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Fomesafen toxicity to bean plants as a function of the time of application and herbicide dose

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ABSTRACT. The objectives of the present study were to evaluate the impact of the time of fomesafen application and its dose on the tolerance of the common bean crop and to investigate the influence of environmental variables on herbicide selectivity. Two field experiments were conducted using the time of fomesafen application and its dose as factors. Crop injury from fomesafen reached 20% when evaluated one week after treatment (WAT). When sprayed at the warmest times of the day when irradiance levels were at their highest (11:00 am and 4:00 pm), fomesafen phytotoxicity was higher compared with other application times. Increased values of these two environmental variables, especially luminosity, was associated with high levels of fomesafen injury to the bean crop. However, assessments made at three WAT demonstrated that the crop had recovered from its initial injuries.

Keywords: environmental variables, air temperature, luminosity, selectivity.

Toxicidade de fomesafen às plantas de feijão em função dos horários de aplicação e das doses do herbicida

RESUMO. Os objetivos do presente estudo foram avaliar o impacto do horário de aplicação de fomesafen e suas doses sobre a tolerância da cultura do feijão e investigar a influência de variáveis ambientais sobre a seletividade do herbicida. Dois experimentos a campo foram conduzidos usando os horários de aplicação de fomesafen e suas doses como fatores. Injúria de fomesafen na cultura chegou a 20% quando avaliada uma semana após o tratamento (SAT). Quando aspergido nos horários mais quentes do dia, quando os níveis de irradiância foram mais elevados (11h00 e 16h00), a fitotoxicidade de fomesafen foi maior comparada aos outros horários de aplicação. Aumento nos valores dessas duas variáveis ambientais, especialmente a luminosidade, foi associado com elevados níveis de injúria de fomesafen ao feijoeiro. No entanto, as avaliações realizadas aos três SAT demonstraram que a cultura havia recuperado de suas injúrias iniciais.

Palavras-chave: variáveis ambientais, temperatura do ar, luminosidade, seletividade.

Introduction

The grains of the common bean (*Phaseolus vulgaris* L.) are important sources of protein for humans, particularly in South America. However, the world production of this crop was only 23.6 million tons in 2012 (FAOSTAT, 2014). Weeds reduce the yield of bean because the plants of this crop have a limited competitive ability. The control of weeds with herbicides is a crucial cultural practice that allows the achievement of high crop productivity.

Selectivity is a characteristic of herbicides that allows them to control weeds without harming the crop. The tolerance of crops to herbicides that inhibit the enzyme protoporphyrinogen oxidase (PROTOX) depends on herbicide detoxification by the plants or on their ability to mitigate the oxidative stress induced by these compounds (GEOFFROY et al., 2002; GULLNER; DODGE, 2000; JUNG et al., 2008; SUGIYAMA; SEKIYA, 2005).

These enzyme-mediated reactions affect the extent of crop sensitivity to the herbicide. Factors that affect crop tolerance include the dose of the herbicide (SOLTANI et al., 2006; WILSON, 2005), the environmental conditions present during its application (FAUSEY; RENNER, 2001; FERREIRA et al., 1998) and the time of spraying (FORNAROLLI et al., 1999; MARTINSON et al., 2002; MOHR et al., 2007; NORSWORTHY et al., 1999; PENCKOWSKI et al., 2003; RAMIRES et al., 1999; SELLERS et al., 2003, 2004; STEWART et al., 2009; STOPPS et al., 2013; WALTZ et al., 2004). Fomesafen is a PROTOX inhibitor that is selective for common bean plants. However, this crop may be injured by fomesafen (SOLTANI et al., 2005, 2006; WILSON, 2005).

The objectives of the present work were to evaluate the influence of the time of fomesafen application on its toxicity to bean plants and to investigate the impact of environmental variables on the selectivity of this herbicide for the bean crop.

