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Article

Susceptibility Among Populations of Crabgrass to Herbicides Inhibiting Photosystem II

Suscetibilidade entre Populações de Capim-Colchão a Herbicidas Inibidores do Fotossistema II

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ABSTRACT - Weed interference reduces the productive potential of crops. Among the control methods used, chemical ones occupy a major position. The repeated application of the same mechanism of action selects tolerant plants. Among the species that affect sugarcane, crabgrass is one of them. This study has aimed to know the tolerance of different populations of this species to herbicides inhibiting photosystem II. Three populations were collected, identified and submitted to the application of ametryn and diuron herbicides in ten different doses, and the experiment was conducted in a experimental design of complete randomized blocks with four repetitions in a factorial arrangement of 10 x 3 (10 doses x 3 populations), being one trial for each herbicide. Sixty days after application, visual control and plants shoot dry biomass were evaluated. The data were submitted to an analysis of variance by the F test and, when significant, their means were compared using the Tukey's test. The averages related to the herbicide doses were submitted to a graphical analysis by applying a log-logistic regression where the herbicide doses required to cause 50 or 80% of injury and reduction of dry biomass of plants were calculated. The resistance factor was calculated to determine the difference in herbicide tolerance among populations. Based on the results, it was observed that the populations exhibit differential sensitivities to herbicides and the one originated in Brazilian city Ribeirão Preto was resistant to ametryn. The population originated in Brazilian city Santa Cruz das Posses was less sensitive to diuron. The RF values ranged between 1.0 and 2.34.

Keywords: *Digitaria horizontalis*, ametryn, diuron, tolerance, resistance.

RESUMO - O controle químico é um dos principais métodos utilizados no manejo de plantas daninhas, porém a aplicação repetida de um mesmo mecanismo de ação pode selecionar indivíduos tolerantes aos herbicidas e formar populações resistentes. O capim-colchão (Digitaria horizontalis) é uma das principais plantas daninhas que afetam a cultura da cana-de-açúcar, e têm sido relatadas falhas no controle dessa espécie após a aplicação de herbicidas inibidores de fotossistema II. Com este trabalho, objetivou-se identificar a resistência de diferentes populações de capim-colchão a inibidores do fotossistema II (ametrina e diuron). Três populações foram coletadas, identificadas e submetidas à aplicação dos herbicidas, sendo os experimentos conduzidos em delineamento experimental inteiramente casualizado em esquema fatorial 10x3, em que 10 foram as doses testadas e 3 as populações, sendo um experimento para cada herbicida, com quatro repetições. Aos 60 dias após a aplicação, avaliaram-se a porcentagem de controle e a massa da matéria seca das plantas. O fator de resistência (FR) foi calculado para se determinar a diferença de controle entre os herbicidas e as populações de capim-colchão. As populações de capim-colchão apresentaram suscetibilidades

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diferenciais para os herbicidas, sendo a população originada de Ribeirão Preto resistente a ametrina. Plantas da população originada de Santa Cruz das Posses mostraram maior tolerância ao diuron. Os valores de FR variaram entre 1,0 e 2,34 entre as populações.

Palavras-chave: *Digitaria horizontalis*, ametrina, diuron, tolerância, resistência.

INTRODUCTION

Weeds are mostly pioneer species that have evolved over time in association with human activities in the various regions of the world. They have become targets of control in agricultural areas due to negatively interfering in human activities (Pitelli and Pavani, 2005).

Control strategies never have consistent results, leading to a selection of plants that somehow escape from the measures employed. Therefore, many plants disappear, while others adapt and are perpetuated, surviving in strong association with man (Pitelli, 1982).

Chemical control has introduced a number of ways of selecting weeds, not only for its herbicidal action, but also by alternative cropping systems to which they are associated, such as tillage and cultivation of genetically modified plants (Vargas, 2003). The high intensity of use of agrochemicals, especially of the same mechanism of action, speeds up this selection.

Sugarcane has selected several species in the last year due to the change of its collection system (Dias et al., 2007). Added to this, in the culture, chemical control has a relevance of 58% with a great use of photosystem II inhibiting herbicides, which may also select certain resistant populations (Franconere, 2010).

