doi: 10.1590/S0100-83582016340300014

Tolerance of Common Bean Plants to Ethoxysulfuron Herbicide and the Mechanism Involved in the Process[1]

Tolerância do Feijoeiro ao Herbicida Ethoxysulfuron e o Mecanismo Envolvido no Processo

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ABSTRACT - The objectives of this study were to determine the effect of herbicide ethoxysulfuron on the development of commom bean (*Phaseolus vulgaris*), to evaluate the impact of the herbicide on the crop grain yield and to determine the mechanism of tolerance of common bean to ethoxysulfuron. Field experiments were carried out with several doses of ethoxysulfuron to determine its effect on the grain yield of two varieties of common bean ("IPR Andorinha", "IPR Tangará"). The mechanism of plant tolerance was evaluated through several methods, including the analysis of the ALS enzyme sensitivity to herbicide and the use of P450 inhibitors (the insecticides malathion + chlorpyrifos) and the use of antidote (mefenpyr-diethyl). When the rate of ethoxysulfuron was 83.2 g ha^{-1}, the grain yield loss reached 25% (IPR Tangará) and 35% (IPR Andorinha). The ALS enzyme activity was severely reduced even at low concentrations ethoxysulfuron (1 µM), indicating that the mechanism of tolerance is not the insensitivity of the herbicide target enzyme. The inhibitors of herbicide detoxification increased the sensitivity of the bean plants to ethoxysulfuron, and the antidote (stimulator of herbicide degradation) mitigated the herbicidal effect. These results strongly support the hypothesis that the mechanism of tolerance of common bean plants to ethoxysulfuron is enhanced herbicide detoxification.

Keywords: *Phaseolus vulgaris*, crop grain yield, ALS enzyme, detoxification.

RESUMO - Os objetivos deste trabalho foram determinar o efeito do herbicida ethoxysulfuron sobre o desenvolvimento do feijoeiro (Phaseolus vulgaris), avaliar o impacto do herbicida sobre o rendimento de grãos e determinar o mecanismo de tolerância do feijoeiro ao ethoxysulfuron. Em campo, foram realizados dois experimentos com doses crescentes de ethoxysulfuron, a fim de determinar o seu efeito sobre o desenvolvimento e o rendimento de grãos de dois cultivares (cvs.) de feijão (IPR Andorinha e IPR Tangará). O mecanismo de tolerância das plantas foi determinado através da análise de sensibilidade da enzima ALS ao herbicida, do uso de inseticidas inibidores da P450 (malathion + chlorpyrifos) e do uso de antídoto (mefenpyr-diethyl). Quando a dose de ethoxysulfuron foi de 83,2 g ha^{-1}*, as perdas de rendimento de grãos atingiram 25% (IPR Tangará) e 35% (IPR Andorinha). A atividade da enzima ALS foi severamente reduzida mesmo em baixas concentrações (1µM) de ethoxysulfuron, sugerindo que o mecanismo de tolerância não é a insensibilidade da enzima alvo do herbicida. Os inibidores da degradação aumentaram a sensibilidade das plantas de feijão ao ethoxysulfuron, e o antídoto (estimulador da detoxificação) mitigou o efeito herbicida. Esses resultados suportam a hipótese de que o mecanismo de tolerância das plantas de feijoeiro ao herbicida ethoxysulfuron é a metabolização das moléculas.*

Palavras-chave: *Phaseolus vulgaris*, rendimento de grãos, enzima ALS, detoxificação.

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[1] Recebido para publicação em 26.10.2015 e aprovado em 7.4.2016.

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INTRODUCTION

The common bean (*Phaseolus vulgaris*) is one of the most important sources of plant protein on the human diet (Luna-Vital et al., 2015). The life cycle of the plants is very short (70-100 days), which limits the crop size and decreases its competitive ability with weeds. Weed competition can reduce the common bean grain yield up to 70% (Kozlowski et al., 2002), besides affecting the harvesting efficiency and the quality of grains. The weed management in bean crop is focused mainly on herbicides. However, there is limited number of herbicide options for the control of broad-leaved weeds in post-emergence in the crop. In Brazil, for instance, it is available herbicides from only three mechanisms of action for the bean crop, namely the inhibitors of photosystem II (FS2), protoporphyrinogen oxidase inhibitors (PROTOX) and inhibitors of acetolactate synthase (ALS) (Brasil, 2015). These facts suggest the need to seek herbicides that have low toxicity to the crop and that provide broad-spectrum weed control.