Material and methods

Two experiments were conducted under field conditions at the Agronomy Farm of the Federal Technological University of Paraná, which is located in Pato Branco, Paraná State, Brazil. For both experiments, the experimental design was a randomized block with a factorial arrangement of the treatments, and four replicates were performed. The first factor consisted of the time at which fomesafen was sprayed (2:00 am, 6:00 am, 11:00 am, 4:00 pm and 9:00 pm), and the second factor consisted of the herbicide doses (0, 100, 137.5, 175, 212.5 and 250 g ha^{-1} for the first experiment and 0, 75, 125, 175, 212.5 and 250 g ha^{-1} for the second experiment).

For the first experiment, the bean cultivar IPR-Tiziu was sown on October 26, 2010. The herbicide was sprayed 24 days after crop emergence (DAE) when the bean plants had four trifoliolates. For the second experiment, the bean cultivar IPR-81 was sown on March 2, 2011. Fomesafen was sprayed 17 DAE when the bean plants had three trifoliolates. The soil was an oxisol with a clay content of 60%. The prevailing environmental conditions present while the spraying of the herbicide was being conducted are presented in Table 1. The herbicide was applied with a CO_2 backpack sprayer with nozzle 80.02 and a spray volume of 200 L ha^{-1}.

Table 1. Environmental conditions at the times of fomesafen applications.

Time	Air temperature (°C)	Relative humidity (%)	PAR[1] (μmol m^{-2} s^{-1})
Experiment 1 – October, 2010.			
2:00 am	18.0	70	-0.10
6:00 am	22.7	60	670.32
11:00 am	30.2	32	1853.58
4:00 pm	31.4	41	1305.56
9:00 pm	23.7	56	-0.10
Experiment 2 – March, 2011.			
2:00 am	14.5	85	-0.06
6:00 am	13.7	88	0.24
11:00 am	23.1	62	1300.35
4:00 pm	23.0	57	332.05
9:00 pm	18.3	71	0.08

[1]Photosynthetically active radiation.

The effect of fomesafen on the bean plants was evaluated one and three weeks after the herbicide treatment (WAT) using the visual scale proposed by Camper (1986), in which ranges from 0 to 100% (no injury to total crop destruction, respectively). An analysis of variance was carried out on the crop injury data. When an interaction between the time of spraying and the herbicide dose was detected, a dose-response curve was adjusted for each application time by fitting the three parameters to the following sigmoidal equation:

$$y= A/(1+\exp(-(d-D_{A50})/b)) \quad (1)$$

where:

A= maximum asymptote for plant injury;

d= fomesafen dose;

D_{A50}= dose for 50% of the maximum asymptote and

b= slope of the curve at its point of inflection.

Comparisons among application times were performed by analyzing the parameters of the equations obtained from each curve and their respective values of standard error. From the equation adjusted to one WAT for each experiment, the fomesafen dose that causes a crop injury of 15% (D_{15}) was calculated. The 5% confidence interval was used to compare this variable among the different times of herbicide application.

The environmental variables (air temperature, relative humidity and photosynthetically active radiation) that were measured at the time at which the herbicide was sprayed were correlated with one another. Additionally, each environmental variable was correlated with D15 using the data from both experiments (n = 10). A conceptual map with the correlation coefficients of each of the environmental variables and D15 was created.

Results and discussion

For both experiments and evaluation periods, a significant interaction between the time of the herbicide spraying and the fomesafen dose was detected. At the first assessment (one WAT) during the first experiment, the maximum asymptote for crop injury was higher when fomesafen was applied at 11:00 am and 4:00 pm compared with the other spray times. This data differed from the maximum asymptote for the nighttime applications (2:00 am and 9:00 pm) (Table 2). Overall, the herbicide injuries on the bean plants were reduced by the three WAT assessment compared with the assessment at one WAT for the first experiment (Table 2). At three WAT, the crop injury was greater when the herbicide was sprayed at 9:00 pm than at other application times (Table 2).

