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Article

SENSITIVITIES OF *Urochloa decumbens* PLANTS TO GLUFOSINATE

Sensibilidade de Plantas de Capim-Braquiária ao Glufosinate

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ABSTRACT - This study aimed to assess the response of *Urochloa decumbens* plants to different doses of glufosinate ammonium, and the sensitivity of plant population to the herbicide. Two studies were conducted, both in greenhouse and repeated at different times. In the dose-response analysis, two experiments were conducted using seven doses of the glufosinate ammonium (0, 50, 100, 200, 400, 800, and 1,600 g a.i. ha^{-1}) with four replications each. In the analysis of sensitivity levels of *U. decumbens* to herbicide, 44 plants were sprayed with a dose of 200 g a.i. ha^{-1} of the herbicide. Tissue ammonium content was determined, and injury percentage was visually assessed. Experiment data were converted to mg of ammonium per kg^{-1} of fresh mass and submitted to analysis of variance, and treatment means were compared by t test ($p\leq0.10$). Control of *Urochloa decumbens* plants by glufosinate might be associated with plant tissue ammonia content, which increased as a function of herbicide application, but not linearly as a function of dose rate. Variability existed in the ammonium content among the individuals of the population of *U. decumbens*.

Keywords: ammonia, glutamine synthetase, weed, variability.

RESUMO - Este trabalho objetivou identificar a resposta de plantas de capim-braquiária a diferentes doses de amônio glufosinate e a sensibilidade de uma população de plantas ao herbicida. Foram realizados dois estudos, ambos implantados em casa de vegetação e repetidos em diferentes momentos. No estudo de curva de dose-resposta, realizaram-se dois experimentos, tendo como tratamentos sete doses de glufosinate (0, 50, 100, 200, 400, 800, 1.600 g i.a. ha^{-1}), com quatro repetições. No que se refere à variação da sensibilidade de capim-braquiária ao herbicida, 44 plantas foram pulverizadas com a dose de 200 g i.a. ha^{-1} do herbicida. Realizaram-se avaliações visuais de fitointoxicação e análises do teor de amônia nos tecidos foliares. Os dados obtidos nos experimentos foram convertidos em mg de amônia kg de massa fresca^{-1} e submetidos à análise de variância, e as médias de tratamento, comparadas pelo teste t ($p\leq0,10$). O controle de plantas de capim-braquiária pelo glufosinate pode ser correlacionado ao teor de amônia nos tecidos vegetais, que aumentou em função da aplicação do herbicida, porém não de forma linear em função da dose. Houve variabilidade quanto ao teor de amônia entre indivíduos de uma população de capim-braquiária.

Palavras-chave: amônia, glutamina sintetase, planta daninha, variabilidade.

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INTRODUCTION

The signal grass (*Urochloa decumbens* Syn. *Brachiaria decumbens*) is originally from Africa and currently distributed in almost all tropical and subtropical regions of the world (Arroyave et al., 2011). This species reproduces by seed and also vegetatively via creeping stems, and it is characterized as a perennial rustic plant, capable of withstanding soil acidity, low fertility, and water deficit. Its seeds present initial dormancy, resulting in irregular germination and viability for up to eight years in the soil (Kissmann, 1997). In Brazil, this species was introduced as forage and, due to its good adaptability, acclimated to acidic soils and low fertility, it became one of the main forage species of the Brazilian *cerrado* (Da Silva et al., 2012).

In addition to its suitability to be used as forage, signal grass is also considered important for the formation of dry biomass for traditional systems of production (Timossi et al., 2007) and/or for spontaneously occurring in agricultural systems, often causing serious competition problems, because of its high aggressiveness and difficult control (Kissmann, 1997), and/or for its allelopathic effects. Thus, even in areas where it has been introduced as forage, signal grass can stand out as an important weed, especially if the area is further destined to other activities. This situation can be commonly found in areas of expansion of eucalyptus (Toledo et al., 2000) and sugarcane (Kuva et al., 2003) in areas that previously had signal grass, and where seed bank on the ground is high. However, in many other crops, such as soybean (Nunes et al., 2009) and maize (Constantin et al., 2008), for instance, this species has been reported to cause finantial losses.

