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MODELING HERBICIDES DOSE-RESPONSE FUNCTIONS TO THE *DIGITARIA HORIZONTALIS* SPECIE

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Abstract: The *Digitaria Horizontalis* specie response to different types of herbicides were studied to obtain dose-response functions using the log-logistic model and the statistical program R. This specie showed to be more susceptible to the combination tembotrione + atrazina and the atrazine herbicide applied alone did not reach 80% of control.

Keywords: Dose-response curves, *Digitaria Horizontalis*, herbicides.

1. INTRODUCTION

The agricultural weed infestation may appear due to several factors, and among them, the ecological imbalance caused by the intervention of man in the environment. This imbalance, conditioned by environment variables, favors the population explosion of some individuals, causing infestation. A common infestation, is the infestation by weed (Shiratsuchi, 2001).

The weed infestation is considered a major problem in modern agriculture, because they generate losses at various levels. Cousens (1985) presents models that relates the yield loss of the agricultural crops to the density of weeds based on the inter-specific competition. Recently, competition was quantified between weeds and soy-beans from index of competitiveness and weeds distribution in crops (Hock et al., 2006).

The process of applying herbicide to control weeds creates significant costs to producers. Over the years, several selective herbicides have been developed and used in crops, following a preventive and restorative solution for weeds control (Rizzardi e Fleck, 2004).

Satisfactory degrees of weed control are often obtained using doses of herbicides below those usually recommended in the product labeling. The doses are usually set aiming at a degree of effective control over a wide range of environmental conditions and management (Rizzardi e Fleck, 2004).

However, the intensive use of chemicals to control weeds may creates a selection of weed species tolerant to a herbicide. The repeated application of one or more herbicides with some mechanism of action, in a population of weeds, selects individuals of species with skill to survive the herbicide treatment (Dias, 2004). This phenomenon characterizes a pressure of selection, caused by intensive use of the herbicides in a population, and contributes to the increasing the proportion of tolerant individuals for the next generation (Dias, 2004).

In weed science research, the most common goal of biological assays is to measure and compare the response of weeds and crops to physical, chemical, biological, or temporal stimuli. Often, biological assays require the use of nonlinear regression models with upper and lower limits, which provide information on the dose required to control the species plant of interest (Stevan et al., 2007).

The matter of weeds is used to illustrate the use of log-logistic model. The log-logistic model has been used extensively to express herbicides dose-responses for many different combinations of herbicides. The log-logistic model is not limited to herbicide-based studies with plants. According to Seefeldt et al. (1995), the log-logistical model should be considered a standard technique for the analysis of dose-response relationships involving antagonism, synergism, selectivity, and resistance, or the effects environment on herbicide activity.

Various authors have used and recommended dose-response functions to determine the susceptibility or resistance of weeds to herbicides applied in different crops (Streibig e Kudsk (1993), Lacerda e Filho (2004), Kim et al. (2006), Haage et al. (2007), Smith et al. (2008) and Merotto et al. (2009)). To obtain the dose-response function, there are several models the Gompertz, the von Bertalanffy, and the Morgan-Mercer-Flodin family (Seber e Wild, 1989) but, the most used by researchers in weed science is the log-logistic model proposed by Seefeldt et al. (1995).

In general, the analysis of data is done with the help of the statistical program R, which provides the parameters for the dose-response curves. **R** is a command-line driven software, and it is relatively user friendly. **R** can simultaneously fit multiple dose-response curves, examine whether a chosen dose-response model is appropriate to discrete data, and calculate biologically relevant quantities such as the effective dose (ED). The user only needs to fit the regression model once and then all parameter combinations of choice can be compared for significance. The *drc* package contains programmed commands for dose-response analysis and enables **R** to plot the distribution of data and regression lines (Stevan et al., 2007).

The objective of this paper was to determine the dose-response function for the crabgrass (*Digitaria Horizontalis*) specie, with the application of nicosulfuron, nicosulfuron + atrazine, tembotrione + aureo, tembotrione + atrazine + aureo e atrazine + oil herbicides. In addiction, there was a mathematical calculation of the dose of the herbicide, as a percentage, of the growth reduction GR of weeds.

