Journal of Plant Protection Research ISSN 1427-4345

ORIGINAL ARTICLE

Tolerance of Brazilian bean cultivars to protoporphyrinogen oxidase inhibiting-herbicides

Antonio Pedro Brusamarello*, Michelangelo Muzell Trezzi,
Fortunato de Bortoli Pagnoncelli Júnior, Paulo Henrique de Oliveira, Taciane Finatto,
Marcos Vinícius Jaeger Barancelli, Bruno Alcides Hammes Schmalz, Patrícia Bortolanza Pereira

Department of Agronomy, Federal Technological University of Paraná (UTFPR), Brazil

Vol. 61, No. 2: 117–126, 2021

DOI: 10.24425/jppr.2021.137018

Received: August 4, 2020
Accepted: November 30, 2020

*Corresponding address:
antoniopedro1991@hotmail.com

Abstract

The high sensitivity of beans to herbicides is one of the limiting factors regarding the management of dicot weeds in bean crops. Protoporphyrinogen oxidase (PPO) inhibition is an important mechanism of action that has unregistered molecules with potential use in bean crops. The objectives of this study were to investigate the tolerance of Brazilian bean cultivars to distinct PPO inhibitors and to determine the existence of cross-tolerance in cultivars to the different PPO inhibitor chemical groups. In the first and second experiments, the BRSMG Talismã, Jalo Precoce, BRS Esplendor, and IPR 81 cultivars were subjected to saflufenacil doses pre- (0, 9.6, 14.1, 20.5, 30.0, and 43.8 g a.i. · ha^{-1}) and post-emergence (0, 0.7, 1.0, 1.5, 2.1, and 3.1 g a.i. · ha^{-1}). In the third experiment, the tolerance of 28 bean genotypes to saflufenacil (20.5 g a.i. · ha^{-1}) in pre-emergence was determined. In the fourth, fifth, sixth and seventh experiments, we investigated the cross-tolerance of bean to the fomesafen, flumioxazin, sulfentrazone, and saflufenacil herbicides, respectively. Even very low saflufenacil doses in post-emergence caused plants of all cultivars to die rapidly; therefore, the tolerance was much lower at this application time than in pre-emergence. There was high tolerance variability to saflufenacil among the 28 cultivars. The bean tolerance to fomesafen, flumioxazin, sulfentrazone, and saflufenacil applied pre-emergence depended on the cultivar and dose. Fomesafen was highlighted owing to its higher selectivity in relation to the different cultivars. No cross-tolerance pattern to the PPO inhibitor chemical groups applied in pre-emergence was observed among the evaluated bean cultivars. The results of this study could be of significance to farmers and technical assistance personnel, as well as for future research on cultivar breeding and the elucidation of biochemical and genetic mechanisms involved in herbicide tolerance.

Keywords: application time, herbicide selectivity, *Phaseolus vulgaris*

Introduction

Brazil is one of the world's largest bean producers, with an annual production of 2.9 million tons (FAO 2020). The presence of weeds is one of the most important factors resulting in low productivity of common beans (*Phaseolus vulgaris*) in Brazil (Barroso *et al.* 2010), which reached only 1,027 kg · ha^{-1} in 2018 (FAO 2020).

The high sensitivity of beans to herbicides, especially those that combat broadleaf weeds, is one of the factors reducing the availability of herbicides registered for use in this crop in Brazil, compared to other cultivated species (Mapa 2020). The difficulty in controlling weeds, resulting from the limited availability of currently registered herbicides, is an obstacle to bean production worldwide (Li *et al.* 2017).

Tolerance to herbicides is a weed and cultivated species' characteristic that is affected by many factors, including anatomical, physiological, and/or morphological mechanisms that hinder the arrival of a lethal herbicide dose to the site of action (Azania and Azania 2014). These mechanisms include leaf morphology and anatomy, absorption, translocation and compartmentalization, and herbicide metabolism differences

(Ferreira *et al.* 2009). The tolerance of cultivated plants may vary depending on the cultivar and the herbicide (Soltani *et al.* 2010; Silva *et al.* 2011; Diesel *et al.* 2014).

Pre- and post-emergence protoporphyrinogen oxidase (PPO)-inhibiting herbicides are registered for weed control in several cultivated species. Broadleaf weeds are the main targets of these herbicides, although some control grasses in the pre-emergence (Mapa 2020). Among PPO inhibitors marketed in Brazil, only fomesafen (Flex®), flumioxazin (Sumisoya®), and saflufenacil (Heat®) are registered for the management of common bean weeds. These herbicides have the potential to manage glyphosate- and acetolactate synthase (ALS)-inhibitor-resistant weed populations and have become fundamental in management programs that aim to prevent and control herbicide resistant and tolerant weed populations (Rumpa *et al.* 2019).

Fomesafen is an herbicide that is widely used for the control of broadleaf weeds in post-emergent beans (Syngenta 2020). This herbicide has low translocation in plants during post-emergence spraying, and has increased efficiency in weeds at an early stage of development and with adequate leaf coverage (Syngenta 2020). In areas with mixed infestations, the use of grass herbicides, such as acetyl coenzyme A carboxylase (ACCase) inhibitors, is also required. Flumioxazin is applied either in the pre- or post-emergence of weeds before sowing beans, and its efficiency can be reduced under high infestations of *Euphorbia heterophylla*, *Bidens pilosa,* and *Ipomoea grandifolia* (Sumitomo 2020), which are important weeds in Brazil.

Saflufenacil has both acropetal and basipetal translocation, unlike most other PPO inhibitors, which have limited translocation via the phloem (Grossmann *et al.* 2011). Its use in common beans is recommended in the pre-harvest stage for the desiccation of the crop or weeds as well as for burndown application on weeds with a minimum interval of 20 days before sowing (BASF 2017). Sulfentrazone is registered for use in some crops, including soybeans, in the pre-emergence of the crop and weeds (FMC 2017).

Saflufenacil and sulfentrazone have potential use in the pre-emergence of beans. In Canada, different bean groups exhibit different tolerance levels to saflufenacil applied pre-emergence (Soltani *et al.* 2014a) and preplanting (Soltani *et al.* 2019) as well as to sulfentrazone (Hekmat *et al.* 2007; Soltani *et al.* 2014b; Taziar *et al.* 2016). The results of a recent study in Brazil demonstrated the existence of differential tolerance among 10 common bean cultivars to saflufenacil applied pre-emergence (Diesel *et al.* 2014); however, no studies have investigated the tolerance variability of Brazilian bean cultivars to sulfentrazone.

