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Article

An Innovative Method to Evaluate the Impact of Temperature on Iodosulfuron-Methyl Selectivity to oat Crop

Método Inovador para Avaliar o Efeito da Temperatura na Seletividade de Iodosulfuron-Methyl na Cultura de Aveia

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ABSTRACT - Temperature affects the selectivity of post-emergence herbicides in a complex manner. The objective of this work was to develop a method to estimate the impact of temperature on herbicide selectivity using the white oat (*Avena sativa*) crop and iodosulfuron-methyl as study models. Greenhouse/growth-chamber experiments were conducted using a completely randomized design with the treatments arranged as a bi-factorial, with three repetitions. The first factor consisted of six temperatures (10, 15, 20, 24, 28 and 32 ^{o}C) to which the plants were submitted during one week after the herbicide spray. The second factor corresponded to seven doses of iodosulfuron-methyl (0, 0.2, 0.4, 0.8, 1.2, 5 and 20 g ha^{-1}). For each temperature, regression curves were fitted to the dose-response data. The rate of herbicide efficacy was computed through the method first proposed in this study. The crop tolerance to the herbicide increased proportionally to the temperature, suggesting the product detoxification is improving crop selectivity. In practical terms, it is predicted that white oat crop tolerance to iodosulfuron-methyl increases in regions of the world with high temperatures. The method developed here also can be used to understand the effect of temperature on herbicide efficacy on weeds.

Keywords: *Avena sativa*, crop tolerance, environmental factors, herbicide detoxification.

*RESUMO - A temperatura impacta a seletividade de herbicidas em pós-emergência de forma complexa. O objetivo deste trabalho foi desenvolver um método para estimar o impacto da temperatura na seletividade de herbicida, utilizando aveia cultivada (**Avena sativa**) e iodosulfuron-methyl como modelos de estudo. Os experimentos foram conduzidos em casa de vegetação/câmaras de crescimento, utilizando-se delineamento inteiramente casualizado, com os tratamentos arranjados como bifatorial, com três repetições. O primeiro fator consistiu de seis temperaturas (10, 15, 20, 24, 28 e 32 ^{o}C), a que foram submetidas as plantas durante uma semana após a pulverização. O segundo fator correspondeu a sete doses de iodosulfuron (0; 0,2; 0,4; 0,8; 1,2; 5; e 20 g ha^{-1}). Para cada temperatura, curvas de regressão foram ajustadas aos dados de dose-resposta. A taxa de eficácia do herbicida foi calculada por método inovador aqui descrito. A tolerância da cultura ao herbicida aumentou proporcionalmente com a temperatura, sugerindo que a detoxificação do produto está beneficiando a seletividade. Em termos práticos, espera-se que a tolerância das culturas de aveia-branca ao herbicida iodosulfuron seja acentuada em regiões do mundo com temperaturas elevadas. O método desenvolvido aqui pode ser usado para determinar o efeito da temperatura na eficácia de herbicidas em plantas daninhas.*

Palavras-chave: *Avena sativa*, tolerância de culturas, fatores ambientais, detoxificação de herbicida.

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INTRODUCTION

The cultivated oat (*Avena sativa*) is an important crop species that is grown over an area of circa 10 million ha (FAOSTAT, 2016). The no-tillage cropping system, on subtropical environments, allows cultivating the oat crop during the winter-spring time and, after its harvest, it is possible to establish the soybean crop (Federizzi et al., 2015). However the oat crop grain yield is decreasing during recent years (Federizzi et al., 2015), in part due to weed competition (Lemerle et al., 1995; Begna et al., 2011). For instance, *Lolium rigidum* competition reduces oat grain yield by 14% (Lemerle et al., 1995), whereas *Chenopodium album* interference decreases this crop grain yield by 10% (Begna et al., 2011).

The herbicides inhibitors of the acetolactate synthase enzyme (ALS) have several benefits to agriculture, including broad-spectrum of weed control, low toxicity to mammals and efficacy at low doses (Powles and Yu, 2010; Queiroz et al, 2013). The iodosulfuron-methyl herbicide is an ALS inhibitor that is registered for the control of mono and dicotyledonous species in several cereal crops, including wheat, rye and barley (Brigante et al., 2005).