2. MATERIAL AND METHODS

The experiment was conducted in a greenhouse with controlled temperature, developed at EMBRAPA - Milho and Sorgo localeted in Sete Lagoas, MG. The experimental layout was a randomized block with five repetitions. The soil used was a red-yellow Oxisol in pots of 600 ml. To application the herbicide, pressurized CO2 backpack sprayer at a flow rate of 150l/ha was used. The species were subjected to treatment in the stage of tiller development.

The effects of the nicosulfuron, nicosulfuron + atrazine, tembotrione + aureo , tembotrione + atrazine + aureo, atrazine + oil (aureo is the oil that comes tembotrione) were studied. A recommended rate D of each herbicide (40 g/ha; 32g + 1000g/ha; 420g + 1,0l/ha; 420g + 1000g + 1,0l/ha e 1000g + 1,0l/ha), respectively, the treatments were: $1/4D$, $1/2D$, $1D$, $2D$, $4D$ and no herbicide. The recommended rate for each herbicide can be found in Table 1.

Table 1 – Recommended rates for herbicides in $ga.i.ha^{-1}$

Herbicide	g a.i. ha^{-1}
Nicosulfuron	40g
Nicosulfuron + atrazine	32g + 1000g
Tembotrione + aureo	420g + 1,01
Tembotrione + atrazine + aureo	420g + 1000g + 1,01
Atrazine + oil	1000g + 1,01

The control percentage was evaluated at 7, 14 and 21 days after application (DAA) of herbicide and dry weight at 21 DAA, with extremes controls from 0 (no control) to 100% (absolute control). To obtain dry biomass the weed was collected by scissors cut, and then placed into paper bag and transported to a camera of air regulated to 65^0C for 72 hours.

The data were submitted to a variance analysis with the statistical program **R** to check if the herbicide doses had significant effect on the response of the weed.

The relation between the dose of herbicide and the response of the plant is the most important issue to understand the effectiveness of the herbicide and its mode of action. The dose-response function is used to quantify the plant sensitivity to the herbicide.

A typical shape of a dose-response curve is sigmoidal, with upper and lower limits, where the upper limit is defined by the response from non treated plants (control), or from plans treated with a very low dose of herbicide, where as the lower limit is determined by the response levels from a high dose of herbicide. The dose corresponding to the midpoint of the plant growth response observed between the upper and lower limits is usually referred to as GR_{50}. One example of such a curve is the log-logistic curve.

The most commonly used model for sigmoidal dose-response curves is the log-logistic model with three or four-parameters. The four parameter log-logistic function which relates the response ρ to the dose u is given by (Seefeldt et al., 1995):

$$\rho = C + \frac{D - C}{1 + exp[b(log(u) - log(GR_{50}))]}, \tag{1}$$

where C is the lowest limit; D is the highest limit; b is the slop, and GR_{50} is the dose to reduct of the 50% response. The log-logistic function is symmetric around the parameter GR_{50}, the inflection point. If $C = 0$, then the four-parameter model reduces to the three-parameter model with the lower limit being zero:

$$\rho = \frac{D}{1 + exp[b(log(u) - log(GR_{50}))]}. \tag{2}$$

Another commonly used model is the four-parameter model given by:

$$\rho = C + D - Cexp{-}exp[b(log(u) - (GR_{50}))]. \tag{3}$$

One of the advantages in using the function described by (1) is that the parameters are biologically meaningful. The highest limit D corresponds to the mean response of the control and the lowest limit C means that the response at very high doses (note that the lowest limit is not necessarily zero). The parameter b describes the slop of the curve around the GR_{50}. The greater the value of b, the steeper the slope of the curve (Seefeldt et al., 1995).

