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AGRONOMIC PERFORMANCE AND CHLOROPHYLL INDICES OF TRANSGENIC SOYBEAN (with *csr1-2* gene), UNDER IMAZAPIC/IMAZAPYR POST APPLICATION

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The transgenic event BPS-CV127-9 (Cultivance® – CV) confers soybean tolerance to imidazoline one herbicides due to the *csr1-2* (or *ahas*) gene from *Arabidopsis thaliana*, which encodes a modified acetolactate synthase (ALS) enzyme. There are few studies on imazapic/imazapyr selectivity in Cultivance® soybean. Therefore, this study was aimed to evaluate chlorophyll indices and agronomic performance of CV soybean under rates of imazapic/imazapyr at V1, V2 and V3 stages. Two experiments were carried out in experimental area of Palotina (Experiment 1) and Brasilândia do Sul (Experiment 2) at Paraná State (PR), Brazil in 2015-16. A randomized block design with four replications was used. Treatments were arranged in a 3 × 5 factorial scheme, with applications at three soybean phonological stages (V1, V2 and V3) using five imazapic/imazapyr rates (0, 39.38/13.13, 78.75/26.25, 118.13/39.38, and 157.5/52.5 g a.e. ha^{-1}). Symptoms of injury to soybean plants, chlorophyll indices, and variables related to agronomic performance (plant height, first pod height, lodging, number of pods per plant, yield, and 100-grain weight) were evaluated. Analysis of variance and F-test ($P < 0.05$) were performed. When significant, the means were compared by the Tukey test ($P < 0.05$) for the factor application stage and subjected to regression analysis ($P < 0.05$) for the factor rates. Imazapic/imazapyr (formulated premix) application up to the rate 157.5/52.5 g a.e. ha^{-1} did not affect chlorophyll indices and agronomic performance of Cultivance® soybean (cultivar BRS-397 CV) at the phonological stages V1, V2 and V3. Thus, the selectivity of imazapic/imazapyr applied in post-emergence was observed for Cultivance® soybean.
Keywords: ALS inhibitors, *Glycine max*, herbicides, imidazolinones, selectivity.

INTRODUCTION

Transgenic event BPS-CV127-9 (Cultivance® – CV) confers tolerance to imidazolinone herbicides in soybean. It was developed by Basf and Embrapa in Brazil and approved for consumption and cultivation in 2009. Tolerance to imidazolinones is conferred by the *csr1-2* (or *ahas*) gene, derived from *Arabidopsis thaliana* (Roux *et al*., 2005; ISAAA 2019), which encodes a modified acetohydroxy acid synthase (AHAS) enzyme (or acetolactate synthase–ALS enzyme), insensitive to herbicides due to amino acid substitution, in which serine is replaced by asparagine at position 653 (EFSA, 2014; Albrecht *et al*., 2018a). The insertion of this gene has no impact on amino acid levels in the plant. Moreover, the nutritional composition of produced grains is equivalent to that of conventional soybean (EFSA, 2018).

Imidazolinone herbicides are inhibitors of ALS, inhibit the synthesis of branched amino acids (leucine, isoleucine, and valine). As a result, protein synthesis is disrupted, which interferes with DNA synthesis and cell growth. After absorption, they are translocated to meristems and apexes, which are areas of active growth; susceptible plants have inhibited growth. Sensitive plants become chlorotic, wither, and die within 7 to 14 days after treatment. Examples of these herbicides include imazaquin, imazethapyr, imazamox, imazapic, and imazapyr. They are systemic herbicides recommended for pre- and post-emergence control of many monocots and eudicots in cereals, soybean, and non-agricultural areas (Oliveira Júnior, 2011; Shaner and O'Connor, 2017; Rodrigues and Almeida, 2018).

Among the imidazolinones, herbicides imazapic/imazapyr (formulated premix) can be applied at pre-emergence and initial post-emergence (up to the V1 stage) of soybean (*csr1-2* transformed) up to a rate of 78.75/26.25 g a.e. ha^{-1} (Rodrigues and Almeida, 2018). However, there are few reports about tolerance and possible effects on chlorophyll indices and agronomic performance of CV soybean under imazapic/imazapyr application. Some undesirable effects arising from the application of this herbicide are reported in other herbicide-tolerant crops (e.g., glyphosate-tolerant crops) such as injury symptoms or changes in chlorophyll indices (Zobiole *et al*., 2010; Albrecht *et al*., 2011; Albrecht *et al*., 2018b). These effects are potentiated in late applications on soybean (V6–R2), with yield reductions for applications at glyphosate rates higher than that recommended (Albrecht *et al*.,2012).