When signal grass occurs undesirably in crop systems, it is considered a weed, and control measures need to be taken. In many productive systems, glyphosate is the most widely used herbicide; However, due to increasing issues of resistance cases, other herbicides have gained importance, among which, glufosinate.

Glufosinate ammonium ([ammonium-DL-homoalanine-4-yl (methyl)]) is a broad-spectrum herbicide used to control large variety of weeds in post-emergence periods (Lydon and Duke, 1999). It is an ammonium salt that acts as a robust inhibitor of glutamine synthetase (GS), and it is the only commercially available herbicide capable of inhibiting the activity of this enzyme (Carbonari et al., 2016). Such inhibition results in reduced production of amino acid glutamine, along with several other amino acids in the plant, and the rapid accumulation of intracellular ammonium levels associated with the rupture of the chloroplast structure, blocking the electron transport chain (Tan et al., 2006; Dayan and Zaccaro, 2012). Therefore, determining ammonium accumulation is an interesting tool to evaluate the level of herbicide stress in the plant (Silva et al., 2016).

Most *Brachiaria* species reproduce by apomixis, wherein the embryo develops from mitotic division of a somatic cell, yielding fertile seeds, without combining the reproductive core pollen grain with the egg cell, resulting in a progeny composed of individuals that are clones of the mother plant (Valle and Savidan, 1996), making it difficult to increase the genetic variability of this genus. On the other hand, the broad natural genetic variability of weeds is one of the main characteristics that allow the adaptation and survival of these species to diverse environmental conditions and different ecosystems.

In the literature, there are few works on the sensitivity of plants of the genus *Brachiaria* to herbicide application (Petter et al., 2011; Correia et al., 2012), especially to glufosinate herbicide, considering the low genetic variability of species of this genus. For this reason, studies that seek to understand the sensitivity of plant populations to herbicides, as well as their effect over time, are important for the development of weed management strategies, and also for predicting the impact of adopting such weed management practices on the infesting population.

Therefore, this study aimed to assess *Urochloa decumbens* plant response to different doses of glufosinate, as well as the susceptibility variation of a population to the herbicide.

MATERIAL AND METHODS

This research included two studies, repeated at different times, where the first was a dose-response analysis of signal grass plants to glufosinate, and the second a plant sensitivity analysis to the herbicide, both were conducted in greenhouse under 27 °C ± 2 °C and natural light.

For such analyses, *Urochloa decumbens* seeds were sown in pots containing approximately 115 mL volume, filled with commercial substrate. Ten days after emergence (DAE), thinning was performed, with only one plant per pot. For applications of glufosinate (Finale SL 200 g i.a. L^{-1} Bayer CropScience Ltd.), we used a stationary spray in enclosed room, equipped with a spray bar with four tips XR 110.02 (Teejet, Jacto Máquinas Agrícolas SA, Pompeia, SP, Brazil), spaced at 0.5 m and positioned at 0.5 m on top of plants, using a spray volume of 200 L ha^{-1}, under constant pressure of 150 pKa, pressurized by compressed air.

Dose-response study in Signal grass

In this present work, two experiments were carried out, both in a completely randomized design with four replicates. The first one aimed to quantify the ammonia content present in the foliar tissues of signal grass plants as a function of glufosinate dose rates; The second experiment aimed to verify the level of control of plants, also in function of herbicide dose rate. In both experiments, treatments were used as herbicide glufosinate seven doses (0, 50, 100, 200, 400, 800, 1,600 g a.i. ha^{-1}).

Signal grass seeds came from the city of Engenheiro Coelho in the Sao Paulo state, Brazil (22°48'S, 47°20'W), and were submitted to the sowing and thinning procedures previously described. At 30 DAE, treatments were applied. In the first experiment, at 2 DAA, the determination of the ammonia content in the plant tissues was performed; in the second, visual evaluations of injury levels were done at 0, 3, 7, 14 and 21 DAA. At the end of the evaluations, a duplicate of both experiments was performed.