The log-logistic model advantages is due to the GR_{50} parameter (growth reduction of 50%). It is the herbicide dose in grams presented in active ingredient per hectare that gives the value of 50% of reduction in the weed's development which indicates the resistant biotype and the level of resistance by the ratio of GR_{50} susceptible/ GR_{50} resistant (Carvalho et al., 2005).

3. RESULTS AND DISCUSSION

A nonlinear regression analysis of dose response data using the log-logistic model was performed by the statistical program R, with the objective of obtain the parameters needed to produce the dose-response functions and determine statistical indicators of accuracy in the collected data.

The data are saved illustrated in Table 2 with the dry weight for the nicosulfuron herbicide. We obtained the parameters b, C, D and GR_{50} of the log-logistic model, which are presented in Table 3. See Appendix for the program lines used in analyze and determine dose-response function parameters.

Table 2 – Data used for determine log-logistic model parameters with dry weight for the nicosulfuron herbicide.

Dose	Timing	Dry weight
0	1	1,36
0	1	0,28
0	1	0,45
0	1	0,47
0	1	0,28
0,25	1	1
0,25	1	0,13
0,25	1	0,17
0,25	1	0,16
0,25	1	0,18
0,5	1	0,93
0,5	1	0,16
0,5	1	0,47
0,5	1	0,14
0,5	1	0,31
1	1	0
1	1	0
1	1	0
1	1	0
1	1	0
2	1	0
2	1	0
2	1	0
2	1	0
2	1	0
4	1	0
4	1	0
4	1	0
4	1	0
4	1	0

Table 3 – Dose-response function parameters for the weed crabgrass using dry weight.

Herbicide	b	C	D	GR_{50}	R^2
Nicosulfuron	9,188	-0,001	0,448	0,63	0,94
Nicosufuron + atrazine	4,07	-0,001	0,567	0,27	0,97
Tembotrione	4,07	-0,001	0,567	0,27	0,97
Tembotrione + atrazine	1,45	-0,004	0,56	0,06	0,82
Atrazine	0,43	0,13	0,57	0,10	0,77

From these parameters the values of GR_{50} and GR_{80} (growth reduction of 80%) for the five herbicides applied could be calculated (Table 4).

The variance analysis R^2 showed values next to 1, indicating high adjustment of the function to the collected data.

The values of C for nicosulfuron, nicosufuron + atrazine, tembotrione e tembotrione + atrazine herbicides were (-0.001, -0.001, -0.001 and -0.004), respectively. Actually, negative percentage reduction does not exist, but Christoffoleti (1999) considers this result normal, if the confidence interval of C parameter is sufficiently large, which includes higher, lower or equal to zero values.

The behavior of the dose-response curves of the crabgrass specie tested with the application of the herbicides listed are showed in Figure 1.

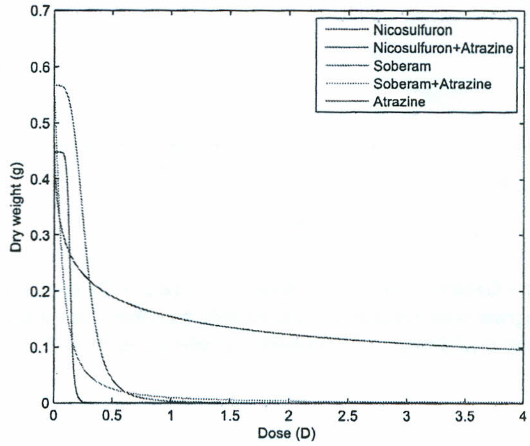

Figure 1 – Dose-response curves for the crabgrass specie with the nicosulfuron, nicosulfuron + atrazine, tembotrione, tembotrione + atrazine and atrazine herbicides treatment in $1/4D$, $1/2D$, $1D$, $2D$ and $4D$ doses using the log-logistic model.

In Table 4 note that the atrazine herbicide does not provide satisfactory results, the control of the crabgrass did not reach 80% using higher doses of herbicide. The nicosulfuron, nicosulfuron + atrazine, tembotrione and trembotrione + atrazine herbicides succeeded in controlling 80% of the crabgrass recommended dose with 0.73, 0.37, 0.37, 0.17, respectively.