The sulfonylurea-tolerant soybean (STS®) (non-transgenic) present no deleterious effects due to the application of ALS inhibitor herbicides (same mode of action of imidazolinones) (Albrecht *et al.*, 2018c; Silva *et al*., 2018; Silva *et al*., 2019). Other technology (Clearfield®- CL) is the cultivation of imidazolinone-tolerant mutagenic genotypes, also non-transgenic, available in maize, sunflower, rice, and wheat. Most studies indicate imazapic/imazapyr selectivity for rice (Galon *et al*., 2012). However, the application of imazapic/imazapyr, in association with seed treatment and clomazone, affected the photosynthetic rate, transpiration rate, and stomatal conductance in rice (Piveta *et al*., 2018).

Specifically, for imidazolinone-tolerant soybean, the pre-emergence application of imazapic/imazapyr (157.5/52.5 g a.e. ha^{-1}) was selective for soybean, without symptoms of injury and effects on yield and photosynthetic parameters (Matte *et al*., 2018). However, few studies can be found in the literature on the post-emergence application of imazapic/imazapyr on CV soybean. In some situations, even in herbicide-tolerant crops (transgenic or not), some undesirable effects from herbicide applications are observed. Thus, this study was aimed to evaluate the chlorophyll indices and agronomic performance of CV soybean under rates of imazapic/imazapyr at the V1, V2, and V3 stages.

MATERIALS AND METHODS

Design and experimental conditions**:** Two experiments were conducted in experimental areas of Palotina (24°20′75″S 53°51′69″W) (Experiment 1) and Brasilândia do Sul (24°05′14″S 53°28′95″W) (Experiment 2), Paraná State (PR), Brazil in 2015-16. The regional climate is the mesothermal humid subtropical (Cfa), according to Köppen classification. Figures 1 and 2 show the climate conditions during the experimental period. For experiment 1, the soil of the experimental area was classified as clayey texture, with 17.5% of sand, 16.25% of silt, and 66.25% of clay. In Experiment 2, soil was classified as sandy texture, with 55% of sand, 15% of silt, and 30% of clay. Table 1 shows the results of soil chemical analysis at a depth of 0–20 cm.

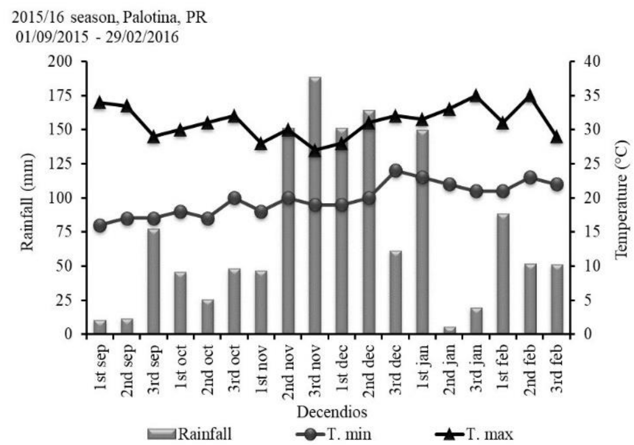

Figure 1. Representation of rainfall, maximum and minimum temperatures for the local conduction of the experiment. 2015-16 season, Palotina, PR, Brazil (experiment 1).

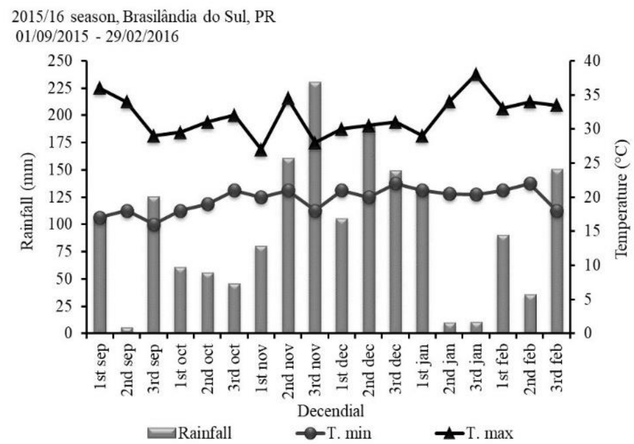

Figure 2. Representation of rainfall, maximum and minimum temperatures for the local conduction of the experiment. 2015-16 season, Brasilândia do Sul, PR, Brazil (experiment 2).