Intraspecific selection is possible due to the high genetic variability that occurs within populations of weeds. Some species considered susceptible to certain herbicides have differentiated individuals that can survive the recommended doses, maintaining growth and reproductive capacity. These individuals, called tolerant biotypes, produce propagules and increase their participation in the diaspore bank. Thus, there is a selection of differentiated populations resistant to the herbicides applied (Gazziero, 1998).

Among the main weeds in which this resistance development process has been reported in sugarcane in Brazil, crabgrass (*Digitaria* spp.) stands out, a plant belonging to the family Poaceae (Gramineae) and the genus *Digitaria*, which includes about 300 species distributed in tropical and subtropical regions (Canto-Dorow and Longhi-Wagner, 2001). These plants are aggressive because they allow its fine stalks to spread with bud sprouting forming new tussocks, besides developing faster than sugarcane in dry periods (Azania et al., 2008; Teixeira, 2011).

Due to the morphological similarity in species of the genus *Digitaria*, the correct taxonomic identification of these plants at the species level becomes difficult and this is a very important point to be considered in inconsistent results obtained in the control of plants of the genus in São Paulo sugarcane fields (Dias et al., 2007). In several regions producing sugarcane, failures in controlling crabgrass are probably no more than an incorrect identification of the species present, where ineffective controls are employed (Toledo et al., 2006).

However, one can not rule out the possibility of selection of resistant biotypes. Toledo et al. (2004) report that several herbicides, specially inhibitors of photosystem II such as tebuthiuron, diuron, hexazinone + diuron, metribuzin, ametryn, have been used in large scale and for a long time in the cane fields of Brazilian States São Paulo and Mato Grosso, with several complaints about flaws in control after the application of these products. Thus, this study has aimed to evaluate the biotypes responses to the action of different photosystem II inhibitors herbicides in studies of dose-response curves of the products ametryn and diuron.

MATERIALS AND METHODS

Crabgrass (*Digitaria horizontalis*) seeds were collected in different producing regions of sugarcane where reports about control failures of this weed with herbicides inhibitors of photosystem II were taking place. The areas selected were georeferenced. To confirm the problem, seeds of this species were acquired from Brazilian company Agro Cosmos (Cosmos Agrícola Produção e Servicos Rurais Ltda.) originating from areas without the application of herbicides and therefore with no reports of failures in the control.

The identification of the species of crabgrass was made by collecting plants that had a fully open inflorescence and mature ears. The inflorescences were separated from the plants and placed in paper bags, properly labeled and identified through observations of morphological characteristics of spikelets, according to the methodology developed by Canto-Dorow (2005).

The biotypes used were collected in the Brazilian state of São Paulo, being P1 (21º10'13,54" S; 48º52'29,07" W – Ribeirão Preto), P2 (21º05'23,07" S; 47º53'23,34" W – Cruz das Posses, a district of Sertãozinho) and P3 (22º29'20,01" S; 47º12'55,51" W, Agro Cosmos – Engenheiro Coelho).

Plastic pots with 3L capacity were filled with 2.8 L of substrate consisting of soil mixture and PlantMax® at the ratio of 3:1, respectively. For this purpose, surface sandy loam texture soil sample was used (Dystrophic Red Latosol – DRL), previously dried in the shade and sieved, with the following chemical characteristics: 5.1 of pH (H_2O); 8.5 mg dm^{-3} of P (resin); V (%) of 41%; and 0.115, 0.75, 0.35, 2.35, 1.215 and 3.56 $cmol_c$ dm^{-3} of K^+, Ca^{2+}, Mg^{2+}, H^+Al^{3+}, SB and T, respectively.

Crabgrass spikelets were randomly sown on the surface of the substrate and up to 20 seeds per container were deposited. A thin layer of soil was added to cover the seeds, provide better germination, and for protection against external enemies. The pots were placed in a greenhouse at monitored temperatures (35/25 ºC day/night) and photoperiod (approximately 14 hours).

On the same days when sowing was carried out, applications of both herbicides inhibitors of photosystem II were made, ametryn (Gesapax, 500 g a.i. L^{-1}) and diuron (Karmex, 800 g a.i. kg^{-1}), in preemergence of weeds for each of the three populations (P1, P2 and P3), maintaining a control free of application. A completely randomized design was used in a 10 x 3 (doses and populations, respectively) double factorial arrangement for each herbicide, with four replications, totaling two experiments with 30 treatments each. The related climatological data are shown in Table 1. The commercial products and the doses used are shown in Table 2. Herbicide doses were standardized, where X equals the commercial dose of each product in the commercial formulation, multiplications (2x, 4x, 8x and 16x) and installment of such doses (x/16, x/8, x/4, x/2).