Table 4 – Values of GR_{50} and GR_{80} recommended by herbicides dose to the crabgrass specie.

Herbicides	GR_{50}(%)	GR_{80} (%)
Nicosulfuron	0,63	0,73
Nicosulfuron + atrazine	0,27	0,37
Tembotrione	0,27	0,37
Tembotrione + atrazine	0,06	0,17
Atrazine	0,10	-

Figures 2 - 6 show the growth reduction at 7, 14 and 21 days to the herbicides application of the crabgrass specie.

Figure 5 shows that $1/2D$ of the tembotrione + atrazine herbicide dose applied to the crabgrass specie significant reduction in the crabgrass green biomass, causing control superior to 80%, while other herbicides require higher doses to achieve the same result. This was the case of the nicosulfuron herbicide doses, which needs dose superior to $3/5D$

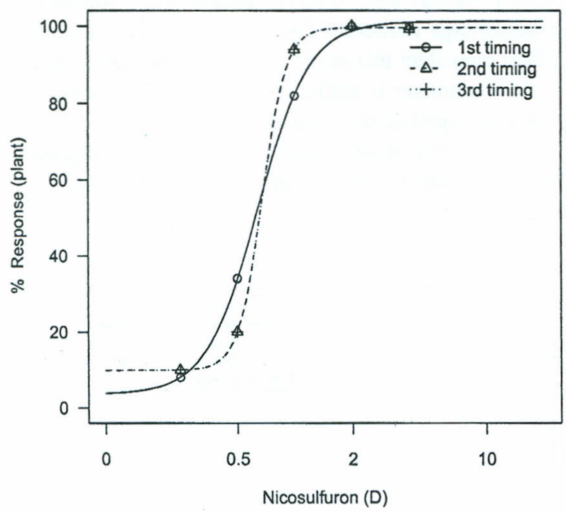

Figure 2 – Growth reduction response at 7, 14 and 21 days for the crabgrass with nicosulfuron herbicide treatment using the log-logistic model with the parameters produced in Table 5.

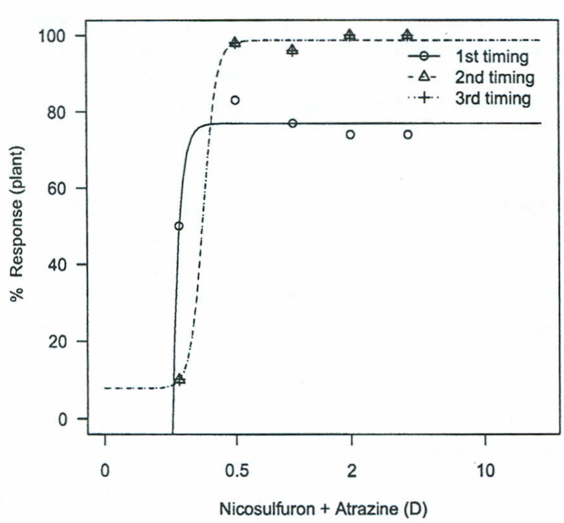

Figure 3 – Growth reduction response at 7, 14 and 21 days for the crabgrass with nicosulfuron + atrazine herbicide treatmentusing the log-logistic model with the parameters produced in Table 5.

to reach around 80% to show an expressive growth reduction (Figure 2).

The nicosulfuron + atrazine and tembotrione herbicides did not show satisfactory results of the dose response for the crabgrass specie. It was found that both herbicides have similar dose-response curves, but for an expressive growth reduction, doses superior to $2D$ doses are needed at timing 7