The temperature affects both plant behavior (growth, physiology and metabolism) and herbicide characteristics and performance (volatility, solubility, absorption and translocation and detoxification of xenobiotics). Hence, the impact of temperature on the crop tolerance to herbicides is not straightforward. Elevated temperature, within biological limits, increases herbicide absorption and efficacy by reducing cuticle wax viscosity and by increasing the chemical diffusion rate through the leaf cuticle (Fuentes and Leroux, 2002). However, elevated temperature increases the tolerance of crops to herbicide because it increases its detoxification rate by plants (McCullough and Hart, 2006). For example, sulfosulfuron (an ALS inhibitor) selectivity to *Agrotis stolonifera* L. was more pronounced at temperatures of 25 °C than at 15 °C (McCullough and Hart, 2008). Likewise, rimsulfuron (another ALS inhibitor) detoxification on maize plants (*Zea mays*) and maximum crop tolerance was observed at a temperature of 30 °C, in contrast to that observed under 10 °C (Koeppe et al., 2000). Therefore, it is necessary to develop a reliable method to evaluate the effect of temperature on herbicide selectivity to crops. The objective of this work was to develop a method to estimate the impact of temperature on crop tolerance to herbicide using the white oat (*Avena sativa*) crop and iodosulfuron-methyl as study models.

MATERIALS AND METHODS

In 2014, experiments were conducted in the greenhouse and growth chambers with controllable temperature, at Federal University at Rio Grande do Sul, located in Porto Alegre, RS.

The experiment was organized as a completely randomized design with the treatments arranged as a bi-factorial, with three replicates. The first factor consisted of six temperatures (10, 15, 20, 24, 28 and 32 °C). The second factor corresponded to seven doses of iodosulfuron-methyl (0, 0.2, 0.4, 0.8, 1.2, 5 and 20 g ha^{-1}). Each experimental unit consisted of 350 mL capacity pots filled with the mixture of soil:sand:compost (1:1:1, v:v:v), with addition 2 g of NPK fertilizer (30-20-10) each. Five seeds from the oat genotype URS Taura (sensitive to iodosulfuron-methyl) were placed in each pot. Two weeks later, excess seedlings were removed, maintaining only two plants per pot. The plants were grown in the greenhouse (temperature 19±5 °C) until the three-leaf growth stage, when the herbicide was sprayed. Iodosulfuron-methyl was applied in spray chamber using compressed air at 200 kPa, spray nozzle 80.02E and spray volume equivalent to 170 L ha^{-1}. A surfactant (Dash®, BASF) was added to all herbicide solutions at the proportion of 0.5% (v:v). Two hours later, when apparently the droplets have dried, the pots were placed in growth chamber with the temperatures adjusted according to the treatments (first factor), and photoperiod of 12h. After one week, the pots were removed from the growth chamber and maintained in the greenhouse until the time of the evaluations.

At 35 days after the herbicide spray (DAT), the effects of the treatments were evaluated through the relative oat plant tolerance (ROPT) and plant height. The ROPT consisted of a visual estimative of the plant injury using a percentual scale, where 0% corresponded to lack of tolerance (plant death) and 100% indicated absence of visible symptoms. Between these extremes, the remaining values corresponded to the magnitude of the following symptoms: reduction of the dimensions of the plant parts, discoloration, chlorosis and necrosis of leaves and meristems.

The data were analyzed using ANOVA. Because there was significant interaction between factors, for each temperature, the three-parameter logistic model (Equation 1) was adjusted between the dependent variable (y) (ROPT and plant height) and the independent variable (x) (herbicide rate),

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

where the parameter a represents the asymptote, b represents the declivity of the curve at the point of inflection, and D_{50} represents the herbicide dose which reduces the value of the dependent variable by 50%. The equations were adjusted using the software SigmaPlot, version 11.0 (Systal Software Corp., USA). Afterwards, data analysis followed the classical curve segmentation model to determine seed germination rate (Soltani et al., 2006; Derakhshan et al., 2014). A detailed description of the curve segmentation models and procedures can be found elsewhere (Soltani et al., 2006; Derakhshan et al., 2014), but, can be summarized as follows: for each dependent variable, a polynomial equation was adjusted between the reciprocal of D_{50} and the temperature at which the plants were grown in the week after application of the product. These correlations yielded the herbicide efficacy rate curve. The data from herbicide efficacy rate and the temperature were plotted and the linear curves were determined. One hypothesis of this study is that the intercepts of these lines on the abscissa axis represent the temperature above which the oat crop achieves maximum tolerance to iodosulfuron-methyl herbicide.