Analysis of signal grass sensitivity to glufosinate

In this present research, the same procedure of sowing and thinning in pots was performed, as previously described, using the same batch of seeds from the previous study. After 30 DAE, 44 plants were sprayed with a dose of 200 g a.i. ha^{-1} herbicide, four plants were maintained without application. The dose of 200 g a.i. ha^{-1} was identified in the previous study to be sufficient to cause injury symptoms and accumulation of ammonia in leaf tissue of signal grass.

At 2 DAA, we determined the ammonia content in the leaves of signal grass plants, and a duplicate of the experiment was performed.

Assessment of analyses

In the dose-response and sensitivity studies of signal grass plants to glufosinate, we determined ammonia content in leaf tissues, according to the protocol below.

Ammonia was extracted from fresh leaf tissue of plants at 2 DAA, immediately after harvesting. Samples were placed in falcon tubes containing 50 mL of water acidified with hydrochloric acid (pH 3.5), which were placed in an ultrasonic bath for 60 minutes. The ammonia content of the solution was determined by spectrophotometry according to methods in the literature (Wendler et al., 1990; Dayan et al., 2015), using a spectrophotometer (Cintra 40, GBC Scientific Equipment Ltd.) of 630 nm wave reading.

In the dose-response study, assessments of injury levels, 0, 3, 7, 14 and 21 DAA were made using visual scale of scores ranging from 0 to 100, where 0 is the total absence of symptoms and 100 the death of plants (SBCPD, 1995).

Data analysis

The analyses of experimental sets showed that the effects of experiments and the treatment-experiment interaction were not significant, allowing the joint analysis of them.

Data accumulation of ammonia analysis, the obtained dose response study experiments were converted to mg kg fresh $weight^{-1}$ ammonia and subjected to analysis of variance, with the treatment means with the aid of comparison t test (p≤0.10). The level of significance was determined for the contrasts between the control treatment and the others, with the use of the t distribution.

As there was a significant correlation, the adjusted Mitscherlich nonlinear regression model was fitted:

$$F = 100\left[1 - 10^{(-c(X+b))}\right]$$

where *b* and *c* correspond to the parameters of the equation. The lateral displacement of the curve corresponds to the parameter *b*, and the concavity of the curve, to the parameter *c*.

In the study of sensitivity to glufosinate in the first generation, the Gompertz model was adjusted, following procedures adapted by Velini (1995).

$$F = e^{\left[a - e^{(-b - c*X)}\right]}$$

where *a*, *b* and *c* correspond to the parameters of the equation. The maximum model asymptote is represented by "and"; the displacement of the curve along the x-axis, by the parameter *b*; and the slope or concavity of the curve in relation to the accumulated frequency, by parameter *c* (Velini, 1995). For better visualization, we chose to present the non-accumulated frequency, which corresponds to the first derivative of the model, according to the equation:

$$F = c * e^{(a - b - c*X - e^{(-b - c*X)})}$$

Also based on the Gompertz model, the position measurements (mode, mean and median) and dispersion (coefficient of variation) of the analyzed data were determined. The accuracy of the data adjustment in the Gompertz model was evaluated by the coefficients of determination (R^2) of the equations.

The analyzes were carried out with the aid of Statistical Analysis System (SAS, portable version 9.2.1), and the graphs were elaborated by Sigmaplot version 12.0.

RESULTS AND DISCUSSION

Dose-response study in Signal grass

In this present research, two experiments were performed to analyze the ammonia content in plant tissues, and two others to assess injury, and showed to be very similar. The analysis of variance showed no significant differences between the two experiments, and there was no difference between the evaluated epochs. Thus, a new analysis was performed, considering data altogether, according to Table 1.

With this new analysis, the contrasts between treatments with application of glufosinate herbicide and the control indicated that there was a difference between them. Plants not receiving application, and those which have been applied in doses of 50 g a.i. ha^{-1} glufosinate, ammonia had significantly lower levels in tissues (p≤0.10) relative to the other; although there was an increase in the level with the application of this dose, it did not differ from the control. Larger accumulations were observed at doses from 100 g a.i. ha^{-1}, indicating that accumulation can be performed in applications related to treatment, but without this linear correlation. Furthermore, the higher content of ammonia 611.03 mg kg^{-1} of fresh weight was also found with the application of the highest dose: 1,600 g a.i. ha^{-1}.