There are relatively few studies on the tolerance of bean crops to herbicides that are either already registered or not registered yet, but are potentially useful, thereby justifying the development of studies that compare different herbicides for use on bean crops. The results of such studies could provide producers with increased options for controlling problematic weeds, including weeds that are resistant and tolerant to other mechanisms of action (Soltani *et al.* 2010).

Therefore, the aim of the present study was to investigate the tolerance of bean cultivars to PPO-inhibiting herbicides and the existence of cross-tolerance to different chemical groups of herbicides.

Materials and Methods

Seven experiments were carried out in a greenhouse (maintained at 25°C, without humidity control or photoperiod) using a completely randomized design. In all experiments, we used pots (experimental units) with a 5 dm^3 capacity, which were filled with soil classified as clayey Oxisol (Embrapa 2018). Based on granulometric analysis, the soil consisted of 83.7% clay, 14.7% silt, and 1.6% sand. The results of chemical analysis were as follows: organic matter (wet digestion): 37.53 (medium); P (Mehlich 1): 1.32 mg · dm^{-3} (low); K (Mehlich 1): 50.83 mg · dm^{-3} (low); pH ($CaCl_2$): 5.50 (medium); Al: 0.00 $cmol_c$ · dm^{-3} (low); H + Al: 3.42 $cmol_c$ · dm^{-3} (medium); Ca: 3.70 $cmol_c$ · dm^{-3} (medium); Mg: 1.20 $cmol_c$ · dm^{-3} (high); SB: 5.03 $cmol_c$ · dm^{-3} (medium); V%: 59.53 (medium); saturation by Al: 0.00% (low); cation exchange capacity (CTC): 8.45. Each pot containing soil which had been sieved through a 2 mm sieve received a correction of fertility equivalent to 270 kg · ha^{-1} of the commercial formulation 2-20-20 (N-P_2O_5-K_2O), in addition to four bean seeds at a depth of 5 cm, with moisture maintained by daily irrigation.

Response of bean cultivars to saflufenacil applied pre- and post-emergence

Two experiments were carried out in a 4 × 6 two-factor scheme with four replications, the first applied, pre--emergence and the second, post-emergence.

In the pre-emergence experiment, the first factor consisted of four bean cultivars (BRSMG Talismã, BRS Jalo Precoce, BRS Esplendor, and IPR 81), and the second factor consisted of six saflufenacil rates (0, 9.6, 14.1, 20.5, 30, and 43.8 g a.i. · ha^{-1}) (Heat®, BASF, São Paulo/São Paulo, Brazil) sprayed pre-emergence. The cultivars and doses used were based on a previous assessment of the tolerance of 10 cultivars to saflufenacil in pre-emergence, carried out by Diesel *et al.* (2014).

The saflufenacil spraying was undertaken immediately after bean sowing, using a CO_2-pressurized backpack sprayer and a spray bar with three flat-type tips (110.02) spaced at 0.5 m and with a spray volume of 200 l · ha^{-1}.

In the post-emergence experiment, the first factor consisted of the same bean cultivars described previously and the second by different saflufenacil rates (0, 0.7, 1.0, 1.5, 2.1, and 3.1 g a.i. · ha^{-1}) (BASF) sprayed post-emergence. After emergence, thinning was carried out, leaving only two seedlings per experimental unit. The herbicide was sprayed at stage V_4 (third fully developed trefoil) together with the adjuvant Dash® HC (Basf, São Paulo/São Paulo, Brazil) at 0.5% (v/v).

The tolerance was determined 35 and 42 days after spraying (DAS) for the applied pre- and post--emergence, respectively. The tolerance was assessed according to the control and injury scale of Frans *et al.* (1986), in which 100% corresponds to complete tolerance (absence of symptoms) and 0% corresponds to the absence of herbicide tolerance (plant death).

Screening of bean cultivars for pre-emergence saflufenacil tolerance

In the third experiment, the tolerance of 28 bean cultivars to saflufenacil sprayed in pre-emergence was evaluated. We used nine cultivars from the black group, 16 from the carioca group, and three from the special group, described in Table 1. Six replicates per cultivar were used, three treated and three not treated with herbicides. A rate of 20.5 g a.i. · ha^{-1} of saflufenacil (BASF) was used as a discriminatory, based on the first experiment.

The implantation and herbicide spraying methods used in this experiment were the same as those used in the first experiment. Tolerance assessment was performed 28 DAS.

Table 1. Description of bean cultivars

Cultivars	Center of origin	Commercial group	Growth habit	Cycle
BRS Talismã	Mesoamerican	carioca	indeterminate type III	semi-early
BRS Pérola	Mesoamerican	carioca	indeterminate type II/III	normal
IPR Gralha	Mesoamerican	black	indeterminate type II	normal
BRS Notável	Mesoamerican	carioca	indeterminate type II/III	semi-early
IPR Uirapuru	Mesoamerican	black	indeterminate type II	normal
IPR Tiziu	Mesoamerican	black	indeterminate type II	normal
BRS Requinte	Mesoamerican	carioca	indeterminate type II	normal
UTF 10	Mesoamerican	carioca	indeterminate type I	normal
IPR Curió	Mesoamerican	carioca	indeterminate type I	early
BRS Esplendor	Mesoamerican	black	indeterminate type II	normal
ANFC 9	Mesoamerican	carioca	indeterminate type II	normal
IPR Andorinha	Mesoamerican	carioca	determined type I	early
ANFP 110	Mesoamerican	black	indeterminate type II	normal
UTF 9	Mesoamerican	black	determined type I	late
IPR Eldorado	Mesoamerican	carioca	indeterminate type II	semi-early
IPR Tuiuiú	Mesoamerican	black	indeterminate type II	normal
BGF 51	Andean	special	determined type I	normal
BGF 14	Andean	special	determined type I	normal
IPR Juriti	Mesoamerican	carioca	indeterminate type II	normal
IPR Campos Gerais	Mesoamerican	carioca	indeterminate type II	normal
IPR Colibri	Mesoamerican	carioca	determined type I	early
IPR 81	Mesoamerican	carioca	indeterminate type II	normal
IAC Imperador	Mesoamerican	carioca	determined type I	semi-early
BRS Esteio	Mesoamerican	black	indeterminate type II	normal
Jalo Precoce	Andean	special	indeterminate type II	semi-early
IPR Tangará	Mesoamerican	carioca	indeterminate type II	normal
BRS Estilo	Mesoamerican	carioca	indeterminate type II	normal
IAC Milênio	Mesoamerican	carioca	indeterminate type III	normal