The enzyme acetolactate synthase (ALS) catalyzes the first step in the biochemical pathway of branched chain amino acids, valine, leucine and isoleucine (Deng et al., 2014). This enzyme is the target of five herbicides chemical groups, sulfonylureas, imidazolinones, triazolopyrimidines, pirimidiltiobenzoatos and sulfonylamine carbonyl triazolinones (Yu and Powles, 2013). The ALS-inhibiting herbicides are widely used in agriculture due to its high selectivity for crops, weed control with low doses and low toxicity to mammals. Ethoxysulfuron is an ALS inhibitor from the chemical group sulfonylureas and it is used for post-emergence control of sedges and some dicotyledonous plants.

The main mechanism of tolerance of cultivated plants to ALS inhibitors is the degradation of the herbicide molecule. Cytochrome monoxygenases (P450s) are one of the largest groups of enzyme families that are responsible for plant tolerance to herbicides (Buker, 2004; Beckie et al., 2012; Yu and Powles, 2013; Hamouzova et al., 2014; Rojano Delgado et al., 2014). The P450 enzymes are present in the endoplasmic reticulum and are involved in synthesis of hormones derived from fatty acids and also with the secondary metabolism of plants (Powles and Yu, 2010). Another group of enzymes capable of detoxifying the ALS-inhibiting herbicides are the glutathione-S-transferases (GST) (Zhen et al., 2012). These detoxifying enzymes can be inhibited by organophosphorus insecticides, which are used as an indirect tool to demonstrate that detoxification is the mechanism of herbicide tolerance in crops (Matzrafi et al., 2014). Mefenpyr-diethyl is called a crop protectant or safener because increases the herbicidal detoxification, resulting in non-toxic compounds in the crop plants (Rosinger and Kocher, 2007). Mefenpyr increases GST activity in wheat (Taylor et al., 2013) or favors the synthesis of P450 in *Alopecurus aequalis* (Hongchun et al., 2013).

The hypothesis tested is that bean plants are selective to ethoxysulfuron thus has potential use for common bean crop and the mechanism of tolerance is based on the detoxification of the herbicide molecule. The objectives of this study were to determine the effect of herbicide ethoxysulfuron on the development of common bean, to evaluate the impact of the herbicide on the crop grain yield and to determine the mechanism of tolerance of common bean to ethoxysulfuron.

MATERIAL AND METHODS

Two experiments were conducted in the field to evaluate the effect of ethoxysulfuron herbicide on the grain yield of common bean. Three laboratory experiments were conducted to identify the mechanism of common bean tolerance to ethoxysulfuron.

Field experiments

Two experiments were conducted between the months of March to May 2014 on fields located in the cities of Mariópolis (26°19'S and 52°39'O and 820 m of altitude) and Renascença (26.09°S and 52°55'O and 670 m of altitude), PR, Brazil. The soil from all places is classified as Distroferric Red Latosol (Oxisol) with the following characteristics: sand 2.50%, silt 39.50%, clay 58.00%, 6.7% organic matter and pH 5.2 (Mariópolis); sand 17.5%, silt 18.75%, clay 63.75%, 3.4% organic matter and pH 5.2 (Renascença).

In all experiments, the experimental design was a randomized block design (RBD) with four replicates. On the experiment at Mariópolis, the common bean varieties (var.) IPR Andorinha was used at the plant density of 200.000 plants ha^{-1}. In Renascença, the var. IPR Tangará was used at the density of 300.000 plants ha^{-1}. In both experiments, each experimental unit had five meters long and two meters wide and the treatments consisted of seven doses of ethoxysulfuron (0, 8.3, 14.6, 20.8, 41.6, 62.5 and 83.3 g ha^{-1}). All the treatments were hand-hoed during the whole season to remove all the weeds, for phytotoxicity assessment of ethoxysulfuron to common bean.

The herbicide was applied with a backpack sprayer pressurized with CO_2, delivering the spray volume of 200 L ha^{-1}, the spray boom had four flat fan nozzles type XR 110.02, spaced 0.50 m apart. The average environmental conditions during the application were relative humidity 65±5% and temperature 26.6±2 °C. The application occurred when the plants reached the four-leaf growth stage. At 25 days after application of the herbicide (DAA), the shoots of 10 plants were randomly collected in each plot and weighed to determine the fresh weight (FW). The grain yield was determined after harvesting all the plants on the central two bean rows, except the ones located at 50 cm at each end. The grain mass were converted to percentage yield reduction in relation to control not sprayed with herbicide. The data was submitted to the analysis of variance (ANOVA) by F test ($p \leq 0.05$) with statistical software GENES (Cruz, 2013). After the ANOVA, the relation between the FW data, averaged for each dose, and the herbicide rate was adjusted to the three-parameters logistic model (Equation 1), as proposed by Seefeldt et al. (1995). The relation between grain yield loss and herbicide rate were adjusted to the hyperbolic model (Equation 2).