To apply the herbicides, a knapsack sprayer at a constant pressure (CO_2) was used, coupled to a two-meter boom with four nozzle tips model XR 110.02, previously calibrated for a spray solution consumption of 200 L ha^{-1}.

Table 1 - Main climatic conditions at the time of spraying

Date	Biotype	Time	Temperature (ºC)		RH (%)	Wind (m/s)
			Air	Soil		
9/30/2009	P1	09:00	26.8	25.2	81.0	1.5
4/30/2010	P2	16:00	25.5	24.9	57.0	Absent
11/29/2010	P3	15:30	32.6	29.4	49.0	2.3

Table 2 - Herbicides and doses used to control crabgrass

Treatment	Dose (L or kg a.i. ha^{-1})								
Active ingredient	x/16	x/8	x/4	x/2	x*	2x	4x	8x	16x
Ametryn	0.15	0.31	0.62	1.25	2.5	5.00	10.00	20.00	40.00
Diuron	0.15	0.30	0.60	1.20	2.4	4.80	9.60	19.20	38.40

* Commercial dose recommended.

In the irrigation following the sowing, enough water was provided for seedling germination and development, avoiding amounts and intensities that could cause the leaching of herbicides. Irrigations throughout the test were made in the containers whenever it was visually necessary.

The evaluation period was extended for 60 days after application. In this period, assessments of visual percentage of weed control were carried out at 15, 30 and 60 days after application (DAA). Assessments followed a percentage scale from 0 to 100%. Grade 0 (zero) was for when there was no control over the weeds and 100 for when there was a total control (SBCPD, 1995). At 60 DAA, at the last evaluation, the remaining weeds in the containers were cut close to the ground, put in paper bags and dried in a forced air circulation oven at 60 °C for 72 hours. Subsequently, the dry matter mass was evaluated on a centigram accuracy balance.

The data were submitted to an analysis of variance by the F test. When significant, they had their means compared by the Tukey's test at 5% probability ($p<0.05$).

The herbicides mean doses obtained in the deployment of the degrees of freedom of the interaction of populations versus doses were submitted to a graphical analysis, applying log-logistic regressions in software Origin, following equation 1:

$$Y = \frac{a}{1+e^{-k\,(x-xc)}} \qquad \text{(eq. 1)}$$

where *Y* is the visual control or the dry matter mass of the shoots expressed as a percentage of the dry matter mass of non-treated plants; *a* corresponds to the higher control value; *k* is the line curvature of the line; and *xc* is the herbicide dose used to control 50% of the population (Kruse et al., 2006; Kajino, 2010).

The herbicide dose necessary to cause 50% or 80% of injury (I_{50} and I_{80}, respectively) and the reduction of the dry matter mass (GR_{50} and GR_{80}, respectively) were determined using equation 1. The resistance factor (RF) obtained by equation 2 was adapted in this case to determine the herbicide resistance difference among the populations. In each case, the test population was compared to the most susceptible one during the application, using values of I_{50} or GR_{50}.

$$RF = I_{50}(R)/I_{50}(S) \text{ or } RF = GR_{50}(R)/GR_{50}(S) \qquad \text{(eq. 2)}$$

where *R* represents the most resistant biotype and *S* represents the most susceptible one.

RESULTS AND DISCUSSION

Through the results of control throughout the visual evaluations, it was noted that there were significant differences in the responses of the different populations to the herbicides used, allowing to carry out the regression analyses (data not shown). At the end of the evaluation, 60 days after application (DAA), differences were significant between the ametryn doses used and the population's responses. The interaction between these factors was also significant (Table 3). These differences occurred both for evaluations of visual control as for mass reductions in the plants dry matter, compared with the control without application. Due to the interaction between factors, only the effect of doses in control and the mass reduction of dry matter of each population are going to be discussed.