During the second experiment, fomesafen that was applied at 11:00 am and 4:00 pm caused greater injury (20%) to the bean crop when compared with the other spray times at one WAT (Table 3). The lowest maximum asymptote for injury was detected when the product was applied at 9:00 pm compared with all of the other spray times (Table 3). On the same evaluation date, the lowest D_{A50} were found when fomesafen was applied at 11:00 am, 4:00 pm, and 9:00 pm, which differed from the D_{A50} detected when the herbicide was sprayed at 6:00 am (Table 3). At three WAT, the fomesafen injury to the bean plants was minimal when the herbicide was sprayed at 2:00 am, 6:00 am, and 9:00 pm (Table 3).

Table 2. Estimated equation parameters that describe fomesafen toxicity to bean plants as a function of herbicide dose and application time, which was evaluated at one and three WAT with the herbicide. October, 2010.

Time	Equation parameters[1]			R^2	P
	A (se[2])	D_{A50} (se)	b (se)		
		One WAT			
2:00 am	17 (2)	183 (13)	33 (8)	0.99	<0.01
6:00 am	22 (6)	200 (25)	39 (12)	0.98	<0.01
11:00 am	29 (2)	166 (9)	41 (6)	0.99	<0.01
400 pm	28 (4)	162 (14)	37 (10)	0.98	<0.01
9:00 pm	19 (2)	188 (15)	45 (7)	0.99	<0.01
		Three WAT			
2:00 am	5 (1)	119 (20)	38 (21)	0.93	0.02
6:00 am	9 (2)	282 (22)	26 (15)	0.93	0.02
11:00 am	7 (2)	120 (31)	45 (31)	0.90	0.03
4:00 pm	19 (2)	167 (9)	29 (7)	0.99	<0.01
9:00 pm	6 (1)	164 (25)	31 (18)	0.92	0.02

[1]Three-parameter sigmoidal equation: $y=A/(1+\exp(-(d-D_{A50})/b))$, where A= maximum asymptote of phytotoxicity; d= fomesafen dose; D_{A50}= dose for 50% of the maximum asymptote and b= slope of the curve at its point of inflection. [2]The standard error of the estimated parameter is in parentheses.

Table 3. Estimated equation parameters that describe fomesafen toxicity to the bean plants as a function of herbicide dose and application time, which was evaluated with the herbicide at one and three WAT. March, 2011.

Time	Equation parameters [1]			R^2	P
	A (se[2])	D_{A50} (se)	b (se)		
		One WAT			
2:00 am	15 (3)	115 (26)	37 (23)	0.90	0.03
6:00 am	16 (2)	138 (17)	25 (14)	0.94	0.02
11:00 am	21 (1)	87 (11)	33 (10)	0.97	<0.01
4:00 pm	21 (1)	88 (12)	11 (9)	0.96	0.01
9:00 pm	9 (1)	94 (12)	19 (9)	0.95	0.01
		Three WAT			
11:00[3] am	43 (96)	290 (234)	63 (38)	0.96	0.01
4:00 pm	12 (2)	190 (17)	41 (9)	0.99	<0.01

[1]Three-parameter sigmoidal equation: $y=A/(1+\exp(-(d-D_{A50})/b))$, where A= maximum asymptote of phytotoxicity; d= fomesafen dose; D_{A50}= dose for 50% of the maximum asymptote and b= slope of the curve at its point of inflection. [2]The standard error of the estimated parameter is in parentheses. [3]The equations at 2h00, 6h00 and 21h00 were not significant ($p > 0.05$) because the crop injury was limited (< 3%).

One hypothesis of the present study was that the bean plants would recover from fomesafen injuries over time. Indeed, lower crop injury was detected at three WAT than at one WAT, regardless of the initial degree of crop injury detected at each time of fomesafen spray (Tables 2 and 3). Other experiments from the literature support this hypothesis. For example, a crop injury of less than 3% was observed when the bean plants were evaluated after three WAT (SOLTANI et al., 2005, 2006; WILSON, 2005). Possible explanations for the recovery of the bean plants from the herbicide symptoms include a lack of movement of the herbicide from the sprayed tissue to the new shoots and the action of glutathione S-transferase (GST) enzymes, which detoxify herbicides (ANDREWS et al., 2005; FREAR et al., 1983; GEOFFROY et al., 2002; KILINC et al., 2011; PASCAL et al., 2000).