$$Y = a/[1 + (x/D_{50})^{b}] \qquad \text{(eq. 1)}$$

where: Y is the response of the dependent variable, x is the dose of the herbicide, a is the maximum asymptote of the curve, b is the slope and D_{50} is the dose required to reduce the dependent variable by 50%.

$$Y = (a * x)/(D_{50} + x) \qquad \text{(eq. 2)}$$

where: Y, a, x and D_{50} are defined as previously described.

The hyperbolic equation (Equation 2) from the data obtained was reorganized using the parameters attained above to estimate the ethoxysulfuron dose based on the yield loss (Equation 3). We propose here that this equation would aid the farmers to decide the herbicide dose considering yield loss caused by the weeds and the one caused by the herbicide.

$$x = (Yl * D_{50})/(a - Yl) \qquad \text{(eq. 3)}$$

Enzymatic test

From bean plants (var. IPR Eldorado and BRS Esplendor) grown in the greenhouse, young leaves were collected and frozen in liquid nitrogen. ALS enzyme extraction followed the method described by Gerwick et al. (1993). The herbicide sensitivity assay conducted in microcentrifuge tubes used 500 µL for each herbicide solution. The final concentrations of ethoxysulfuron-ethyl herbicide were 0 (100% enzyme activity), 1, 3, 6, 12, 24, and 48 µM. A volume of 500 µL distilled water was used for positive control (equivalent to 100 % of ALS activity). For the negative control treatment (equivalent to 0% of the ALS enzyme activity), 250 µL of 1.8 N H_2SO_4 was added to the microcentrifuge tubes. In each microcentrifuge tube, it was added 500 µL of the enzyme extract, which were incubated for a period of 90 min at 37 °C and the final product of this reaction was acetolactate. After this period, the reactions were stopped with 250 µL of 1.8 N H_2SO_4, except on the negative control. All treatments were performed with three replicates.

A second incubation for 15 min at 60 °C was conducted after addiction of 700 µL of 2 N sodium hydroxide solution, containing 0.25% of creatinine and 2.5% of naphthol for the reaction of formation of the colored complex acetoin. The amount of this compound was determined by absorbance readings on a spectrophotometer (Shimadzu UV-1800) at 535 nm. The ALS activity values were expressed in enzyme unit per mg (U mg^{-1}), where one unit of ALS is defined as the amount of enzyme able to produce 0.1 absorbance unit per minute, expressed as a function of total protein concentration (specific activity). The results of inhibition of enzyme activity were converted to percentage values, considering as 100% activity in the absence of the herbicide.

After the ANOVA (Cruz, 2013), a regression between the ALS enzyme activity and the herbicide concentration was adjusted to the exponential-decay model (Equation 4):

$$y = c + [a * \exp(-b * x)] \qquad \text{(eq. 4)}$$

where: Y, a and b were described previously, c is the minimum asymptote of the curve and x is the herbicide concentration.

Effect of detoxification inhibitors and herbicide antidote (safener)

During the spring of 2015, two experiments were conducted at the greenhouse using a completely randomized design (CRD) with four replicates, and the treatments were arranged in a factorial scheme. Pots with the capacity of 4500cm³ were filled with soil, whith the fertility corrected according to chemical analysis. At weekly intervals, 10 mL of Hoagland solution was added to each pot to maintain optimal fertility for the plant development. The day/night temperatures at the greenhouse were 25±5 °C/19±4 °C.

On the first experiment, the common bean var. BRS Esplendor was used and the herbicide was sprayed when the plants were at the two-leaf growth stage. The factor A consisted of seven doses of ethoxysulfuron (0, 3, 7, 15, 25, 50 and 75 g ha^{-1}). The factor B consisted of two conditions (without and with) of inhibitors of herbicide detoxification (malathion at 1.000 g ha^{-1}, and chlorpyrifos, at 1.125 g ha^{-1}) (Beckie et al., 2012), which were applied 3 hours before the herbicide application. Herbicide and inhibitors were applied with CO_2 pressurized backpack sprayer, calibrated as described previously. At 25 DAA, the shoots were collected and dried to determine its mass (DW). Data were converted to the percentage mass reduction in relation to the control not sprayed with herbicide or herbicide+detox-inhibitors. After the ANOVA (Cruz, 2013), the data was adjusted to the three parameters logistic model (Equation 1).