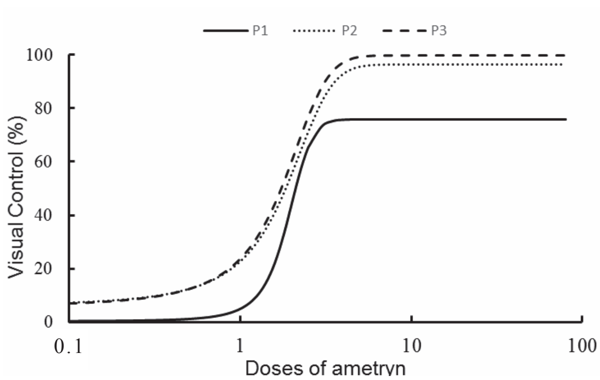
Table 3 - Summary of the analysis of variance with F values for the variables control and reduction of the dry matter mass of crabgrass in relation to the control, according to the population and ametryn dose at 60 days after the application

Factor of variance	Degrees of freedom	Control	P	Red. mass	P
Population	2	105.34 **	< 0.0001	5.96 **	0.0037
Dose	9	581.2 **	< 0.0001	341 **	< 0.0001
P x D	18	5.67 **	< 0.0001	3.71 **	< 0.0001

** Significant at 1% probability by the Tukey's test; P: populations; D: doses of the herbicide.

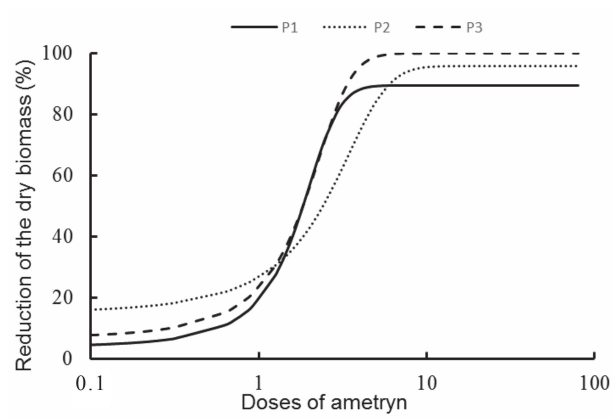
In the visual control, when analyzing the doses of ametryn and their effects on the three populations, it was noted that in P1 the plants showed higher tolerance to the herbicide than the others in all doses tested (P2 and P3). The latter had a similar behavior in response to the herbicide. Only in controls higher than 50% population P2 shall require higher herbicide rates than P3 (Figure 1).

For mass reduction of the dry matter, at low doses population P1 continued to be more tolerant. In median controls (around 50%), population P2 was more tolerant. However, for greater than 80% control, considered satisfactory, populations P1 and P2 have shown again to be more tolerant. So much so that population P1 did not have its dry matter mass reduced to levels higher than 90%. It is noteworthy that the differences among visual assessments and dry matter mass are due to the fact that even with satisfactory control (for example, for P3), there is a plant mass that increases the values observed, downplaying the differences of visual control observed (Figure 2).



P1 – plants from Ribeirão Preto; P2 – Cruz das Posses and P3 – Engenheiro Coelho.

Figure 1 - Percentage of visual control (%) on a logarithmic scale of different populations of crabgrass with the application of ametryn in ten different doses, 60 days after the application.



P1 – plants from Ribeirão Preto; P2 – Cruz das Posses; and P3 – Engenheiro Coelho.

Figure 2 - Percentage of dry matter mass reduction of different populations of crabgrass with the application of ametryn in ten different doses, 60 days after the application.

Confirmation of these different responses can be explained by the values of the RFs calculated. In the visual inspection for 50% of control, it was noted that population P1 required higher concentration of the herbicide. These values were 1.13 and 1.20 times higher than those for populations P2 and P3, respectively. The value of 80% was not calculated because P1 has not reached this control (Table 4; Figure 1).

Table 4 - Parameters of the log-logistic equation used to calculate the concentration of ametryn herbicide required to 50 and 80% of control or reduction of the dry matter mass (I and GR, respectively) of populations of crabgrass

Population	$R^{2(1)}$	a	x_c	K	I_{50}	$RF^{(2)}$	I_{80}	RF
Control (%)								
P1	0.95	75.87	1.05	3.00	1.05	1.00	–	–
P2	0.97	96.26	0.92	1.48	0.92	1.13	1.44	1.00
P3	0.99	99.83	0.87	1.59	0.87	1.20	1.31	1.09
Dry matter mass in relation to the control (%)								
P1	0.97	89.41	0.91	1.82	0.91	1.32	1.44	1.56
P2	0.89	95.84	1.21	0.73	1.21	1.00	2.25	1.00
P3	0.97	99.77	0.91	1.42	0.91	1.32	1.40	1.60

(1) Coefficient of determination; (2) Resistance factor. Regression: Y = a/1 + e (-k (x-xc)), where Y is the visual control or the dry matter mass of the shoots expressed as a percentage of the dry matter mass of non-treated plants; a corresponds to the higher control value; k is the line curvature of the line; and xc is the herbicide dose used to control 50% of the population.