For both experiments, the lowest D_{15} was detected when fomesafen was applied at the warmest times of the day (11:00 am and 4:00 pm) compared with other times. Another hypothesis of this work was that the time of fomesafen application would affect the crop's tolerance to the herbicide. In fact, for both experiments the crop injury was greater when fomesafen was applied at the warmest times of day that also exhibited the highest irradiance values (11:00 am and 4:00 pm) (Tables 2 and 3; Figure 1).

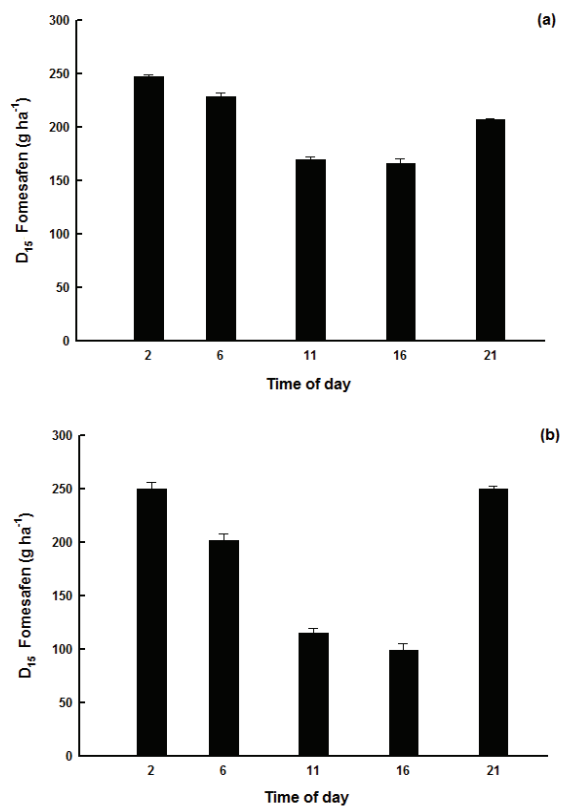

Figure 1. Toxicity of fomesafen to bean plants as a function of application time using the fomesafen rate that causes a bean crop injury of 15% (D15). (a) Experiment 1 and (b) Experiment 2. The vertical bars represent the confidence intervals ($p < 0.05$) of the estimated doses.

Several studies in the literature have also documented the effect of the time of spray of PROTOX inhibitors on the selectivity of soybean plants (FAUSEY; RENNER, 2001; FERREIRA et al., 1998). For example, increased toxicity of fluthiacet and flumiclorac (both PROTOX inhibitors) to the crop occurred when these two herbicides were applied at 6:00 am compared with 2:00 pm and 10:00 pm (FAUSEY; RENNER, 2001). In contrast, lactofen (another PROTOX inhibitor) injury was more intense when the herbicide was sprayed at 5:00 pm and 10:00 pm compared with other spray times (FERREIRA et al., 1998). One possible reason for this paradox is that the environmental variables interact with one another to determine the effect of the herbicide on the plants (SIVESIND et al., 2011).

Multiple (with two or three variables) linear correlations were not significant ($p > 0.05$) (data not shown). Overall, each environmental variable was correlated with each of the other variables and also with D_{15}. Air temperature was negatively correlated ($p < 0.01$) with relative humidity and positively correlated ($p < 0.01$) with photosynthetically active radiation (Table 4, Figure 2). Relative humidity was negatively correlated ($p < 0.01$) with photosynthetically active radiation (Table 4, Figure 2). D_{15} was positively correlated ($p < 0.17$) with the relative humidity. The other two environmental variables were negatively correlated with D_{15} (Table 4). The conceptual map indicates that photosynthetically active radiation had the greatest impact on D_{15} (Figure 2).