Cycle: early (<75 days); semi-early (75–85 days); normal (85–95 days); late (>95 days) (Agronorte 2020; Embrapa 2020; IAPAR 2020; UTFPR 2020)

Tolerance of bean cultivars to PPO-inhibiting herbicides

The fourth, fifth, sixth and seventh experiments consisted of PPO-inhibiting herbicides belonging to four distinct chemical groups: saflufenacil (pyrimidinediones) (BASF), flumioxazin (N-phenylphthalamides) (Flumyzin®, Sumitoto, São Paulo/São Paulo, Brazil), sulfentrazone (triazolinones) (Boral® 500 SC, FMC, Campinas/São Paulo, Brazil), and fomesafen (diphenyl ethers) (Flex®, Syngenta, São Paulo/São Paulo, Brazil), respectively. The experiments were conducted in a two-factor arrangement 4 × 8, with four replications. The first factor consisted of four rates of each herbicide; saflufenacil (0, 14.1, 20,5, and 30 g a.i. · ha^{-1}), flumioxazin (0, 70, 110, and 140 g a.i. · ha^{-1}), sulfentrazone (0, 400, 600, and 800 g a.i. · ha^{-1}), and fomesafen (0, 280, 560, and 840 g a.i. · ha^{-1}) rates were defined according to the results of Diesel *et al.* (2014), Soltani *et al.* (2005), Hekmat *et al.* (2007), and Sikkema *et al.* (2009), respectively. The second factor included eight bean cultivars (in decreasing order of tolerance for saflufenacil observed in the third experiment: BRSMG Talismã, BRS Requinte, IPR Curió, IPR Tuiuiú, ANFP 110, IPR Juriti, BRS Esteio, and IAC Milênio).

The implantation and herbicide spraying methods used were the same as those used in the first experiment. Tolerance and fresh mass (FM) assessments were performed 28 DAS. To determine the FM, the plants were cut close to the ground, weighed on a precision scale, and the weight values were converted to percentages in relation to the untreated control.

Statistical analysis

The data were subjected to analysis of variance by the *F* test ($p < 0.05$) and the means of the qualitative factors were subjected to a means comparison test ($p < 0.05$), with the aid of the computer program RStudio (RStudio Team 2016), using the ExpDes.pt package (Ferreira *et al.* 2011). For the quantitative factors, three-parameter logistic nonlinear equations (equation 1) were adjusted with the aid of the SigmaPlot 10.0 program (SigmaPlot 2006).

$$y = \frac{a}{1 + \left[\frac{x}{x_0}\right]^b}, \qquad (1)$$

where: y – represents the response of the dependent variable, x – dose of the herbicide, a – maximum asymptote of the curve, b – slope of the curve, x_0 – dose required to reduce the dependent variable by 50% (I_{50}).

Results

Response of bean cultivars to saflufenacil applied in pre- and post-emergence

The cultivar BRSMG Talismã demonstrated a high level of tolerance to saflufenacil applied in pre-emergence even at the highest dose (43.8 g a.i. · ha^{-1}), while Jalo Precoce had the lowest tolerance and IPR 81 and BRS Esplendor had intermediate tolerance levels (Fig. 1).

The higher tolerance levels of the BRSMG Talismã and IPR 81 cultivars were reflected in their I_{50} values, which required doses of more than 43.8 g a.i. · ha^{-1} to reduce the tolerance level by 50%, while the I_{50} of BRS Esplendor and Jalo Precoce required doses of 32.8 g a.i. · ha^{-1} and 26.3 g a.i. · ha^{-1}, respectively (Table 2). Even very low saflufenacil rates applied post--emergence caused plants of all cultivars to die rapidly (data not presented).

Screening of bean cultivars for pre-emergence saflufenacil tolerance

There was a wide variation in the tolerance of the 28 bean cultivars to saflufenacil applied in pre-emergence. The cultivars BRSMG Talismã, BRS Pérola, IPR Gralha, BRS Notável, IPR Uirapuru, IPR Tiziu, BRS Requinte, UTF 10, IPR Curió, and BRS Esplendor were more tolerant to saflufenacil (20.5 g a.i. · ha^{-1}) than the other cultivars, while Jalo Precoce, BRS Estilo, and IAC Milênio had the lowest tolerance levels (Fig. 2).

Tolerance of bean cultivars to four PPO inhibitors in pre-emergence

The interaction between the herbicide rate and the cultivar occurred only for the herbicides flumioxazin and fomesafen, which is depicted in Figure 3. The cultivar

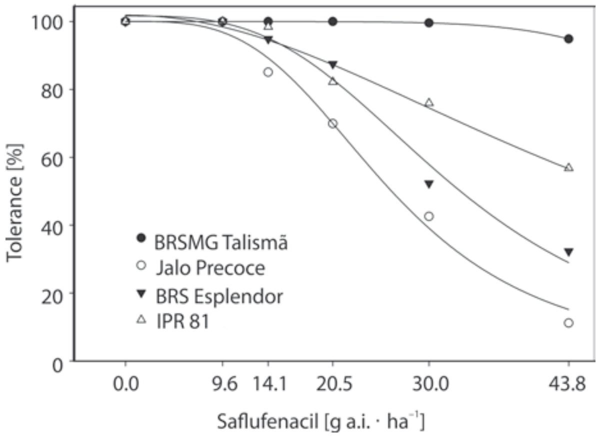

Fig. 1. Average tolerance of four cultivars to six saflufenacil rates applied in pre-emergence

Table 2. Parameters of the adjusted equations for the tolerance of four bean cultivars to saflufenacil applied in pre-emergence

Cultivars	Parameters			R^2	*p*-value
	a	*b*	I_{50}		
BRSMG Talismã	100.00 (0.007)	6.84 (0.107)	>43.8 (0.446)	0.99	<0.0001
Jalo Precoce	100.01 (2.652)	3.38 (0.429)	26.3 (1.100)	0.99	<0.0001
BRS Esplendor	101.67 (2.802)	3.16 (0.490)	32.8 (1.542)	0.98	0.0020
IPR 81	101.87 (2.631)	2.08 (0.414)	>43.8 (4.123)	0.96	0.0050

Logistic equation of three parameters: $y = a/(1+ (x/x_0)^b)$, where: *y* – dependent variable; *x* – concentration of the herbicide; *a* – maximum asymptote of the curve; *b* – slope of the curve; x_0 – dose of herbicide required to reduce the dependent variable by 50% (I_{50}); R^2 – coefficient of determination; *p*-value – probability values of the I_{50} values underestimated by the logistic model of the three parameters because the curve did not exceed 50% of the *y* axis, and are shown as ">". In parentheses is the standard error