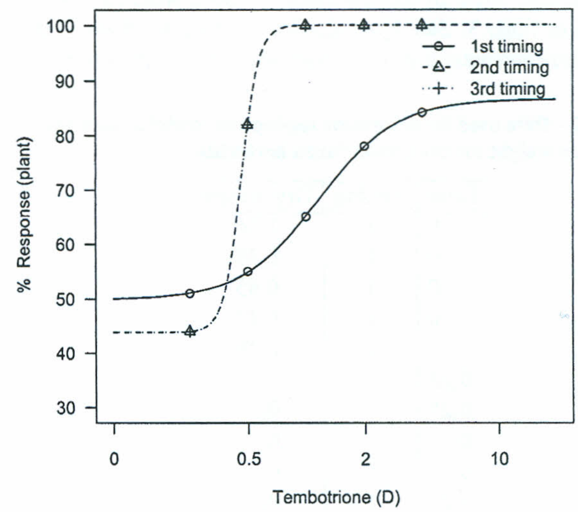

Figure 4 – Growth reduction response at 7, 14 and 21 days for the crabgrass with tembotrione herbicide treatment using the log-logistic model with the parameters produced in Table 5.

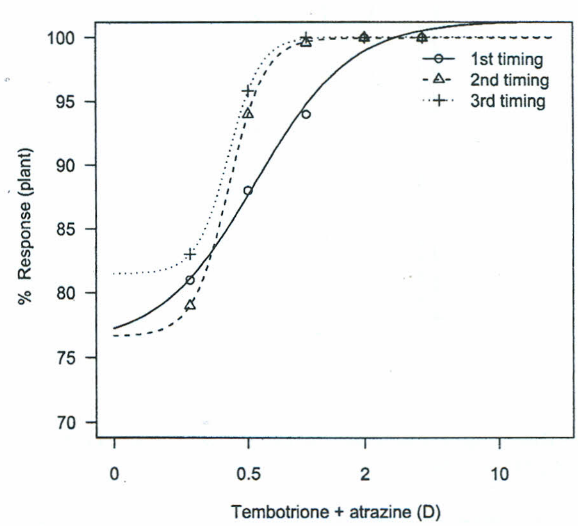

Figure 5 – Growth reduction response at 7, 14 and 21 days for the crabgrass with tembotrione + atrazine herbicide treatment using the log-logistic model with the parameters produced in Table 5.

DAA (Figures 3 and 4).

The classification of the crabgrass susceptibility in relation to herbicides application using the GR_{50}, adjusted for the log-logistical model is found in Table 6.

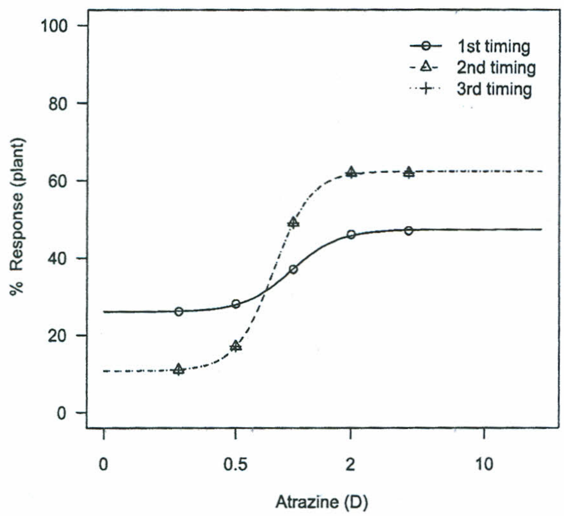

Figure 6 – Growth reduction response at 7, 14 and 21 days for the crabgrass with atrazine herbicide treatment using the log-logistic model with the parameters produced in Table 5.

Table 5 – Dose-response function parameters for the weed crabgrass using growth reduction response (%) for the timing.

Herbicide	Timing	b	C	D	GR_{50}
Nicosulfuron	1	-3.21	3.54	101.22	0.64
	2	-6.88	9.90	99.62	0.67
	3	-6.88	9.90	99.62	0.67
Nicosulfuron + Atrazine	1	-1.25	-1.46	0.77	0.18
	2	-1.24	7.80	0.98	0.33
	3	-1.24	7.80	0.98	0.33
Tembotrione	1	-2.17	49.85	86.60	1.16
	2	-9.66	43.85	100.01	0.46
	3	-9.66	43.85	100.01	0.46
Tembotrione + Atrazine	1	-1.75	76.00	101.29	0.54
	2	-4.68	76.62	99.97	0.39
	3	-5.23	81.47	100.04	0.39
Atrazine	1	-3.53	25.95	47.32	0.97
	2	-4.39	10.71	62.41	0.78
	3	-4.39	10.71	62.41	0.78

Table 6 – Classification of herbicides on the GR_{50} parameter for the crabgrass specie.