On the second experiment, the common bean var. was BRS Eldorado. The factor A consisted of seven doses of ethoxysulfuron (0, 3, 7, 15, 30, 60 and 120 g ha^{-1}). The factor B consisted of two conditions (without and with) of herbicide antidote mefenpyr-diethyl (18 g ha^{-1}), which was applied three hours before the herbicide spraying. The antidote was applied with a CO_2 back-pack sprayer with one XR 110.01 VS nozzle, calibrated to deliver the volume of 100 L ha^{-1}. The herbicide was sprayed as described previously. At 25 DAA the distance between the third and fourth node of the plants was measured. Data were converted to percentage inter-node reduction in relation to the control not sprayed with herbicide or herbicide+antidote. After the ANOVA (Cruz, 2013), the data was fitted to the sigmoidal model (Equation 5) and four-parameters logistic model (Equation 6). The D_{50} values obtained in each equation was used to calculate the tolerance factor (TF). The TF determines how the plants are more tolerant without applying metabolism inhibitors, or by applying the antidote.

$$Y = d + \{a/[1 + \exp\ -((x - D_{50})/b)]\} \qquad \text{(eq. 5)}$$

$$Y = d + \{a/[1 + (x/D_{50})^{b}]\} \qquad \text{(eq. 6)}$$

where: Y, x, b and D_{50} were described previously, a is the difference between the minimum and maximum asymptote of the curve, and d can represent the minimum or the maximum asymptote of the curve depending on the estimated signals of the a and b parameters.

RESULTS AND DISCUSSION

Field experiments

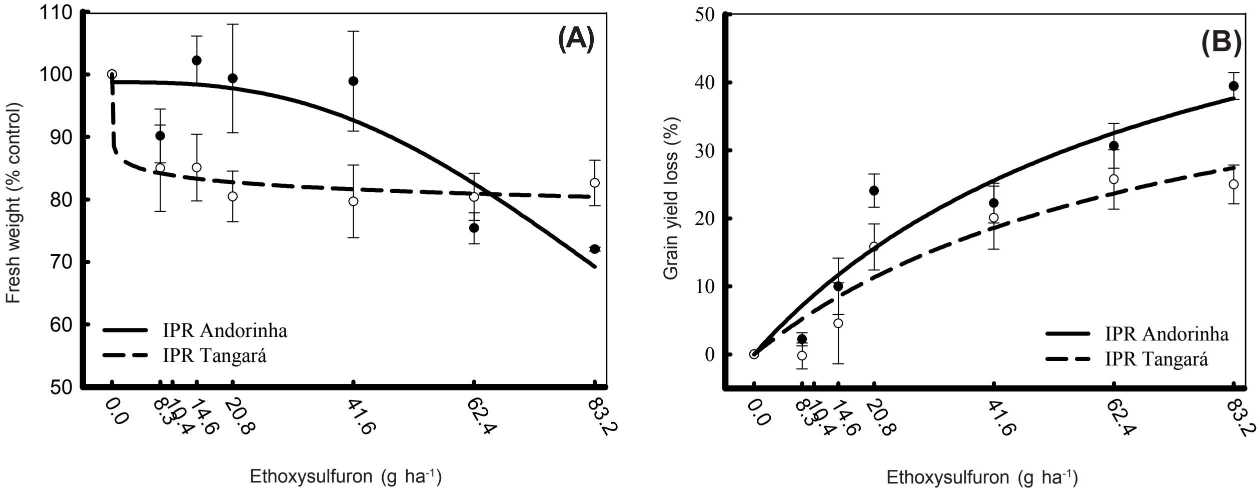
The three-parameter logistic equation fit ($p \leq 0.05$) the regression between FW and herbicide rate (Table 1). At reduced ethoxysulfuron doses, the plants from var. IPR Andorinha are less affected by the herbicide than plants from the var. IPR Tangará (Figure 1A). At the highest dose evaluated (83.2 g ha^{-1}), the FW from plants of both var. was reduced to 70-80%, in relation to untreated control.

The rectangular hyperbolic model adjusted ($p \leq 0.01$) the regression between the yield loss and ethoxysulfuron rates (Table 2).