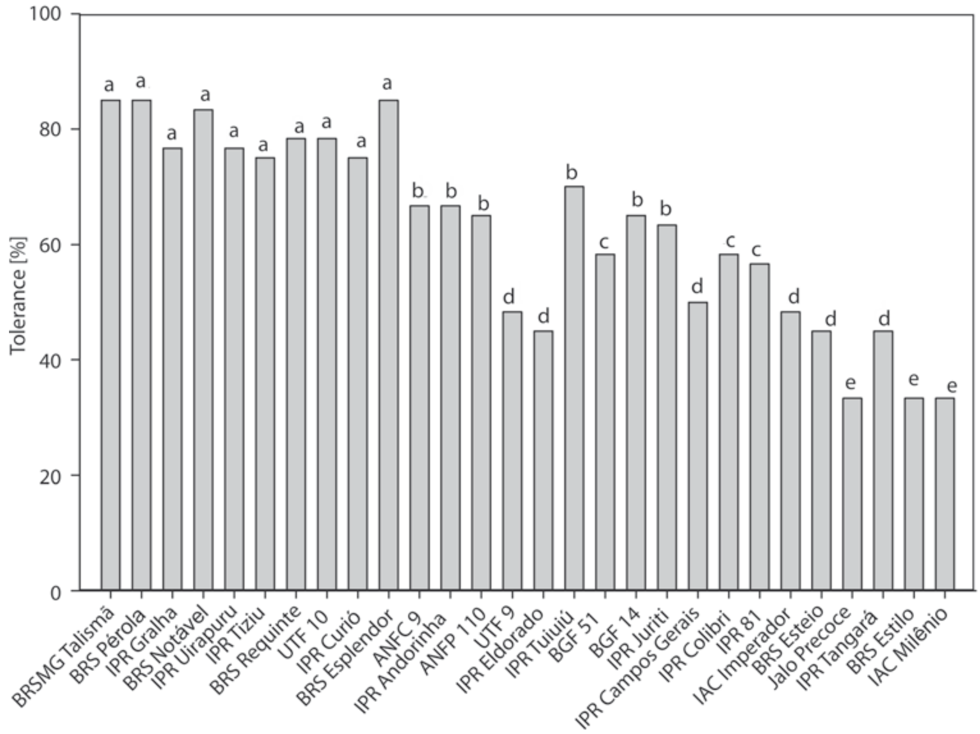

Fig. 2. Tolerance of bean cultivars to 20.5 g a.i. · ha^{-1} of saflufenacil applied in pre-emergence. Means followed by the same letter did not differ by the Scott-Knott test ($p < 0.05$)

BRS Esteio stood out from the others owing to its high level of tolerance to flumioxazin doses of 70 g a.i. · ha^{-1} and 110 g a.i. · ha^{-1} (>78% relative to the untreated control). The 140 g a.i. · ha^{-1} flumioxazin dose had a drastic, negative impact on cultivar tolerance (Fig. 3A).

Fomesafen had the greatest selectivity among all herbicides, with tolerance levels above 68% for all cultivars. The cultivars BRSMG Talismã and IPR Juriti had tolerance levels above 83% even with the use of the highest fomesafen rate (840 g a.i. · ha^{-1}) (Fig. 3B). For tolerance, there was no interaction between the herbicide rate and the cultivar for saflufenacil and sulfentrazone and the results observed for each cultivar in the average herbicide doses were very similar to fresh mass, so only the fresh mass results were presented.

On average, the highest FM after saflufenacil application was obtained in BRSMG Talismã (100% in relation to the untreated control), which was superior to the other cultivars in this respect, with the exception of ANFP 110 (91%) (Fig. 4). The cultivars IPR Curió and IAC Milênio had the highest FM (89%) after the flumioxazin application, differing only from BRS Requinte (72%) and IPR Tuiuiú (74%). The FM of the cultivars BRSMG Talismã and IPR Juriti were 93% and 89%, respectively, after the application of sulfentrazone; these cultivars stood out from the rest and differed only from ANFP 110 (68%) and BRS Esteio (70%). After