Table 1. Soil chemical analysis of experimental areas.

Palotina, PR									
pH	**Ca^{+2}**	**Mg^{+2}**	**K^{+}**	**Al^{3+}**	**$H^{+}+Al^{3+}$**	**CEC**	**V**	**P**	**O.M.**
$CaCl_2$		---------------------- $cmol_c\ dm^{-3}$ ----------------------					%	mg kg^{-1}	g kg^{-1}
5.2	5.73	2.24	0.91	0.00	5.76	14.64	60.66	32.24	37.1
Brasilândia do Sul, PR									
pH	**Ca^{+2}**	**Mg^{+2}**	**K^{+}**	**Al^{3+}**	**$H^{+}+Al^{3+}$**	**CEC**	**V**	**P**	**O.M.**
$CaCl_2$		---------------------- $cmol_c\ dm^{-3}$ ----------------------					%	mg kg^{-1}	g kg^{-1}
5.3	2.67	0.95	0.30	0.00	2.95	6.87	57.06	30.44	14.5

CEC: cation exchange capacity, V: Base saturation, O.M.: organic matter.

Before the setup of experiments, the area of Experiment 1 was cultivated with wheat, and the area of Experiment 2 was cultivated with second crop maize. The soybean cultivar BRS-397 CV (Embrapa, Brasília, DF, Brazil), classified as early maturing group (6.2), indeterminate growth habit, cycle around 110 to 120 days, was used. This cultivar was sown on 1 December 2015, with a density of 333,000 seeds ha^{-1} and interrow spacing of 0.5 cm. Plots consisted of six rows with five meters, but only four central rows with two meters in length were harvested for evaluation (borders were discarded), totalling a useful area of experimental units of 3.0 m^2.

The experimental design was a randomized block design with four replications. Treatments were arranged in a 3 × 5 factorial scheme, with applications at three soybean phonological stages (V1, V2 and V3) and five imazapic/imazapyr rates (0, 39.38/13.13, 78.75/26.25, 118.13/39.38, and 157.5/52.5 g a.e. ha^{-1}). The determination of the phonological stages followed the classification of Fehr *et al.* (1971). The formulated premix herbicide Soyvance® Pré (imazapic/imazapyr 525/175 g a.e. kg^{-1}, Basf S.A., São Paulo, SP, Brazil) was used. The experimental units consisted of plots with six rows of 5 m in length.

Sprayings were carried out according to each level of the factor phonological stage using a CO_2-pressurized sprayer with a constant pressure of 29 PSI and flow rate of 0.65 L min^{-1}, equipped with a boom with six flat fan spray nozzles (XR 110.02). Applications were performed at 0.5 m from the target, speed of 1 ms^{-1}, 0.5 m spacing between nozzles, and 200 L ha^{-1} spray volume.

Herbicide application in Experiment 1 was carried out on 14, 21 and 28 October 2015, under a mean temperature of 26, 28and 29°C, wind speed of 5, 6 and 7 km h^{-1}, and relative air humidity of 64, 62 and 61%, respectively. For Experiment 2, applications were performed on 16, 23 and 29 October 2015, with a mean temperature of 28, 29 and 30°C, wind speed of 4, 6 and 7 km h^{-1}, and relative air humidity of 65, 62 and 60%, respectively.

Evaluations**:** Symptoms of injury were evaluated visually by assigning percentage scores to each experimental unit (0% for no symptoms and 100% for plant death), considering, in this case, significantly visible symptoms according to plant development (Velini *et al.*, 1995). This evaluation was performed at 60 days after emergence (DAE).

The chlorophyll index was also performed at 60 DAE. For this, five plants were randomly selected in the useful area of plots. Chlorophyll indices type A, B, and total were measured using an electronic chlorophyll meter (clorofiLOG – CFL1030, Falker Automação Agrícola Ltda., Porto Alegre, RS, Brazil). This equipment determines Falker chlorophyll (FC) indices (Barbieri Júnior *et al*., 2012). The chlorophyll index was always evaluated on the central leaf of the first fully developed trefoil.

Variables related to agronomic performance (plant height, first pod height, lodging, number of pods per plant, yield, and 100-grain weight) were also evaluated. Heights and number of pods were evaluated at full maturation (R8 stage) (Fehr *et al*., 1971) by counting the number of pods in 10 plants randomly selected in the useful area of each plot. The variable lodging were evaluated by assigning visual scores, as follows: 1 = all plants upright, 2 = some plants inclined or slightly lodged, 3 = all plants moderately inclined or 25 to 50% lodged, 4 = all plants severely inclined or 51 to 80% lodged, and 5 = all plants lodged (Carvalho *et al*., 2010).