Table 1 - Equation parameters, coefficient of determination (R²), residual mean square (RMS) and probability of significance of the equation (*p*) to determine the relationship between ethoxysulfuron rates and the fresh weight evaluated on two bean varieties at 25 days after the herbicide application

Varieties	Parameters[1/]			R²	RMS	*p*
	a	*b*	D_{50}			
IPR Andorinha	98.76 (3.83)[2]	2.70 (1.63)	114.07 (29.15)	0.70	47.33	< 0.05
IPR Tangará	100.02 (2.12)	0.11 (0.07)	> 83	0.91	4.49	< 0.05

[1/] Three-parameter logistic equations. [2/] Values in parentheses indicate the standard error of the estimative of the parameter.



Each dot corresponds to the average of four repetitions and the bars represent its standard error. Equation and its parameters on Table 1 and 2.

Figure 1 - Effect of ethoxysulfuron on (A) fresh weight (%), measured at 25 days after treatment; and (B) grain yield loss (%), of two bean varieties, IPR Andorinha and IPR Tangará.

Table 2 - Equation parameters, coefficient of determination (R²), residual mean square (RMS) and probability of significance of the equation (*p*) to determine the relationship between ethoxysulfuron rates vs. common bean grain yield loss

Varieties	Parameters[1/]		R²	RMS	*p*
	a	D_{50}			
IPR Andorinha	71.39 (30.22)[2/]	74.58 (54.71)	0.89	23.45	< 0.01
IPR Tangará	52.32 (24.93)	75.49 (62.09)	0.88	15.46	< 0.01

[1/] Hyperbolic equation $y = (a * x) / (x + D_{50})$ based on normalized data to percentage of yield loss in relation to the untreated control, where a = maximum asymptote of the curve; x = ethoxysulfuron doses (independent variable); D_{50} = required dose of ethoxysulfuron to reduce a by 50%. [2/] Values in parentheses indicate the standard error of the estimative of the parameter.

Independent of the var. evaluated, the grain yield loss (Figure 1B) increased proportionally to ethoxysulfuron rate. In the rate of 83.2 g ha^{-1}, the maximum yield loss detected for plants of the var. IPR Tangará was 25%, while in the var. IPR Andorinha was 35%.

The tolerance to ethoxysulfuron in common bean plants from the var. IPR Andorinha and IPR Tangará was dependent on the herbicide rate. The grain yield loss of plants from the var. IPR Andorinha were more sensitive to ethoxysulfuron than plants from the var. IPR Tangará. Considering the life cycle of plants of var. IPR Andorinha is 73 days and of var. IPR Tangará is 87 days, it suggests that ethoxysulfuron tolerance on common bean plants can be related to plant life cycle. One possible explanation for this result is that plants with longer life cycle exhibit higher morphological plasticity (Lamego et al., 2004; Bianchi et al., 2011), which may decrease the effects of the herbicide.

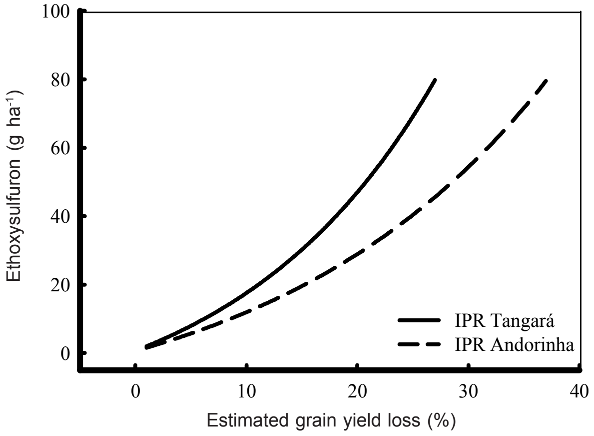
Figure 2 may be useful to help to define the herbicide dose considering the level of yield losses caused by the weeds. For example, assuming the yield loss caused by the weeds is 20% either on the common bean crop var. IPR Tangará or var. IPR Andorinha, then the maximum dose of ethoxysulfuron to be used should not exceed 46 g ha^{-1} and 29 g ha^{-1} on each var., respectively. The impact of the herbicide ethoxysulfuron on the crop when used below these rates would be smaller than the yield losses assumed by the weeds.

One possible form to reduce the crop injury from the herbicide is to adjust the herbicide rate (Figure 2). However, to avoid the decrease on the weed control, synergistic mixtures with herbicides from other mechanisms of action should be considered. This tactic also would be helpful to decrease the risk of evolution of herbicide resistance in weeds associated with low doses (Powles and Yu, 2010).