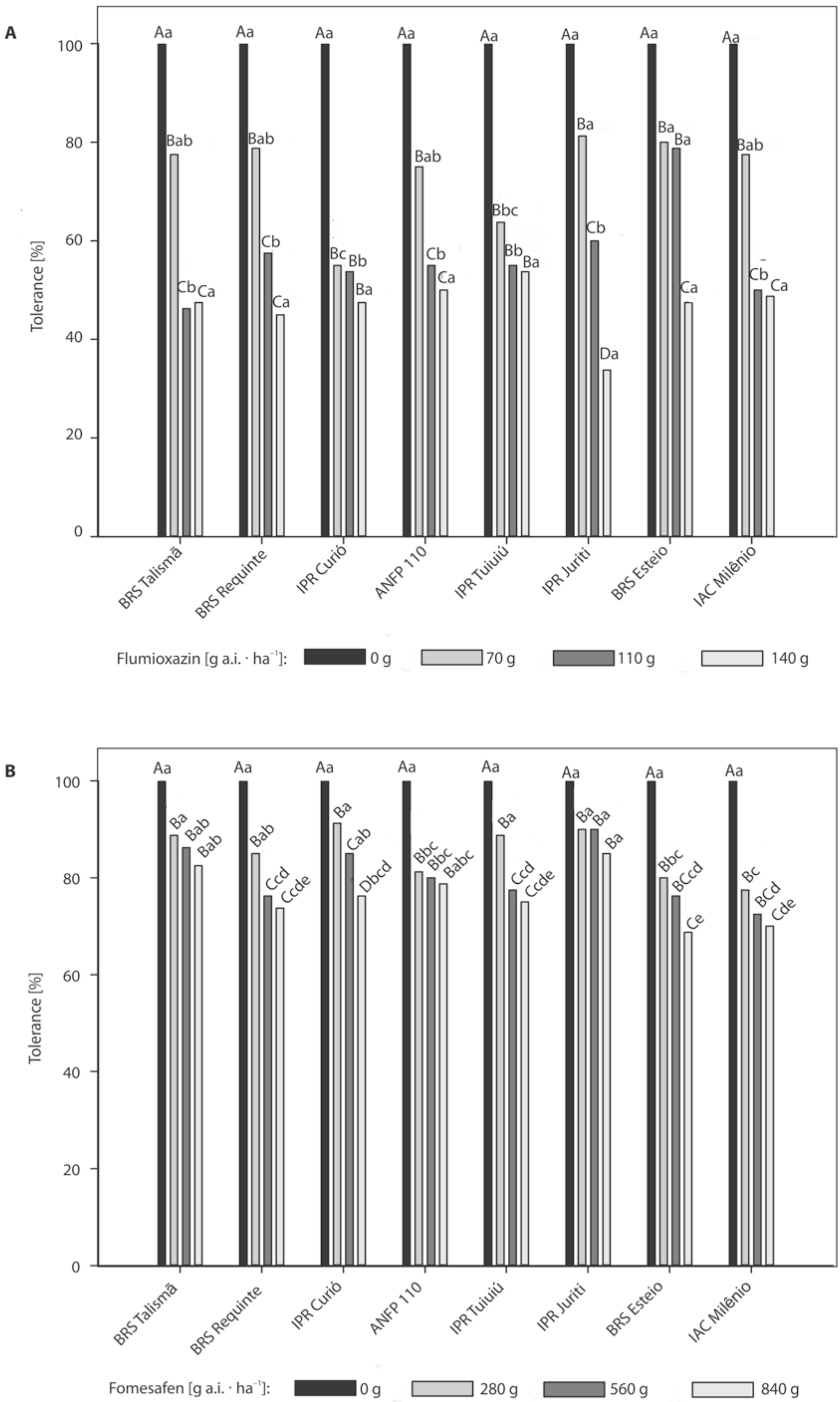

Fig. 3. Tolerance of bean cultivars to flumioxazin (A) and fomesafen (B) applied in pre-emergence, considering rates and cultivars. Means followed by the same capital and lowercase letter compare rates and cultivars, respectively, and did not differ by Duncan's test ($p < 0.05$)

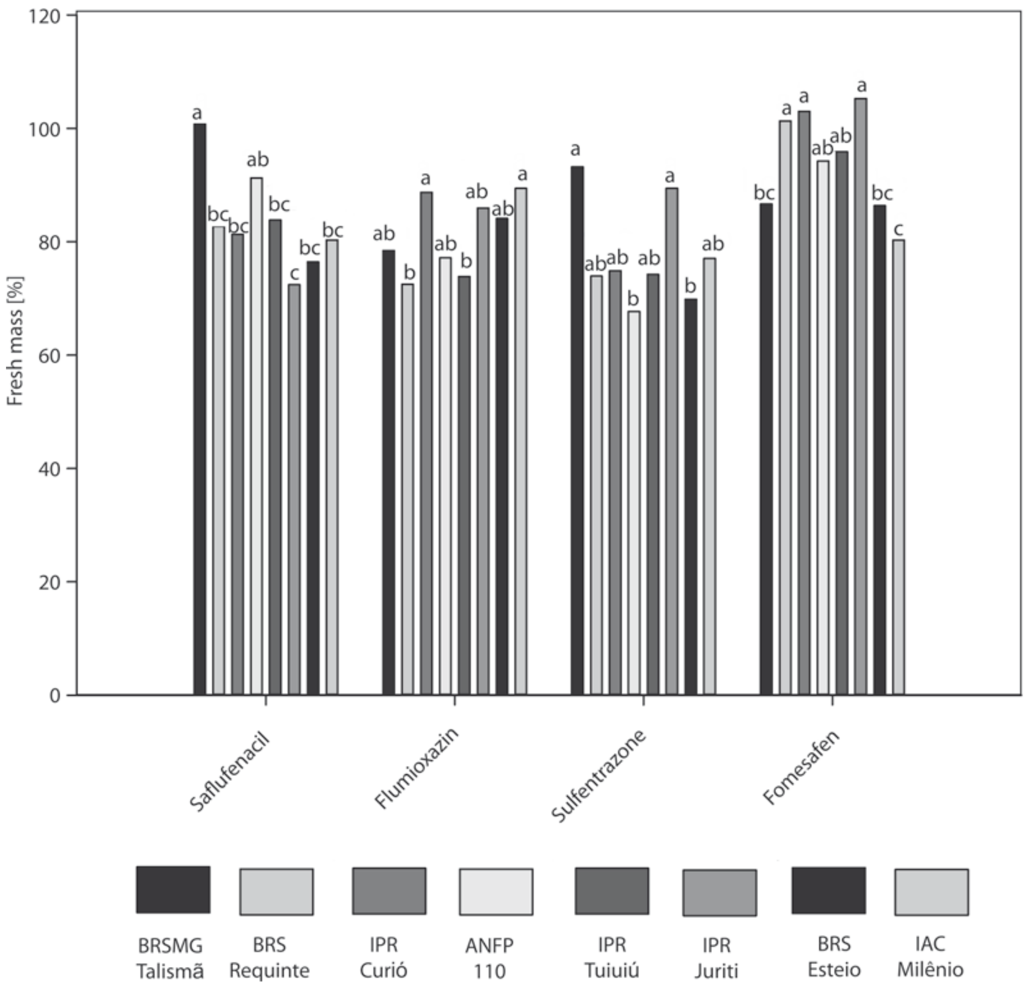

Fig. 4. Fresh mass (% compared to the untreated control) of bean plants determined at 28 days after spraying (DAS) of saflufenacil, flumioxazin, sulfentrazone, and fomesafen applied in pre-emergence. Differences between cultivars under the average herbicide rates. Means followed by the same letter did not differ by Duncan's test ($p < 0.05$)

the application of fomesafen, the cultivars IPR Juriti, IPR Curió, and BRS Requinte had the highest FM values (105, 103, and 101%, respectively), differing only from BRSMG Talismã (87%), BRS Esteio (86%), and IAC Milênio (80%).

Discussion

The experiments investigating the response of common bean cultivars to the herbicide saflufenacil applied pre- and post-emergence revealed greater bean tolerance in pre-emergence and greater tolerance differences among cultivars at this application time, in relation to post-emergence spraying. In post-emergence, saflufenacil doses that were smaller than those recommended by commercial product labels, were sufficient to trigger characteristic effects of this herbicide, which are described in the literature as the rapid loss of cell membrane integrity, tissue necrosis, and plant death (Hao *et al.* 2011; Aldridge *et al.* 2019). The action of some of the PPO-inhibiting herbicides can be enhanced when they are applied to the seedling shoots, combined with the addition of adjuvants (Castro *et al.* 2013).