RESULTS AND DISCUSSION

The effect of the iodosulfuron on the ROPT depended on the temperature (P<0.01). At each temperature, the logistic model was appropriate to express the regression between the herbicide rate and the ROPT (Figure 1 and Table 1). When iodosulfuron-methyl was used up to 1.2 g ha^{-1}, high crop tolerance (ROPT>80%) was achieved without differences among temperatures ranging from 20 to 32 ^{o}C. However, when the temperature was 10 or 15 ^{o}C, minimum ROPT was observed for iodosulfuron-methyl sprayed at 5 and 20 g ha^{-1} (Figure 1).

There was a temperature-dependent effect (P<0.01) of herbicide rate on the oat plant height (% of the untreated), evaluated at 35 DAA. The logistic model also was appropriate to estimate the impact of iodosulfuron-methyl rate on the oat plant height (Figure 2 and Table 2). It was observed a higher sensitivity of plants at low temperatures (10 and 15 ^{o}C) in contrast to 32 ^{o}C. When iodosulfuron-methyl was used at the rate labeled for the wheat crop (5 g ha^{-1}), there was no reduction of the plant height when the temperature was 32 ^{o}C. This result contrasts with the ones observed on the other temperatures, where the crop height was reduced.

The D_{50} estimated using the logistic equation for the variable ROPT increased at an exponential proportion (R^2 = 0.95) to the temperature (Figure 3A). In other words, with the increment of the temperature it was necessary a higher rate of the herbicide to achieve 50% of herbicide tolerance on oat plants (Table 1 and Figure 3A). Likewise, the D_{50} calculated through the logistic correlation between the oat plant height and temperature, also increased exponentially (R^2 = 0.94) with the temperature (Figure 3A).

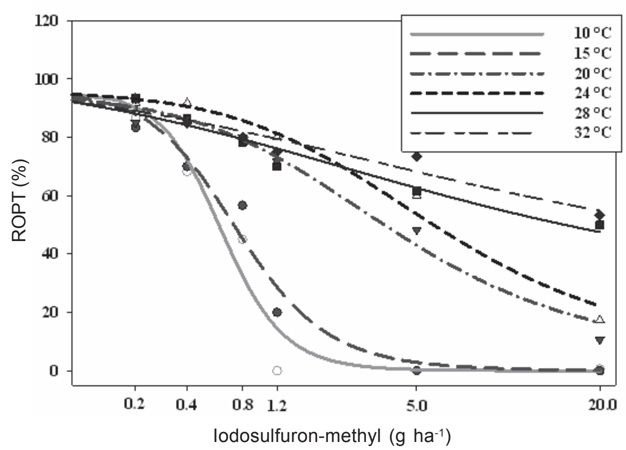

Figure 1 - Relative oat plant tolerance (ROPT) in response to iodosulfuron-methyl rates and different temperatures, when evaluated 35 days after herbicide application. Equations on Table 1.

When the D_{50} data of the dependent variable and the temperature were plotted, it was observed that there was no line segmentation. The herbicide efficacy rate estimated for the variables ROPT and oat plant height decreased linearly (R^2 = 0.88 and 0.95, respectively) with the increment of the temperature (Figure 3B). We speculate this work has detected the temperature-dependent herbicide-detoxification capability of the oat plant. This result indicates that with increasing

Table 1 - Parameters of the equations[(1)] that describe the effect of iodosulfuron-methyl rates and temperatures on the relative tolerance of oat plants, when evaluated 35 days after the herbicide application

Temperature (°C)	Regression equation parameters				
	a	b	$^{(4)}D_{50}$	$^{(5)}R^2$	$^{(6)}P$
10	95.26 (8.76)[(2)]***[(3)]	2.61 (0.90)**	0.62 (0.10)***	0.94***	<0.01
15	95.81 (7.36)***	1.84 (0.54)**	0.76 (0.12)***	0.96***	<0.01
20	94.90 (4.57)***	0.98 (0.17)***	4.12 (0.89)***	0.97***	<0.01
24	95.74 (4.10)***	1.04 (0.20)***	6.32 (1.28)***	0.96***	<0.01
28	101.69 (4.43)***	0.43 (0.08)***	14.83 (5.49)*	0.94***	<0.01
32	100.41 (4.14)***	0.41 (0.08)***	> 20[(7)]	0.92***	<0.01