Tolerance mechanism

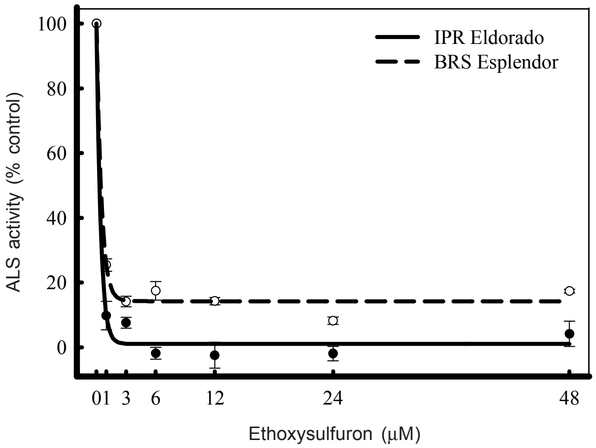
Independent on the var. evaluated, the activity of the ALS enzyme was severely reduced by the herbicide ethoxysulfuron (Figure 3). The D_{50} on either var. did not differ (Table 3), which suggests that the ALS enzyme from plants of both var. are sensitive to ethoxysulfuron.

The three-parameter logistic model adjusted ($p \leq 0.01$) the regression between DW and ethoxysulfuron rate (Figure 4A, Table 4). The DW responded in an inverse proportion to the increment of the herbicide rate. The D_{50}



Equation ER = (YL * D_{50})/(a-YL). Equation parameters on Table 2.

Figure 2 - Ethoxysulfuron rates (ER) estimated according to assumed weed-caused grain yield loss (YL) of common bean for cultivars IPR Tangará and IPR Andorinha.



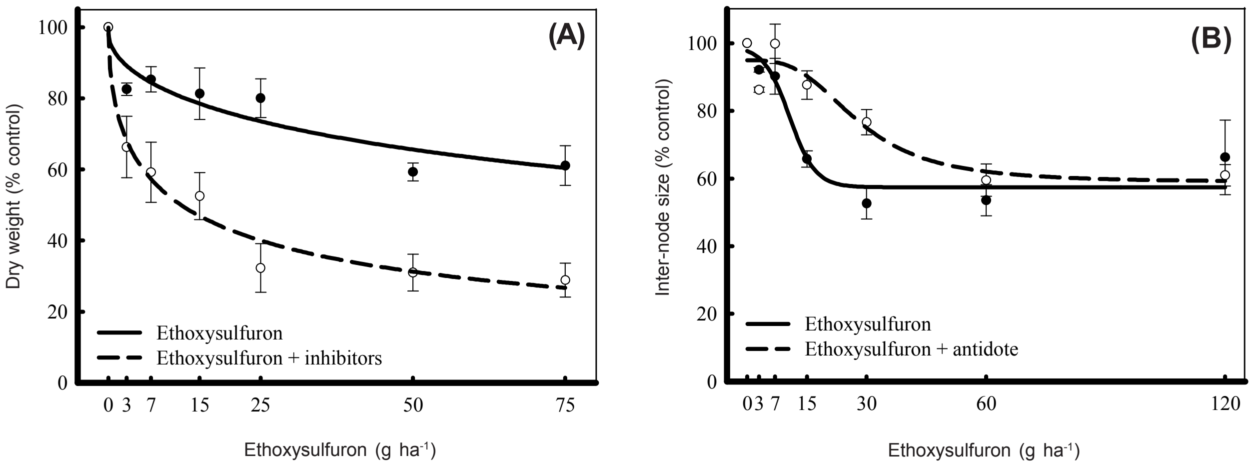
Each dot corresponds to the average of three repetitions and the bars represent its standard error. Equation and its parameters on Table 3.

Figure 3 - Effect of ethoxysulfuron on the ALS enzyme activity of two common bean varieties (IPR Eldorado and BRS Esplendor).

Table 3 - Equation parameters, coefficient of determination (R²), residual mean square (RMS) and probability of significance of the equation (p) to determine the relationship between ethoxysulfuron concentration and the activity of the ALS enzyme (% in relation to the untreated), for two common bean varieties

Varieties	Parameters[1/]			D_{50}	R²	RMS	p
	a	b	c				
IPR Eldorado	98.96 (4.93)[2/]	2.41 (0.55)	1.03 (2.02)	0.29	0.98	20.28	< 0.01
BRS Esplendor	85.77 (4.11)	2.04 (0.37)	14.23 (1.69)	0.42	0.99	14.08	< 0.01

[1/] Three-parameter exponential decay. [2/] Values in parentheses indicate the standard error of the estimative of the parameter.