A saflufenacil phytotoxic effect applied post-emergence occurs at a much lower dose than necessary to generate pre-emergence phytotoxicity; however, the results of our study indicated that, even in pre-emergence, doses that can be considered low for other crops on which the product is used, could cause a strong reduction in bean tolerance.

Mechanisms that confer tolerance to PPO inhibitors include: the reduction of absorption and translocation (Kilink *et al.* 2011), the increased activity of antioxidant enzymes (Xavier *et al.* 2018), and the rapid herbicide metabolism (Dayan *et al.* 1997; Kilink *et al.* 2011). In pre-emergence application, specific mechanisms capable of interfering with the absorption, translocation and metabolism of herbicidal compounds in plant tissues may contribute to increasing plant tolerance. For example, the anatomical structures of the emerging aerial parts (epicotyl and hypocotyl) as well as endoderm differences, are considered important barriers for the absorption and translocation of herbicides (Vidal 2002; Oliveira Jr. *et al.* 2011). The selectivity of saflufenacil in pre-emergence occurs through the positioning of the molecules in relation to the structures of the initial development and through faster metabolism (BASF 2017). Dicotyledons allow for greater absorption and translocation and reduced metabolism of saflufenacil than monocotyledonous species, such as corn (Grossmann 2011). In addition, part of the saflufenacil applied in pre-emergence is adsorbed onto soil colloids (Gannon *et al.* 2014), thereby contributing to less herbicidal activity in this application mode.

The saflufenacil tolerance variability among bean cultivars in pre-emergence corroborates the results of Diesel *et al.* (2014), who detected differential saflufenacil tolerance levels among 10 common bean cultivars in pre-emergence, with the BRSMG Talismã and Jalo Precoce cultivars standing out for their higher and lower tolerance, respectively. Soltani *et al.* (2014a) reported that Adzuki beans (*Vigna angularis*) had a similar tolerance level to that of the control group and higher than that of the cultivars of kidney, black, and white *P. vulgaris*.

The tolerance variability of different bean cultivars to saflufenacil was more evident in the applied pre- than in the post-emergence, which suggests the existence of different tolerance mechanisms between these modalities. In post-emergence, the morphological characteristics of the leaf surface (trichomes and wax plates) and the cuticle are considered to be the main barriers to herbicide absorption (Vidal 2002). Thus, the absence of significant barriers for the absorption and translocation of saflufenacil applied to the shoots of bean cultivars could explain the absence of tolerance among the evaluated cultivars. In addition, the rapid absorption and translocation of this herbicide in shoots causes the rapid production of free radicals after sun exposure (Grossmann 2011; Vidal *et al.* 2014), which can compromise the action of other tolerance mechanisms and mask differences between cultivars that were detected in pre-emergence.

Differentiated tolerance levels among cultivars were also observed for the herbicides flumioxazin, fomesafen, and sulfentrazone, in pre-emergence applications. In addition to the cultivar, tolerance was affected by the herbicide dose. Flumioxazin is selective for applications before bean sowing at a dose of 25 g a.i. · ha^{-1} (Sumitomo 2020). As noted in this study, pre-emergence applications resulted in high flumioxazin phytotoxicity at doses higher than 70 g a.i. · ha^{-1}. Fomesafen, which is recommended in 250 g a.i. · ha^{-1} doses for beans in post-emergence (Syngenta 2020) was the least phytotoxic of the tested herbicides. The tolerance of beans to sulfentrazone in pre-emergence has also been observed in several studies, at doses of 105 g a.i. · ha^{-1}, 140 g a.i. · ha^{-1}, 280 g a.i. · ha^{-1} (Soltani *et al.* 2014b), 210 g a.i. · ha^{-1} (Taziar *et al.* 2016), 420 g a.i. · ha^{-1} (Hekmat *et al.* 2007; Taziar *et al.* 2016), and 840 g a.i. · ha^{-1} (Hekmat *et al.* 2007). It is likely that the tolerance mechanisms to sulfentrazone that have been reported in other cultivated species may explain the differences between the bean cultivars examined in this study. In some potato cultivars, tolerance has been attributed to the differential root uptake and the differential root translocation to the shoot (Bailey *et al.* 2003). In soybeans, tolerance is attributed to the lower absorption of the herbicide by the leaves at the early stages of development (Li *et al.* 2000).

The aim of the present study was to determine the existence of variability among cultivars within each PPO inhibitor and to clarify whether the order of herbicide selectivity within a cultivar would be the same for the other cultivars. In this regard, for each cultivar used, a different herbicide selectivity order was obtained. For example, the cultivar BRSMG Talismã exhibited higher levels of tolerance to the herbicides saflufenacil and sulfentrazone, thereby exhibiting cross--tolerance to the pyrimidione and triazolinone chemical groups, respectively, and a lower level of tolerance to flumioxazin (n-phenylphthalimides group). However, the cultivar BRS Esteio was in the group with the highest level of tolerance to the herbicide flumioxazin (n-phenylphthalimides), but also belonged to the group with the lowest level of tolerance to the herbicides saflufenacil (pyrimidinediones) and sulfentrazone (triazolinones). Therefore, there was no cross-tolerance pattern to herbicides among all the evaluated bean cultivars.

The existence of a cross-tolerance pattern among cultivars would facilitate bean management by growers and also the development of research to improve the tolerance of bean cultivars to herbicides. It would improve a cultivar's tolerance to a particular herbicide, and its response to different herbicides with the same action mechanism.

It is important to note that, in the present study, we did not detect a relationship between the tolerance level of 28 cultivars to saflufenacil and specific characteristics described in Table 1, such as the center of origin (Mesoamerican or Andean), commercial group (black, carioca, or special), and cycle (early, semi-early, or late) (Fig. 2). There was also no relationship between the tolerance level of eight cultivars to four PPO inhibitors (Fig. 4) and the characteristics of the commercial group, growth habit, and cycle (Table 1). Among the mechanisms considered important for the tolerance of plants to PPO inhibitors are the metabolism of herbicides to less toxic compounds (Dayan *et al.* 1997; Kilink *et al.* 2011), the antioxidative enzyme activity (Xavier *et al.* 2018), and the herbicide absorption and translocation differences (Kilink *et al.* 2011). Probably the mechanisms of tolerance to PPO--inhibiting herbicides mentioned above are not associated with specific morphophysiological characteristics or the center of origin of bean cultivars. Plant tolerance to herbicides is affected by numerous factors, including the genetic makeup of each cultivar and the physicochemical characteristics of the herbicide (Soltani *et al.* 2010; Oliveira Jr. *et al.* 2011; Silva *et al.* 2011; Diesel *et al.* 2014), which interact with each other, resulting in multiple responses.