Plants from the two central rows were manually harvested (R8 stage), discarding the first and last meter of the plot, totalling a harvested area of $3m^2$. Pods were then threshed in a thresher for experiments, cleaned, and packed in paper bags for further evaluation. Grains produced in each plot had their weight measured and moisture corrected to 13%; the yield was calculated in kg ha^{-1} from these data. The 100-grain weight was determined from the mean weight of two subsamples of 100 grains per plot and had their moisture corrected to 13%.

Statistical analysis**:** The data were analysed according to Pimentel-Gomes and Garcia (2002) using the statistical program Sisvar 5.6 (Ferreira, 2011). Analysis of variance (ANOVA) and F-test (P<0.05) were performed. When significant, the means were compared by the Tukey (1949) test (P<0.05) for the factor application stage and subjected to regression analysis (P<0.05) for the factor rates.

RESULTS

Crop injury and chlorophyll indices: No injury symptoms were found in soybean plants for the application of imazapic/imazapyr (up 157.5/52.5 g a.e. ha^{-1}) at phonological stages (V1, V2 and V3) for both experiments. Also, for chlorophyll indices A, B, and total no significant effect was observed for the factors rates or stage, no interaction was found between factors (P>0.05) for chlorophyll indices A, B, and total (Table 2). This corroborates with what was observed for crop injury.

Agronomic performance: The same behaviour was observed for variables related to agronomic performance: first pod height, lodging, number of pods per plant, 100-grain weight, and yield - with means of 3,321 kg ha^{-1} (exp. 1) and 3,661kg ha^{-1} (exp. 2). No significant effect was observed for the factors rates or stage, no interaction was found between factors (P > 0.05) (Tables 3 and 4). Thus, the selectivity of imazapic/ imazapyr was found for post-emergence application (V1, V2 and V3) for the CV soybean.

Except for the total plant height (Table 3) at experiment 2, which had a significant effect on stage (P <0.05). Application of imazapic/imazapyr rates at V3 provided lower height (86 cm) to CV soybean plants when compared to application at V1 (94 cm), not differing from the application at V2 (89 cm).

Table 2. **Chlorophyll A, B and total indices of soybean plants under imazapic/imazapyr rates in 2015-16 season.**

Treatments	Chlorophyll A (FC)		Chlorophyll B (FC)		Total chlorophyll (FC)	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Stages						
V1	33	34	10	12	43	45
V2	34	34	11	12	45	46
V3	33	34	11	12	44	45
Rates (g a.e. ha^{-1})						
0	34	33	10	11	44	44
39.38/13.13	34	34	11	12	44	46
78.75/26.25	33	34	10	12	43	46
118.13/39.38	34	35	11	12	44	47
157.5/52.5	33	33	10	12	44	45
CV (%)	7.61	6.44	12.27	15.90	8.39	8.19
F-test						
Stage (S)	NS	NS	NS	NS	NS	NS
Rates (R)	NS	NS	NS	NS	NS	NS
S x R	NS	NS	NS	NS	NS	NS

FC: Falker chlorophyll; NS: non-significant, means do not differ from each other by the F-test ($P < 0.05$).

Table 3. **First pod height (cm), height (cm) and lodging of soybean plants under imazapic/imazapyr rates in 2015-16 season.**

Treatments	FPH (cm)		PH (cm)		Lodging	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Stages						
V1	22	20	105	94 a	4	3
V2	20	19	103	89 ab	3	3
V3	20	17	98	86 b	3	3
Rates (g a.e. ha^{-1})						
0	22	18	104	90	3	3
39.38/13.13	20	19	101	91	3	3
78.75/26.25	21	18	104	89	3	3
118.13/39.38	19	18	102	88	3	3
157.5/52.5	20	18	100	89	4	3
CV (%)	11.80	10.40	7.21	4.30	26.03	25.55
F-test						
Stage (S)	NS	NS	NS	*	NS	NS
Rates (R)	NS	NS	NS	NS	NS	NS
S x R	NS	NS	NS	NS	NS	NS

PH: plant height, FPH: first pod height. *Means followed by the same letter in column are not different by Tukey's (1949) test ($P < 0.05$). NS: non-significant, means do not differ from each other by the F-test ($P > 0.05$).