To reduce 50% of the dry matter mass, P2 required 1.32 times more herbicides than populations P1 and P3. In the reduction of 80%, 1.56 and 1.60 times more herbicides were necessary comparing to populations P1 and P3, respectively (Table 4).

It is noted therefore that plants showed differential susceptibility to ametryn. Populations P1 and P2 showed more difficulty to control compared to P3. RF values observed were low (less than 2) and varied between the control and the dry matter mass of the plants.

Therefore, even with such low levels of resistance of less than 2, there are indications that there will be resistance to ametryn in population P1. The recommended dose of the product, ranging from 2.5 to 4.0 kg a.i. ha^{-1}, has satisfactorily controlled population P3. The same was not true for P1: with 2.5 kg a.i. ha^{-1}, this population control was 68.75. With 8.0 kg a.i. ha^{-1}, population P1 was controlled in 70%. And with 20.0 kg a.i. ha^{-1} (a dose five times higher than the maximum recommended) in 75%.

This resistance could be linked to different resistance mechanisms, including those generated by enzymatic modifications, such as mutations in the gene psbA, found in *Poa annua*. There may still be mechanisms that are not a target for resistance, such as detoxification of the herbicide, identified in *Abutilon theophrasti*, or problems of uptake and translocation of the herbicide also observed in *P. annua* (Yuan et al., 2007; Perry et al., 2012; Svyantek et al., 2016). Other species have similar levels of resistance to inhibitors of photosystem II (Perez-Jones et al., 2005; Ribeiro et al., 2008; Carvalho et al., 2012; Heap, 2016). The presence of these mutations should be investigated in this species, because most of the time it is also responsible for generating resistance to other herbicides in the same mechanism of action (Yu et al., 2013).

For diuron herbicide, population responses to the different doses applied were also varied. At the end of the period assessed (60 DAA), it was noted that both the populations as the herbicides obtained different responses of control and reduction of dry matter mass, as well as the interaction between these factors (Table 5).

Table 5 - Summary of the analysis of variance with F values for the variables control and reduction of the dry matter mass of crabgrass in relation to the control, according to the population and diuron dose at 60 days after the application

Factor of variance	Degrees of freedom	Control	P	Red. mass	P
Population	2	4.27*	0.0168	10.64**	< 0.0001
Dose	9	1011.67**	< 0.0001	227.29**	< 0.0001
P x D	18	2.77**	0.0008	2.54**	0.0020

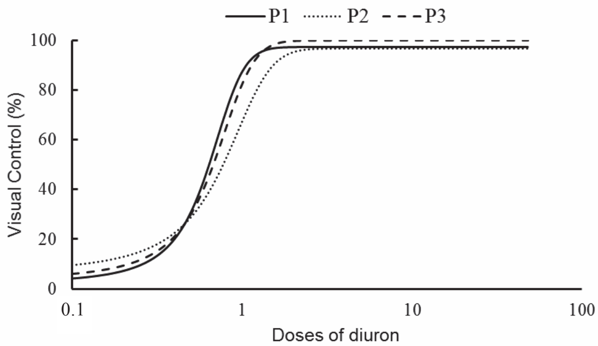
*, ** Significant at 5% and 1% probability, respectively; P: populations; D: doses of the herbicide.

With regard to the control, it was noted that only the control of populations P2 and P3 differed, with P2 being more sensitive than P3 (Figure 3). To reduce the dry matter mass, on average population P2 was less sensitive than P1 and P3, but these averages changed according to the dose. The regression for plants control showed that population 2 (P2) is more tolerant than the others when the control is between 50% and 90%. Thereafter, they resemble diuron in their susceptibility (Figure 3), (Table 6).

This difference may be best observed in the data for the dry matter mass (Figure 4). Population P2, originating from Cruz das Posses, showed higher tolerance to different doses of diuron.