Each dot corresponds to the average of four repetitions and the bars represent its standard error. Parameters on Table 4.

Figure 4 - Effect of ethoxysulfuron on (A) dry weight of commom bean plants (var. BRS Esplendor) in the presence or absence of herbicide detox-inhibitors (chlorpyrifos+malathion) evaluated 25 days after application; and (B) inter-node size of common bean plants (var. IPR Eldorado) in the presence or absence of the herbicide antidote (mefenpyr), evaluated 25 days after application.

Table 4 - Equation parameters, coefficient of determination (R^2), tolerance factor (TF), residual mean square (RMS) and probability of significance of the equation (*p*) to determine the relationship between dry weight (BRS Esplendor) as a function of ethoxysulfuron rate, with or without the use herbicide detox-inhibitors; and the inter-node size (IPR Eldorado) as a function of the herbicide rate, with or without antidote. Data evaluated 25 days after ethoxysulfuron application

Treatments		Parameters				R²	RMS	TF	*p*
		a	*b*	D_{50}	*d*				
Inhibitors[1/]	Without [3/]	98.11 (5.73)[6/]	0.57 (0.20)	172.07 (91.73)	-	0.83	34.62	14.34	< 0.05
	With[3/]	99.89 (5.06)	0.55 (0.09)	12.00 (3.15)	-	0.96	25.76	-	< 0.01
Antidote[2/]	Without[4/]	41.67 (10.24)	-3.21 (2.22)	10.64 (3.22)	57.44 (3.93)	0.88	43.55	-	< 0.05
	With[5/]	-36.07 (9.28)	-3.06 (2.73)	27.96 (8.47)	94.94 (4.35)	0.82	49.67	2.62	< 0.05

[1/] Inhibitors of herbicide degradation (malathion + chlorpyrifos). [2/] Antidote (mefenpyr-diethyl). [3/] Three-parameter logistic equation. [4/] Four-parameter sigmoidal equation. [5/] Four-parameter logistic equation. [6/] Values in parentheses indicate the standard error of the estimative of the parameter.

for the treatment with and without herbicide detox-inhibitors were 172 and 12 g ha^{-1}, respectively. In other words, the common bean plants were 14 fold more tolerant to ethoxysulfuron in the absence of the inhibitor of ethoxysulfuron detoxification, when compared to the treatments in the presence of the inhibitors (Table 4).

The four-parameter logistic model fit the regression between the herbicide rate and the inter-node size on common bean plants (Figure 4B, Table 4). The differences between the curves with and without the antidote mefenpyr were only evident for ethoxysulfuron rates between 15 and 30 g ha^{-1} (Figure 4B). The D_{50} values for the treatment with and without herbicide safener were were 27.9 and 10.6 g ha^{-1}, respectively. This results indicate that in the presence of the safaner mefenpyr the plants were 2.6 fold more tolerant to ethoxysulfuron than in its absence (Figure 4B, Table 4).

Taken togheter, these results support the hypothesis that the detoxification of ethoxysulfuron is the selectivity mechanism of the compound to common bean plants. In fact, there is several evidences that herbicide detoxification is responsible for many cases of tolerance of crop plants to herbicides, including corn to nicosulfuron (Liu et al., 2015), cotton to pyrithiobac (Snipes and Seifert, 2003), corn to mesotrione (Ogliari et al., 2014), and soybean to sulfentrazone (Walsh et al., 2015).

There is evidence of tolerance of common bean to other ALS inhibitors (Procopio et al., 2009). The grain yield losses caused by the herbicide chlorimurom-ethyl on common bean plants from several varieties ranged from 0 to 55%. The herbicide imazethapyr has the potential to be selective to the common bean crop, despite crop yield losses up to 18% (Procopio et al., 2009).

In this study the contribution of P450 activity to the enhanced detoxification of ethoxysulfuron in common bean was supported either with the use of detox-inhibitors or inducer of P450 enzymes involved in sulfonylurea metabolism This results were in accordance with other authors (Matzrafi et al., 2014). This research highlights the need to avoid using tank mixtures of ethoxysulfuron with organophosphate insecticides such as malathion and chlorpyrifos to prevent crop injury by the herbicide.

ACKNOWLEDGEMENTS

To CAPES, CNPq and UTFPR, for the financial support of this research.

REFERENCES

Beckie H.J. et al. Basis for herbicide resistance in canadian populations of wild oat (*Avena fatua*). **Weed Sci.** 2012;60:10-8.