Knowledge of the tolerance variability of the different cultivars examined in this study will be important for producers and technical assistance personnel, as it may assist in selecting cultivars that are more tolerant to herbicides. Such knowledge will be extremely useful for selecting herbicides with registration potential, for which there is currently limited information in terms of their selectivity for a large number of cultivars. In addition, such information is important for the development of research which will investigate the biochemical and genetic mechanisms involved in herbicide tolerance.

The results of the present study highlight the importance and encourage the design of further studies on the evaluation of cultivars under different environmental conditions (soils, climate, etc.) and stressful situations for plants. This will generate more knowledge and ensure greater safety regarding the selectivity of these herbicides at the field level.

In summary, the tolerance of bean cultivars to saflufenacil used in doses compatible with the management of weeds, occurred only partially when applied pre-emergence, as very low doses in post-emergence caused the rapid death of the plants. The pre-emergence tolerance to saflufenacil, flumioxazin, sulfentrazone, and fomesafen depended on the cultivar and dose used. Fomesafen stood out for its higher selectivity levels. No cross-tolerance pattern for PPO-inhibiting herbicides was observed among the evaluated bean cultivars.

Acknowledgements

The authors would like to acknowledge the Coordination for the Improvement of Higher Education Personnel (CAPES) and National Conuncil of Scientific and Technological Development (CNPq) for providing scholarships, and the Federal University of Technology Paraná for supplying financial support and infrastructure.

References

Agronorte. 2020. Available on: http://www.agronorte.com.br/. [Accessed: 15 January 2020]

Aldridge R.B., Jennings K.M., Chaudhari S., Monks D.W., Everman W.J., Mehra L.K. 2019. Tolerance of southern highbush and rabbiteye blueberry cultivars to saflufenacil. Weed Technology 33 (3): 475–480. DOI: https://doi.org/10.1017/wet.2018.115

Azania C.A.M., Azania A.A.P.M.A. 2014. Seletividade de herbicidas. p. 217–233. In: “Aspectos da Biologia e Manejo de Plantas Daninhas” [Aspects of biology and weed management] (P.A. Monquero, ed.). São Carlos: Rima Editora. (in Portuguese)

Bailey W.A., Hatzios K.K., Bradley K.W., Wilson H.P. 2003. Absorption, translocation, and metabolism of sulfentrazone in potato and selected weed species. Weed Science 51 (1): 32–36. DOI: https://doi.org/10.1614/0043-1745-(2003)051[0032:ATAMOS]2.0.CO;2

Barroso A.A.M., Yamauti M.S., Alves P.L.C.A. 2010. Competitive interaction between commom black bean cultivars and *Euphorbia heterophylla*. Bragantia 69 (3): 609–616. DOI: https://doi.org/10.1590/1983-40632018v4851669

BASF (Badische Anilin Und Soda-Fabrik). 2017. Heat®. Available on: http://www.adapar.pr.gov.br/arquivos/File/defis/DFI/Bulas/Herbicidas/heat160218.pdf. [Accessed: 10 January 2020]

Castro M.J.L., Ojeda C., Cirelii A.F. 2013. Advances in surfactants for agrochemicals. Environmental Chemistry Letters 12 (1): 85–95. DOI: https://doi.org/10.1007/s10311-013-0432-4

Dayan F.E., Weete J.D., Duke S.O., Hancock H.G. 1997. Soybean cultivars differences in response to sulfentrazone. Weed Science 45 (5): 634–641. DOI: https://doi.org/10.1017/S0043174500093255

Diesel F., Trezzi M.M., Oliveira P.H., Xavier E., Pazuch D., Pagoncelli F. Jr. 2014. Tolerance of dry bean cultivars to saflufenacil. Ciência e Agrotecnologia 38 (4): 352–360. DOI: http://dx.doi.org/10.1590/S1413-70542014000400005

Embrapa (Empresa Brasileira de Pesquisa Agropecuária). 2018. Sistema Brasileiro de Classificação de Solos. [Brazilian System of Soil Classification]. 5th ed. Revista e Ampliada, Brasília, Brasil, 356 pp. (in Portuguese)

FAO (Food and Agriculture Organization of the United Nations). 2020. Available on: http://www.fao.org/faostat/en/#data/QC. [Accessed: 11 May 2020]

Ferreira E.A., Concenço G., Vargas L., Silva A.A. 2009. Manejo de plantas daninhas tolerantes ou resistentes ao glyphosate no Brasil. [Management of glyphosate-tolerant or resistant weeds in Brazil]. p. 357–400. In: “Glyphosate” (E.D. Velini, D.K. Meschede, C.A. Carbonari, M.L.B. Trindade, eds.). Botucatu: Fepaf, 493 pp. (in Portuguese)

Ferreira E.B., Cavalcanti P.P., Nogueira D.A. 2011. ExpDes: Experimental Designs package R package version 1.1.1.

FMC (FMC Química do Brasil Ltda). 2017. Boral® 500 SC. Available on: http://www.adapar.pr.gov.br/arquivos/File/defis/DFI/Bulas/Herbicidas/boral500sc250418.pdf. [Accessed: 10 January 2020]

Frans R., Talbert R., Marx D., Crowley H. 1986. Experimental design and techniques for measuring and analyzing plant responses to weed control practices. p. 29–46. In: “Research Methods in Weed Science” (N.D. Camer, ed.). 3rd ed. Champaign, Southern Weed Science Society, USA, 486 pp.