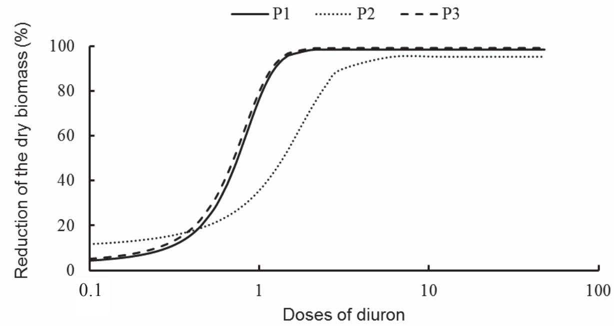
As for RF, for 50% of control of the populations, 1.22 and 1.14 times more herbicides were needed in the control of populations P1 and P3, respectively, in relation to P2. For 80% these amounts were 1.35 and 1.26%. As for the reduction of the dry matter mass relative to the control, it was found that population P2 demanded 1.86 and 1.94% more of the herbicidal dose than the dose required to control 50% of populations P1 and P3, respectively (Figures 3 and 4).

When the value considered was 80%, these values went to 1.25 and 2.34 times more herbicide. In this case, it can be said that population P2 showed a significant difference in the need of herbicide



P1 – plants from Ribeirão Preto; P2 – Cruz das Posses; and P3 – Engenheiro Coelho.

Figure 3 - Percentage of control of different populations of crabgrass with the application of diuron in ten different doses, 60 days after the application.



P1 – plants from Ribeirão Preto; P2 – Cruz das Posses; and P3 – Engenheiro Coelho.

Figure 4 - Percentage of dry matter mass reduction of different populations of crabgrass with the application of diuron in ten different doses, 60 days after the application.

for its control. However, one can not say that it is a case of agronomic resistance because the recommended dose of the product, which is between 1.6 and 3.2 kg a.i. ha^{-1}, has satisfactorily controlled both populations (Figure 4).

Although not showing resistance to diuron in this study, finding the difference in susceptibility among plants is important, since problems may occur in the field, such as the application of underdoses that are going to select individuals and consequently more resistant populations, reducing the effectiveness of control over of time (Neve et al., 2014).

When searching the Integrated Weed Management, one should take advantage of the fact that the reproduction of the species is more restricted at long distance compared to others, such as sourgrass (Santi, 2014) and perform fast control of populations that are more demanding of herbicide to be controlled.

Among the measures to be taken to control these populations, such as P1 and P3, is the use of combinations of herbicides, such as, ametryn + clomazone or other products, such as metribuzin, highlighted in other studies (Carvalho et al., 2010; Nicolai et al., 2010). The improved efficacy of other herbicides can be explained by the residual higher amount of each product (Inoue, 2007).

It may be concluded that the populations present differential susceptibilities for the herbicides inhibiting FSII and the population in Ribeirão Preto may be resistant to ametryn. The population originated in Ribeirão Preto is more tolerant to ametryn and the one in Santa Cruz das Posses to diuron.

Table 6 - Parameters of the log-logistic equation used to calculate the concentration of herbicide diuron required for 50 and 80% of control or reduction of dry matter mass (I and GR, respectively) of populations of crabgrass

Population	$R^{2(1)}$	a	x_c	k	I_{50}	$RF^{(2)}$	I_{80}	RF
Control (%)								
P1	0.99	97.47	0.50	5.86	0.50	1.22	0.71	1.35
P2	0.97	96.78	0.61	3.36	0.61	1.00	0.96	1.00
P3	0.99	100.01	0.53	4.77	0.53	1.14	0.76	1.26
Dry matter mass in relation to the control (%)								
P1	0.98	98.64	0.59	4.80	0.59	1.86	0.83	2.25
P2	0.91	95.31	1.07	1.62	1.07	1.00	1.87	1.00
P3	0.99	99.29	0.56	4.78	0.56	1.94	0.80	2.34

(1) Coefficient of determination; (2) Resistance factor. Regression: Y = a/1 + e (-k (x-xc)), where Y is the visual control or the dry matter mass of the shoots expressed as a percentage of the dry matter mass of non-treated plants; a corresponds to the higher control value; k is the line curvature of the line; and xc is the herbicide dose used to control 50% of the population.

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