Table 4. Correlations of environmental variables with one another and with the fomesafen rate that causes 15% injury to the bean plants (D_{15}) using the data from both experiments when evaluated one week after the herbicide was sprayed.

Variable y	Variable x	Equation	R^2	P	n[1]
AT[2]	RH	y=42.48-0.33x	0.96	<0.01	10
AT	PAR	y=18.08+0.01x	0.67	<0.01	10
RH	PAR	y=73.00-0.02x	0.63	0.01	10
D_{15}[3]	AT	y=297.43-4.76x	0.26	0.13	10
D_{15}	RH	y=101.86+1.47x	0.22	0.17	10
D_{15}	PAR	y=216.94-0.04x	0.30	0.10	10

[1] Number of data pairs used in the analysis. [2] AT = air temperature; RH = relative humidity and PAR = photosynthetically active radiation. [3] Fomesafen dose that causes 15% injury to the bean crop.

In the experiments of the present study, the strong correlations between the environmental variables (Table 4, Figure 2) suggest that the final impact of the time of day on the efficacy of the herbicide is dependent on the environmental conditions. Therefore, the strongest correlations of D_{15} with air temperature and photosynthetically active radiation (Table 4, Figure 2) indicate that the effect of fomesafen on bean plants is favored by increase in these two variables. This result is consistent with the observation of the highest crop injury when fomesafen was applied at 11:00 am and 4:00 pm (Tables 2 and 3, Figure 1). Of these two environmental variables, photosynthetically active radiation was the most important variable for determining the impact of fomesafen on the bean crop (Figure 2). This result may be related to the mode of action of the herbicide. In fact, it has been demonstrated that high irradiance favors the performance of PROTOX inhibitors on weed control (FAUSEY; RENNER, 2001; HESS, 2000; HWANG et al., 2004; LEE; DUKE, 1994; VANSTONE; STOBBE, 1979). If the conditions that govern crop absorption and the effect of PROTOX inhibitors on the bean crop are general rules in plants, the data of this work suggest that the best effect of fomesafen on weeds can be achieved when the product is sprayed at high temperatures and irradiance levels.

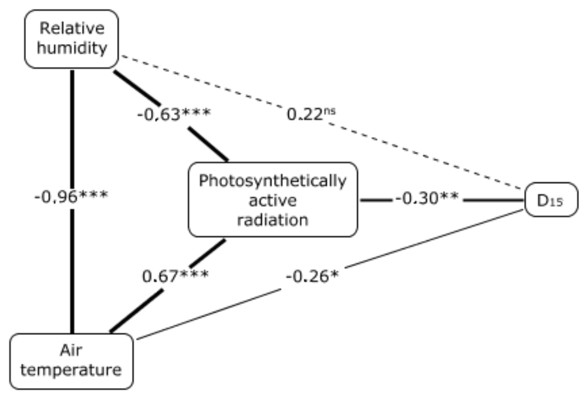

Figure 2. Conceptual map showing the correlation coefficients when the environmental variables were compared with one another at the fomesafen dose that causes 15% injury to bean plants (D15). The dashed lines indicate a lack of significance, and thicker lines indicate correlations with a higher degree of significance as follows: *($p < 0.15$), **($p < 0.10$) and ***($p < 0.01$).

Conclusion

The selectivity of fomesafen for bean plants depends on the herbicide dose, the time of the herbicide spray, and the assessment date. A high rate of injury to the bean crop occurred when the highest herbicide dose was applied, but fomesafen phytotoxicity was negligible when assessed at three WAT. The greatest injury to the bean plants by fomesafen occurred when the compound was sprayed at 11:00 am and 4:00 pm. Air temperature and irradiance levels were the most influential environmental conditions that affected the herbicide's impact on the crop.

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