[(1)] Three-parameter logistic equation $y=a/(1+(X/X_0)^b)$. [(2)] Values in parentheses correspond to standard error of the estimative of the parameter. [(3)] Significance by the 't' test: * 10%; ** 5% and *** 1%; ns indicates not significant. [(4)] Iodosulfuron-methyl dose which reduces the relative tolerance by 50% (except at 32 °C, when the value was superior to the dose tested). [(5)] Coefficient of determination of the model. [(6)] Probability of the equation significance by 't' test. [(7)] Actual value estimated for the equation is 31.61 (14.05), which is above the herbicide rate tested.

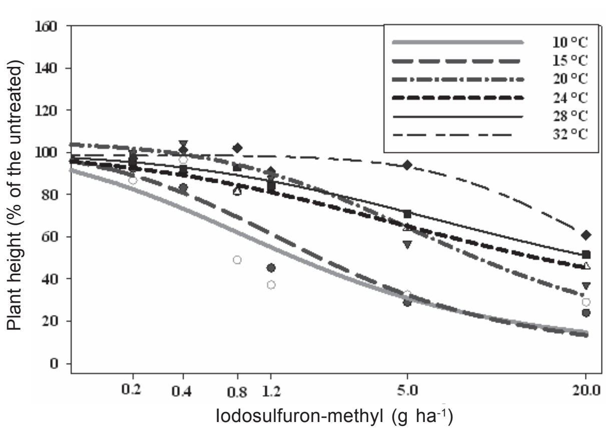

Figure 2 - Oat plant height (% of the untreated) in response to iodosulfuron-methyl rates and temperatures, evaluated 35 days after herbicide application. Equations on Table 2.

temperatures, among the ones tested, the crop became more tolerant to the herbicide, thus requiring a higher iodosulfuron dose to attain 50% crop injury. In summary, the minimum efficacy rate of iodosulfuron-methyl occurs at 24.5 and 26.4 °C for the variables oat plant height and ROPT, respectively (Figure 3B).

The results from the method presented here support the hypothesis that the intercepts of the D_{50} x temperature lines on the abscissa axis (24.5 and 26.4 °C for the variables oat plant height and ROPT, respectively) represent the temperature above which the oat crop achieves maximum tolerance to iodosulfuron-methyl herbicide. Also, the results of this work support the hypothesis that oat crop tolerance to iodosulfuron-methyl is temperature-dependent. Probably, this result is a balance among several antagonistic processes, such as absorption-translocation vs. detoxification. The impact of temperature on herbicide detoxification by the crop has been demonstrated in several papers. For instance, temperatures of 25 and

Table 2 - Estimated parameters of equations[(1)] describing the effect of iodosulfuron-methyl rates and temperatures on oat plant height, assessed 35 days after herbicide application

Temperature (°C)	Regression equation parameters				
	a		$^{(4)}D_{50}$	$^{(5)}R^2$	$^{(6)}P$
10	104.83 (17.43)***	0.68 [(2)](0.31)*[(3)]	1.39 (1.07)[ns]	0.68**	0.04
15	103.46 (11.18)***	0.81 (0.25)**	1.91 (0.88)*	0.86***	<0.01
20	105.20 (5.65)***	0.92 (0.22)**	8.20 (2.19)**	0.92***	<0.01
24	100.49 (2.09)***	0.58 (0.05)***	14.25 (1.98)***	0.99***	<0.01
28	100.37 (2.02)***	0.64 (0.07)***	> 20[(7)]	0.98***	<0.01
32	98.69 (2.10)***	1.68 (0.67)*	> 20[(7)]	0.91***	<0.01

[(1)] Three-parameter logistic equation $y=a/(1+(X/X_0)^b)$. [(2)] Values in parentheses correspond to standard error of the estimative of the parameter. [(3)] Significance by the 't' test: * 10%; ** 5% and *** 1%; ns indicates not significant. [(4)] Iodosulfuron-methyl dose which reduces the relative tolerance by 50% (except at 32 and 28 °C, when the value was superior to the dose tested). [(5)] Coefficient of determination of the model. [(6)] Probability of the equation significance by 't' test. [(7)] Actual values estimated for the equation are 21.17 (2.93) and 26.58 (4.40), for 28 and 32 °C, respectively. These values are above the herbicide rate tested.