DISCUSSION

The pre-emergence application of imazapic/imazapyr (157.5/52.5 g a.e. ha^{-1}) was selective for CV soybean, with no symptoms of injury, effects on yield and photosynthetic parameters (Matte *et al.*, 2018), as observed in this study for the post-emergence application on soybean (V1, V2 or V3). CV soybean was also tolerant to the residual effect of imazapic/imazapyr when applied in post-emergence on imidazolinone-tolerant rice (Agostinetto *et al*., 2018). These results demonstrate the tolerance of CV soybean to the application of imazapic/imazapyr under different management situations. Non-imidazolinone-tolerant soybean cultivars showed their agronomic performance negatively affected by the pre-emergence application of imazapic/imazapyr (Agostinetto *et al*., 2018). Ulbrich *et al.* (2005) also observed a residual period of 87–88 days for imazapic/imazapyr application (105/35 g a.e. ha^{-1}) for soybean (non-tolerant to imidazolinones). Imidazolinones are widely used for weed control in tolerant mutagenic cultivars such as rice, wheat, and corn, especially rice (Clearfield® technology – CL) (Bzour *et al*., 2018; Rangel *et al*., 2018). In this context, these and the present study position

Table 4. **Number of pods, 100-grain weight and yield of soybean plants under application of imazapic/imazapyr rates in 2015-16 season.**

Treatments	Pods		100-grain weight (g)		Yield (kg ha^{-1})	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Stages						
V1	37	42	17	17	3,406	3,589
V2	37	39	16	17	3,267	3,631
V3	37	39	16	17	3,289	3,762
Rates (g a.e. ha^{-1})						
0	37	39	16	17	3,332	3,656
39.38/13.13	38	40	16	16	3,305	3,539
78.75/26.25	38	41	16	17	3,288	3,678
118.13/39.38	36	38	17	17	3,590	3,668
157.5/52.5	36	40	16	17	3,089	3,761
CV (%)	9.44	8.29	6.06	4.33	10.98	14.04
F-test						
Stage (S)	NS	NS	NS	NS	NS	NS
Rates (R)	NS	NS	NS	NS	NS	NS
S x R	NS	NS	NS	NS	NS	NS

NS: non-significant, means do not differ from each other by the F-test ($P > 0.05$).

imidazolinone-tolerant soybean as safe for succession to CL crops.

Regarding agronomic performance, no negative effect of imazapic/imazapyr rates on soybean was observed for application at the V1, V2, and V3 stages. However, plant height showed a reduction for application at V3 when compared to V1. Similarly, Hungria *et al*. (2015) observed that imazapic application (70 g a.e. ha^{-1}) or insertion of a resistance gene (compared to conventional soybean isoline) did not affect negatively on soybean yield and biological nitrogen fixation. Imidazolinones have great potential for weed management, being important in preventing the selection of biotypes resistant to glyphosate and other herbicides (Barnes *et al*., 2017; Underwood *et al*., 2018; Hedges *et al*., 2019), in different chemical control programs of weeds. Imazapic/imazapyr application was also effective in weed control, such as *Bidens pilosa*, *Raphanus raphanistrum* (Santos *et al*., 2012), *Echinochloa crus-galli*, *Cyperus esculentus* (Helgueira *et al*., 2018), *Digitaria insularis* (Melo *et al*., 2017) and voluntary maize (Piasecki and Rizzardi, 2016). In this context, given the effectiveness of the imidazoline one herbicides reported in other studies and selectivity for imazapic/imazapyr application on soybean up to V3 and a rate of up to 157.5/52.5 g a.e. ha^{-1}, CV soybean has been characterized as an alternative in weed management whether by the use of imazapic/imazapyr or in succession to CL crops, as highlighted by Agostinetto *et al*. (2018) and Matte *et al*. (2018).

The possibility of using other herbicides stands out for CV soybean cultivation, such as ALS-inhibiting herbicides of the imidazolinone family. Herbicide rotation with different mechanisms of action is essential in preventing the selection of resistant biotypes and effectively controlling weeds. Imazapic/imazapyr (formulated premix) application up to a rate of 157.5/52.5 g a.e. ha^{-1} did not affect chlorophyll indices and agronomic performance of Cultivance® soybean (cultivar BRS-397 CV) at the phonological stages V1, V2 and V3. Thus, the selectivity of imazapic/imazapyr (up to 157.5/52.5 g a.e. ha^{-1}) was found optimum for post-emergence application (up to V3) for the Cultivance® soybean.

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