet®, Wheaton, USA), positioned at 50 cm height from the target, moving at a speed of 1 m s^{-1}, resulting in a 50-cm wide coverage per nozzle and in an application volume corresponding to 200 L ha^{-1}.

In the Area 1, the treatments were applied on 30 October 2015, under average temperature, wind speed, and relative air humidity of 25.6 °C, 5 km h^{-1}, and 63.7%, respectively. In Area 2, the treatments were applied on 6 November 2015, under average temperature, wind speed, and relative air humidity of 27 °C, 6 km h^{-1}, and 62%, respectively. The plants were manually harvested when they reached full maturity: 95% mature pods, based on their colour (R8 stage).

Data collection

The variables evaluated were plant height, number of pods per plant, chlorophyll index (*a*, *b*, and total), grain yield, and 100-seed weight. Phytotoxicity was evaluated visually by quantifying interveinal chlorosis and necrosis on the plants of each plot at 7, 14, 21, and 28 days after application (DAA) of the herbicide product, based on injury symptoms, using a scale of grades from 0 (absence of injuries) to 100 (plant death) (SBCPD, 1995).

Ten plants were randomly selected in the evaluation area of each plot and evaluated for first pod height and final height. First pod height (cm) was measured at full maturity of the plants in both locations. Chlorophyll index was measured at 54 DAA (R1 reproductive stage), using a chlorophyll meter (ClorofiLOG; Falker, Porto Alegre, Brazil); the result was expressed as average of five plants. The number of pods per plant was evaluated at the R8 reproductive stage. The 100-seed weight was determined by counting and weighing 8 subsamples of 100 seeds, and grain yield was estimated in kg ha^{-1}; the grain moisture content was adjusted to 13% for these two variables.

Statistical analyses

The data of all treatments were subjected to analysis of variance (ANOVA) at 5% significance level by the F test, calculating the *p*-value, and to regression analysis at 5% probability, according to Pimentel-Gomes and Garcia (2002), using the Sisvar program(Ferreira, 2011). The sites were evaluated separately. Subsequently, the data were fit to non-linear regression models, according to Seefeld et al. (1995), using the SigmaPlot program, according to Equation 1.

$$y = Pmín + \frac{a}{\left[1+\left(\frac{x}{b}\right)^{c}\right]} \quad (1)$$

Where:

y = response of the variable;

x = herbicide product rate (kg ha^{-1})

$Pmín$ = lower limit;

a = amplitude between the lower and upper value of the variable;

b = herbicide rate which provides 50% response of the variable;

c = slope.

Conclusion

The imidazolinone-resistant soybean plants showed tolerance to imazapyr + imazapic herbicide in both locations evaluated in the present study. The 2015-2016 crop season had regular, well-distributed rainfall, which favoured the crop development and the recovery of plants from injuries caused by the herbicide. The use of high rates of the evaluated herbicide are not recommended, especially under irregular and infrequent rainfall conditions. Further studies about this soybean technology over longer periods and under different soil characteristics are needed.

The use of AHAS-transgene soybean plants is an alternative for the rotation of mechanisms of action of herbicides, enabling a preventive control of selection of weeds resistant to herbicides.

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