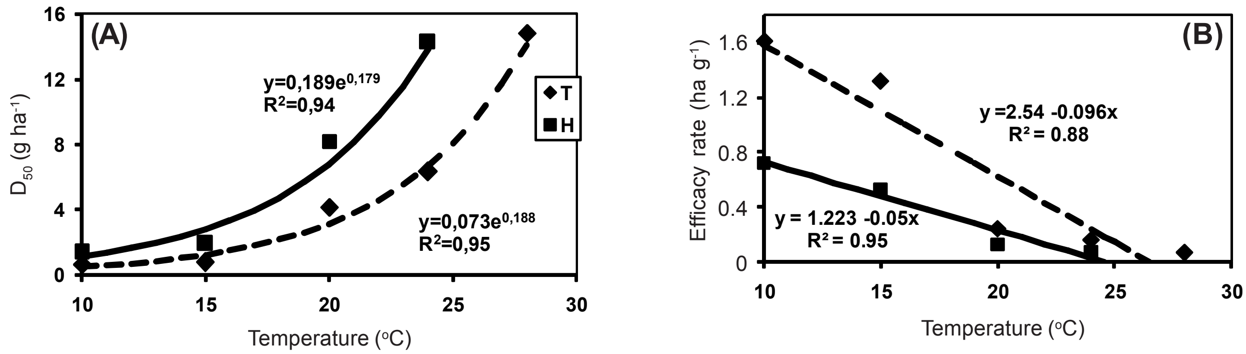

Figure 3 - Effect of air temperatures during the week after the herbicide treatment on the correlation between the temperature and (A) D_{50} or (B) iodosulfuron-methyl efficacy rate, measured on two variables (relative oat plant tolerance = T and plant height = H).

30 °C yielded higher rimsulfuron detoxification in maize (*Zea mays*) plants, when compared to 10 °C (Koeppe et al., 2000). Also, the sulfonylurea sulfosulfuron injury to wheat plants was higher at 21/7 °C (day/night) than at 10/5 °C (Geier et al., 1999), in part, because at lower temperatures plants have reduced leaf area, thus reduced absorption and translocation of the herbicide (Olson et al., 2000).

Another point to consider is the possibility that the herbicide target enzyme and the degradation enzymes (when present) could have different temperature-dependent activities (Mahan et al., 2004). For instance, experiments conducted with transgenic *Gossypium hirsutum* (tolerant to glufosinate-ammonium an inhibitor of glutamine synthetase) indicate the optimum temperature for the activity of the detoxifying enzymes are sub-optimal for the activity of the herbicide target enzyme, thus, helping to increase the tolerance to the herbicide (Mahan et al., 2006).

At least two practical applications can be foreseen from this work. First, the predicted planet warming due to anthropogenic climate change (Jacobeit et al., 2014) may be beneficial, at least in part, to the oat production because herbicides from new mechanisms of action can became available to selective weed control on the crop. Second, current trends to expand the cultivation of the oat crop to warmer parts of the world (central Brazil and India) suggest increased tolerance to iodosulfuron-methyl with high temperatures would allow additional opportunity for weed management on this crop. However, it is imperative that the industry evaluates the level of herbicide residues on the oat grains and register the herbicide on this crop.

The research method presented in this paper is an innovative approach to determine the impact of the temperature on the herbicide selectivity to crops. The method was adapted from the classical method to determine cardinal temperature for seed germination (Soltani et al., 2006; Derakhshan et al., 2014). But, it is important to highlight that the impact of temperature on seed germination and on herbicide efficacy are different. In seed germination, the major condition which controls the process is temperature (Derakhshan et al., 2014). However, in herbicide efficacy, temperature affect both plant and herbicide processes, thus, it is possible that at the same time, temperature has opposite effects on plant and on herbicide.

The tolerance of *Avena sativa* to iodosulfuron-methyl is dependent on the temperature and herbicide doses. The oat plants tolerance to iodosulfuron-methyl decreases with low temperatures, but increased temperatures reduce the sensitivity of oat plants to iodosulfuron-methyl.

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