Regarding the correlation of the injury percentage with the ammonia content in the leaf tissues, it was possible to adjust the nonlinear regression model of Mitscherlich. It was found, therefore, that plants with ammonium levels near or above 180 mg ammonia kg fresh $weight^{-1}$ corresponded to the injury level of 100% at 21 DAA (Figure 1).

With the action of the glufosinate herbicide applied to plants, the enzyme glutamine synthetase is inhibited and thus fails to exert its function and convert the ammonia and glutamate

Table 1 - Analysis of variance of ammonia content in plant tissue, as a function of herbicide glufosinate ammonium dose rates applied to signal grass plants

Dose rates (g a.i. ha^{-1})	Ammonia content (mg kg fresh $weight^{-1}$)	F	P Value
0	19.42	-	1
50	72.25	0.49	0.488
100	148.87	2.94	0.094
200	170.10	3.98	0.052
400	408.13	26.50	<0.001
800	428.93	29.42	<0.001
1,600	611.03	61.39	<0.001
F treatments		16.78	<0.001
F experiments		0.09	0.762
F (treatments x experiments)		0.55	0.37
LSD (t $p \leq 0.10$)	127.00		
VC (%)	56.87		

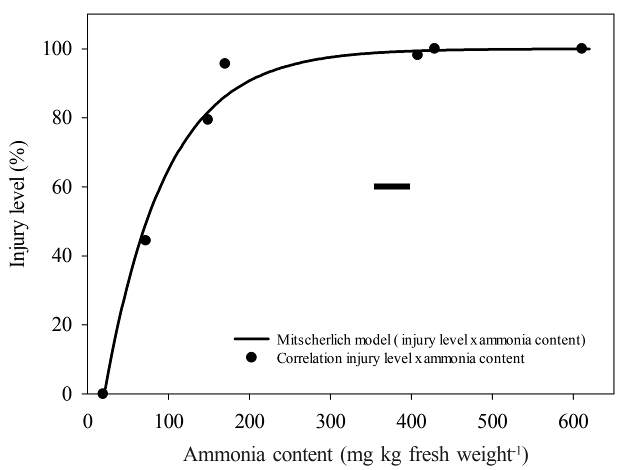

Figure 1 - Correlation injury level due to ammonia content in leaf tissue, analysis of signal grass plant ao amonio glufosinate. dose-response to glufosinate ammonium.

substrates to glutamine. Moreover, ammonia accumulation in the tissues typically occurs, and it can be quantified (Wendler et al., 1990) and further used as an indicator for the correlation of the performance of the herbicide by several studies in the literature (Wild et al., 1987; Carbonari et al., 2016; Silva et al., 2016).

Analysis of signal grass sensitivity to glufosinate

In order to observe the behavior of several individuals of a signal grass population before glufosinate application, the herbicide sensitivity study was performed. The non-linear Gompertz equation model was adjusted with the data obtained, with the parameter estimates and the position and dispersion measurements presented in Table 2.

It was possible to verify, according to the model adjusted in this study (Figure 2), the population variability of the two experiments to the application of glufosinate. The plants had 88 clustered contents ranging from about 0 to 700 mg ammonia kg fresh $weight^{-1}$, demonstrating that despite reproduce by apomixis probably seeds were from different clones.

The measures of position, mean, median, and data trend, shown in Table 2, are different from each other, and the mean and median values are higher than the modal value. Such data justify the positive asymmetric distribution found in the non-accumulated frequencies curve, corresponding to the first derivative of the Gompertz model (Figure 2). Thus, it was possible to identify that the curve showed a tendency to rise rapidly and to descend more slowly (Araldi et al., 2013).

Our study could show the differentiation within the same population, allowing to identify groups of individuals quite differentiated. In a study of *Convolvulus arvensis*, it was found that a single population contained five biotypes with different levels of sensitivity to glyphosate, showing levels of visual injury ranging from about 30 to 100%, using scale from 0 to 100% (DeGennaro and Weller, 1984).