Bianchi M.A et al. Interference of *Raphanus sativus* in soybean cultivars yield. **Planta Daninha**. 2011;29:783-92.

Buker R.S. et al. Physiological basis for differential tolerance of tomato and pepper to rimsulfuron and halosulfuron: site of action study. **Weed Sci.** 2004;52:201-5.

Cruz C.D. GENES - a software package for analysis in experimental statistics and quantitative genetics. **Acta Sci.** 2013;35:271-6.

Deng W. et al. Different cross-resistance patterns to AHAS herbicides of two tribenuron-methyl resistant flixweed (*Descurainia sophia L.*) biotypes in China. **Pestic Biochem Physiol.** 2014;112:26-32.

Gerwick B.C. et al. Rapid diagnosis of ALS/AHAS inhibitor herbicide resistant weeds. **Weed Technol.** 1993;7:519-24.

Hamouzova K. et al. Mechanisms of resistance to acetolactate synthase-inhibiting herbicides in populations of *Apera spica-venti* from the Czech Republic. **Pestic Manage Sci.** 2014;70:541-8.

Hongchun W. et al. The role of cytochrome P450 monooxygenase in the different responses to fenoxaprop-P-ethyl in annual bluegrass (*Poa annua* L.) and short awned foxtail (*Alopecurus aequalis* Sobol.). **Pestic Biochem Physiol.** 2013;107:334-42.

Kozlowski L.A. et al. Período crítico de interferência das plantas daninhas na cultura do feijoeiro-comum em sistema de semeadura direta. **Planta Daninha**. 2002;20:213-20.

Lamego F.P. et al. Tolerance to interference of competing plants and suppressive ability by soybean genotypes – II. Response of yield variables. **Planta Daninha**. 2004;22:491-8.

Luna-Vital D.A. et al. Biological potential of protein hydrolysates and peptides from common bean (*Phaseolus vulgaris* L.): A review. **Food Res Inter.** 2015;76:92-104. 2015.

Liu X. et al. RNA-Seq transcriptome analysis of maize inbred carrying nicosulfuron-tolerant and nicosulfuron-susceptible alleles. **Inter J Molec Sci.** 2015;16:5975-89.

Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Agrofit: sistema de agrotóxicos Fitossanitários. [acessado em: 26 out. 2015]. Disponível em http://extranet.agricultura.gov.br/agrofit_cons/principal_agrofit_cons.

Matzrafi M. et al. Evolution of herbicide resistance mechanisms in grass weeds. **Plant Sci.** 2014;229:43-52.

Ogliari J. et al. Análise do perfil eletroforético de proteínas citoplasmáticas para verificação do processo de desintoxicação do herbicida mesotrione em plantas de *Zea mays*. **Planta Daninha**. 2014;32:161-72.

Powles B.S., Yu Q. Evolution in action: plants resistant to herbicides. **Ann Rev Plant Biol.** 2010;61:317-47.

Procopio S.O. et al. Potencial de uso dos herbicidas chlorimuron-ethyl, imazethapyr e cloransulam-methyl na cultura do feijão. **Planta Daninha**. 2009;27:327-36.

Rojano-Delgado A.M. et al. Mechanism of imazamox resistance of the Clearfield® wheat cultivar for better weed control. **Agron Sustain Develop.** 2014;35:639-48.

Rosinger C., Kocher H. Safener for Herbicides. In: KRAMER W. e SCHIRMER, U. **Modern Crop Protection Compounds**. 1. ed. Wiley-VCH Verlag, 2007. p.259–282.

Seefeldt S.S. et al. Log-logistic analysis of herbicide dose-response relationship. **Weed Technol.** 1995;9:218-27.

Snipes C.E., Seifert S. Influence of Malathion Timing on Cotton (*Gossypium hirsutum*) Response to Pyrithiobac. **Weed Technol.** 2003;17:266-8.

Taylor V.L., et al. Protective responses induced by herbicide safeners in wheat. **Environ Exp Bot.** 2013;88:93-9.

Walsh K.D. et al. Biologically effective rate of sulfentrazone applied pre-emergence in soybean. **Canadian J Plant Sci.** 2015;95:1-6.

Yu Q., Powles B.S. Resistance to AHAS inhibitor herbicides: current understanding. **Pest Manage Sci.** 2013;70:1340-50.

Zhen H. et al. Difference and mechanism of tolerance of various wheat varieties to florasulam. **Chinese J Pestic Sci.** 2012;14:260-6.