Gannon T.W., Hixson A.C., Keller K.E., Weber J.B., Knezevic S.Z., Yelverton F.H. 2014. Soil properties influence saflufenacil phytotoxicity. Weed Science 62 (4): 657–663. DOI: https://doi.org/10.1614/WS-D-13-00171.1

Grossmann K., Hutzler J., Caspar G., Kwiatkowski J., Brommer C.L. 2011. Saflufenacil (KixorTM): biokinetic properties and mechanism of selectivity of a new protoporphyrinogen IX oxidase inhibiting herbicide. Weed Science 59 (3): 290–298. DOI: https://doi.org/10.1614/WS-D-10-00179.1

Hao G.F., Zuo Y., Yang S.G., Yang G.F. 2011. Protoporphyrinogen oxidase inhibitor: an ideal target for herbicide discovery. Chemistry in China 65 (12): 961–969. DOI: https://doi.org/10.2533/chimia.2011.961

Hekmat S., Shropshire C., Soltani N., Sikkema P.H. 2007. Responses of dry beans (*Phaseolus vulgaris* L.) to sulfentrazone. Crop Protection 26 (4): 525–529. DOI: https://doi.org/10.1016/j.cropro.2006.05.002

Kilink O., Grasset R., Reynaud S. 2011. The herbicide aclonifen: the complex theoretical basis of sunflower tolerance. Pesticide Biochemistry and Physiology 100 (2): 193–198. DOI: https://doi.org/10.1016/j.pestbp.2011.04.001

Li Z., Van Acker R., Robinson D., Soltani N., Sikkema P. 2017. Managing weeds with herbicides in white bean in Canada: a review. Canadian Journal of Plant Science 97 (5): 755–766. DOI: https://doi.org/10.1139/cjps-2017-0030

Li Z., Wehtje G.R., Walker R.H. 2000. Physiological basis for the differential tolerance of *Glycine max* to sulfentrazone during seed germination. Weed Science 48 (3): 281–285. DOI: https://doi.org/10.1614/0043-1745(2000)048[0281:PBFTDT]2.0.CO;2

Mapa (Ministério da Agricultura Pecuária e Abastecimento). 2020. Agrofit: Sistema de agrotóxicos fitossanitários. [Agrofit: Phytosanitary Pesticide System]. Available on: http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons. [Accessed: 12 January 2020]. (in Portuguese)

Oliveira Jr. R.S., Constantin J., Inoue M.H. 2011. Biologia e Manejo de Plantas Daninhas. [Weed Biology and Management]. Omnipax, Curitiba, Brasil, 348 pp. (in Portuguese)

RStudio Team. 2016. Integrated Development for R. RStudio, Boston, MA.

Rumpa M.M., Krausz R.F., Gibson D.J., Gage K.L. 2019. Effect of PPO-inhibiting herbicides on the growth and sex ratio of a dioecious weed species *Amaranthus palmeri* (Palmer Amaranth). Agronomy 9 (275): 1–14. DOI: https://doi.org/10.3390/agronomy9060275

Sigmaplot. 2006. Exact Graphy for Exact Science. Version 10.0.

Sikkema P., Shropshire C., Soltani N. 2009. Response of dry bean to pre-plant incorporated and pre-emergence applications of s-metolachlor and fomesafen. Crop Protection 28 (9): 744–748. DOI: https://doi.org/10.1016/j.cropro.2009.05.011

Silva D.V., Santos J.B., Silveira H.M., Carvalho F.P., Neto M.D.C., Ferreira E.A., Silva A.A., Cecon P.R. 2011. Tolerância de cultivares de mandioca aos herbicidas fomesafen e fluazifop-p-butil. [Tolerance of cassava cultivars to fomesafen and fluazifop-p-butyl herbicides]. Revista Brasileira de Herbicidas 10 (3): 219–231. DOI: https://doi.org/10.7824/rbh.v10i3.125. (in Portuguese)

Soltani N., Bowley S., Sikkema P.H. 2005. Responses of dry beans to flumioxazin. Weed Technology 19 (2): 351–358. DOI: https://doi.org/10.1614/WT-04-146R1

Soltani N., Shropshire C., Sikkema P.H. 2010. Sensibility of leguminous crops to saflufenacil. Weed Technology 24 (2): 143–146. DOI: https://doi.org/10.1614/WT-09-029.1

Soltani N., Shropshire C., Sikkema P.H. 2014a. Sensitivity of dry bean to dimethenamid-p, saflufenacil and dimethenamid-p/saflufenacil. American Journal of Plant Science 5 (21): 3288–3294. DOI: http://dx.doi.org/10.4236/ajps.2014.521343

Soltani N., Shropshire C., Sikkema P.H. 2014b. Response of dry bean to sulfentrazone plus imazethapyr. International Journal of Agronomy 2014 (3): 1–6. DOI: http://dx.doi.org/10.1155/2014/287908

Soltani N., Shropshire C., Sikkema P.H. 2019. Sensitivity of dry bean to herbicides applied preplant for glyphosate-resistant horseweed control in a strip-tillage cropping system. Weed Technology 33 (1): 178–184. DOI: https://doi.org/10.1017/wet.2018.107

Sumitomo (Sumitomo Chemical do Brasil Representações Ltda). 2020. Sumisoya®. Available on: http://www.adapar.pr.gov.br/arquivos/File/defis/DFI/Bulas/Herbicidas/sumisoya0719.pdf. [Accessed: 15 January 2020]

Syngenta (Syngenta Proteção de Cultivos Ltda). 2020. Flex®. Available on: http://www.adapar.pr.gov.br/arquivos/File/defis/DFI/Bulas/Herbicidas/flexdez2016.pdf. [Accessed: 15 January 2020]

Taziar A.N., Soltani N., Shropshire C., Robinson D.E., Long M., Gillard C.L., Sikkema P.H. 2016. Response of four dry bean market classes to pre-emergence applications of pyroxasulfone, sulfentrazone and pyroxasulfone plus sulfentrazone. American Journal of Plant Sciences 2016 (7): 1217–1225. DOI: http://dx.doi.org/10.4236/ajps.2016.78117

UTFPR (Universidade Tecnológica Federal do Paraná). 2020. Available on: http://portal.utfpr.edu.br/. [Accessed: 25 January 2020]

Vidal R.A. 2002. Ação dos Herbicidas: Absorção, Translocação e Metabolização. [Herbicide Action: Absorption, Translocation and Metabolization]. Evangraf, Porto Alegre, Brasil, 89 pp. (in Portuguese)

Vidal R.A., Merotto Jr. A., Schaedler C.A., Lamego F.P., Portugal J., Menendes J., Kozlowski L.A., Trezzi M.M., De Prado R. 2014. Mecanismos de ação de herbicidas. [Herbicide mechanisms of action]. p. 235–256. In: "Aspectos da Biologia e Manejo das Plantas Daninhas" (P. Monquero, ed.). [Aspects of Weed Biology and Management]. Rima, São Carlos, Brasil, 400 pp. (in Portuguese)

Xavier E., Trezzi M.M., Oliveira M.C., Vidal R.A., Brusamarello A.P. 2018. Activity of antioxidant enzymes in *Euphorbia heterophylla* biotypes and their relation to cross resistance to ALS and Protox inhibitors. Planta Daninha 36 (1): 1–13. DOI: https://doi.org/10.1590/s0100-83582018360100138