The differences observed in the population of signal grass plants may be related to several factors, among them the plant phenotype, the individual morphological characteristics of the leaves and even the different amounts of the deposited herbicide. This fact can be justified by foliar morphology, which in a way hinders the deposition of herbicides. According to Silva et al. (2007), the morphology of the plant directly influences the amount of herbicide deposited. Among the aspects related to morphology, the angle or orientation of the leaves in relation to the spray jet, as well as specialized structures, such as trichomes, stand out.

Table 2 - Estimates of parameters, position measures and dispersion of Gompertz models adjusted for the data obtained in the ammonia content analysis, in first generation signal grass plants

Model		1st generation
Parameter estimates	a	4.6052
	b	-2.8703
	c	0.0165
Average		215.15
Medium		196.17
Mode		173.96
R^2		0.9985
VC (%)		41.55

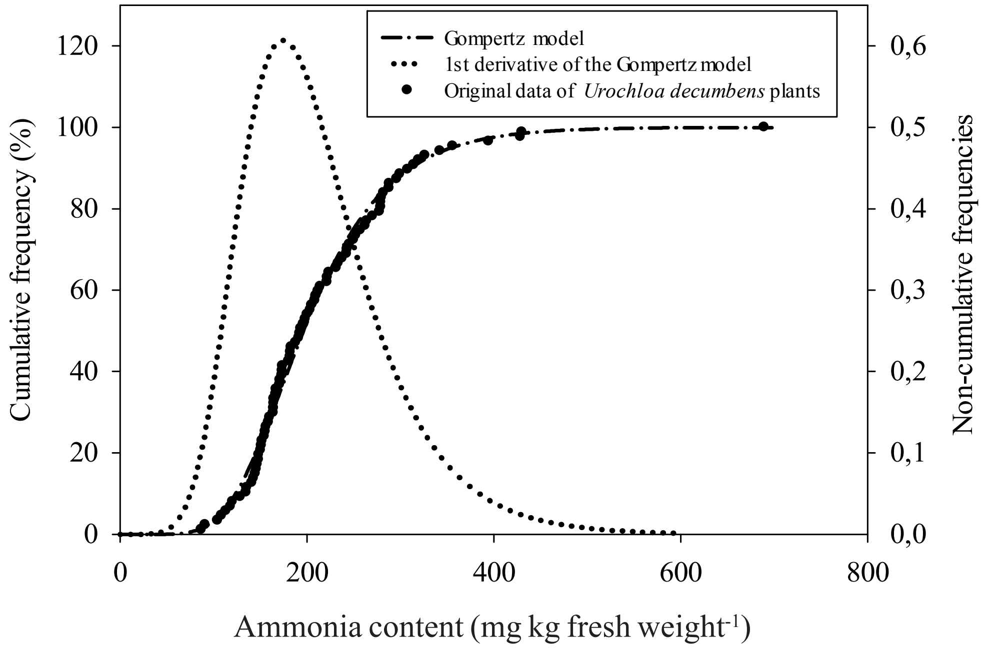

Figure 2 **-** Cumulative Frequency (%) of plants in the population and non-cumulative frequencies corresponding to the first derivative of the Gompertz model for the dispersion of ammonia content from the analysis of signal grass plant sensitivity to glufosinate ammonium.

Nevertheless, there is a possibility that the behavior is related to the genetic variability of the population. Although not selective, glufosinate may cause different sensitivity responses in weeds, mainly due to their wide genetic variability. The causes can be explained by differences in translocation, uptake and metabolism (Pline et al., 1999; Skora-Neto et al., 2000; Everman et al., 2009).

The existing genetic variability of a population is the result of the natural evolution process of the species, which derives mainly from mendelian variation, interspecific hybridization, and polyploidy (Winkler et al., 2002). It is unclear how these processes contribute to herbicide resistance and, more specifically, how they could contribute to the development of resistance to glufosinate in weed populations susceptible to this herbicide.

The control of signal grass plants is associated with the glufosinate dose rate applied and, similarly with the accumulation of ammonia in plant tissues. Plant sensitivity to this herbicide may vary naturally within its population.

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