Herbicides	$GR_{50}(\%)$
Tembotrione + atrazine	6
Atrazine	10
Nicosulfuron + atrazine	27
Tembotrione	27
Nicosulfuron	63

4. CONCLUSION

After determining the dose-response functions it was concluded that the crabgrass specie is more sensitive to the atrazine + tembotrione herbicide, therefore showed the lowest GR_{50} (0.06), which means that to obtain a control of 50% in crabgrass specie only 6% of the recommended dose is required, while the atrazine, nicosufuron + atrazine, nicosulfuron, tembotrione herbicides showed to be necessary 10%, 27%, 27% and 63%, respectively, as the recommended rate of herbicides. However, the atrazine herbicide applied alone did not reach 80% of control.

Thus, we can conclude that the tembotrione + atrazine herbicide inhibited the development after the 7 DAA with a small dose, showing to be an interesting management option the crabgrass specie reaching satisfactory results, since, with very small doses, presenting as an interesting option for the management of this specie.

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APPENDIX

R Program lines used in analyze and determine the dose-response function parameters.
The text inside brackets are comments and should not be include in the R Program.

Table 7 – R program lines used in analyze and determine the dose-response function parameters.

Line	R Program input	Comments
01	library (drc)	This function loads and activate the drc package in R.
02	datafile<-'C:dir//subdir//filename.csv'	Location of the data file.
03	dataname<-read.csv(file=datafile, header=TRUE, sep=";", dec=",")	Assign a name to the data file e use the arguments to alone (,) as decimal separator.
04	head(dataname) Dose Timing Dryweight 1 0.00 1 1.36 2 0.00 1 0.28 3 0.00 1 0.45 4 0.00 1 0.47 5 0.00 1 0.28 6 0.25 1 1.00	Show data. This line prints the first six lines of the data.
05	IMGDM<-multdrc(Response $\sim Dryweight, Timing, fct = l4()$, dataname = datafile)	Commands multdrc and l4() are used to fit a fours-parameter log-logistic model.
06	summary(IMGDM)	Provide a summary of the parameter estimates.
07	anova(IMGDM)	The command anova is used to extract the lack-of-fit test for the fitted four-parameter log-logistic model.
08	ED(IMGDM,c(80))	The ED (effective doses) command calculate values for GR_{80}.
09	ED(IMGDM,c(50))	The ED command calculate values for GR_{50}
10	plot(IMGDM,conName="0", conLevel=0.1, xlim=c(0,20), xlab="Herbicide)", ylab= " Dry Weight ", col = c(1,1,1), pch=c(1,2,3), legendText = c("1st timing", "2nd timing", "3rd timing"))	Comments to plot the growth reduction curves with color and symbols, correspond to with points for the extension .csv or .txt.

Table 8 – Output for four-parameter model:

Output for four-parameter model:					
	Model fitted: Log-logistic (ED50 as parameter) (4 parms)				
	Parameter estimates:				
		Estimate	Std. Error	t-value	p-value
	b:	9.18	26.09	0.35	0.72
	c:	-0.001	0.07	-0.01	0.98
	d:	0.44	0.08	5.16	2.16e-05
	e:	0.63	0.42	1.48	0.14
	Residual std error: 0.27				
	Lack-of-fit test				
	ModelDf	RSS	Df	F value	p value
One-way ANOVA	24	1.8016			
DRC model	26	1.9457	2	0.95	0.39
Estimated effective doses					
	Estimative	Std. Error			
80	0.73	1.04			
50	0.63	0.42			