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VARIATION IN THE SENSITIVITY OF WANDERING JEW PLANTS TO GLUFOSINATE AMMONIUM[1]

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ABSTRACT - This study aimed to identify the response of wandering jew *(Commelina benghalensis* L.) plants to different doses of glufosinate ammonium and the sensitivity of plants populations to the herbicide. Two studies were conducted, both in a greenhouse, and were repeated at different times. In the first study, two experiments were conducted to examine the dose-response curve using seven different doses of the glufosinate ammonium herbicide (0, 50, 100, 200, 400, 800, and 1600 g a.i. ha^{-1}) with four replicates each. In the second study, which examined the range in sensitivity of wandering jew plants to glufosinate ammonium, 26 plants were sprayed with a dose of 200 g a.i. ha^{-1} herbicide. Visual assessments of percent injury and measurements of leaf tissue ammonium content were conducted. The use of untreated wandering jew control plants allowed for the correlation of glufosinate ammonium treatment with the ammonium concentrations in treated plant tissues; the ammonium concentration increased as a function of herbicide application, albeit not linearly with the dose. Ammonium content varied among individuals of the wandering jew plant population.

Keywords: Ammonia. *Commelina benghalensis* L.. Glutamine synthetase. Weed.

VARIAÇÃO DA SENSIBILIDADE DE PLANTAS DE TRAPOERABA AO AMÔNIO GLUFOSINATE

RESUMO - Esse trabalho teve como objetivo identificar a resposta de plantas de trapoeraba (*Commelina benghalensis* L.) 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 primeiro, referente à curva de dose-resposta, realizou-se dois experimentos, tendo como tratamentos sete doses do herbicida amônio glufosinate (0; 50; 100; 200; 400; 800; 1600 g i.a. ha^{-1}), com quatro repetições cada. Já no segundo, referente à variação da sensibilidade da trapoeraba ao amônio glufosinate, 26 plantas foram pulverizadas com a dose de 200 g i.a. ha^{-1} do herbicida. Realizou-se avaliações visuais de injúria e análises do teor de amônia nos tecidos foliares. O controle de plantas de trapoeraba pelo amônio glufosinate pode ser correlacionado ao teor de amônia nos tecidos vegetais, que aumenta 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 trapoeraba.

Palavras-chave: Amônia. *Commelina benghalensis* L.. Glutamina sintetase. Planta daninha.

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INTRODUCTION

The glufosinate ammonium is a synthetic version of phosphinothricin, a bialaphos degradation product produced by the bacterial species *Streptomyces viridochromogenes* and *S. hygroscopius* (DAYAN, DUKE, 2014). It is a non-selective herbicide recommended for the control of a broad spectrum of weeds that has been used as an alternative to glyphosate, especially when reactive management has been established (after the onset of resistance; BRUNHARO; CHRISTOFFOLETI; NICOLAI, 2014). Glufosinate ammonium acts by inhibiting the enzyme glutamine synthetase (GS) that is involved in ammonium assimilation and nitrogen regulation in plants (SUNDAR; SAKTHIVEL, 2008).

As a consequence of GS inhibition, glutamine synthesis via the glutamate pathway is blocked, resulting in a reduction in the assimilation of ammonium, which is accumulated in the leaves of treated plants (CARBONARI et al., 2016). This herbicide may also affect the synthesis of some amino acids, increase the production of free radicals, and block photosynthesis (CARVALHO, 2013). Visual symptoms, including rapid chlorosis and necrosis, appear rapidly after glufosinate ammonium application and cause plant death within a few days. The rapid accumulation of high ammonia levels caused by inhibition of GS enzyme activity causes cell destruction (SENSEMAN, 2007). Therefore, increased ammonia levels are used as an indicator of glufosinate performance (PETERSEN; HURLE, 2001).

Commelina benghalensis L., commonly known as wandering jew, is one of the most important weeds in agricultural areas in Brazil where it causes productivity losses, hinders harvesting operations, and acts as a host for a range of viruses and nematodes (ZAPPAROLI et al., 2007). This species has been prominent because of its aggressive characteristics, including aerial and subterranean seed production, dormant seeds, and reproduction under water stress conditions (WEBSTER; GREY, 2008).

The stems of the wandering jew form several branches that easily develop into new plants when cut. Rhizomes increase the ability of weeds, like wandering jew, to persevere in agricultural areas when combined with their underground fruiting bodies (BLANCO, 2010). Moreover, glyphosate-resistant populations of wandering jew have been evolutionarily selected through their differential tolerance to glyphosate (MACIEL et al., 2011) that may be caused by differences in their glyphosate absorption and metabolism rates (MONQUERO et al., 2004).

In this context, given the control difficulties associated with the use of the glyphosate herbicide, glufosinate ammonium represents a promising alternative that may be used to control both resistant weeds and those that are difficult to control, such as wandering jew. As tolerance to an herbicide may vary between populations of the same species (PAZUCH et al., 2013), different tissue levels of ammonia and, consequently, visual symptoms of injury, are likely to be found among plants of the same population.

In this study, therefore, we aimed to identify the response of individual wandering jew plants to different glufosinate ammonium doses and examine sensitivity to this herbicide across a plant population.

MATERIAL AND METHODS

Two studies were carried out between January and July 2014, repeated at different times. The first was a dose-response study of wandering jew plants to glufosinate ammonium, while the second was a study of the sensitivity of wandering jew plants to the herbicide. For both studies, plants were grown under a natural light source in a greenhouse at a temperature of 27°C ± 2°C.

Wandering jew seeds were sown in pots with an approximate volume of 115 mL, filled with commercial substrate. Thinning was performed 10 d after emergence (DAE) with only one plant retained per pot. A stationary sprayer was used to apply glufosinate ammonium (Finale SL, 200 g a.i. L^{-1}, Bayer CropScience Ltd.) in a closed room equipped with a spray bar with four XR 110.02 nozzles spaced 0.5 m apart and placed 0.5 m above the plants, using 200 L ha^{-1} spray volume under a constant pressure of 150 kPa and pressurized by compressed air.

Dose-response study in wandering jew plants

In this study, two experiments were carried out following a completely randomized design and four replicates each. The first aimed to quantify leaf ammonia content in wandering jew plants as a function of the glufosinate ammonium dose applied. The second experiment aimed to assess plant injury depending on the herbicide dose. In both experiments, seven doses of the glufosinate ammonium herbicide (0, 50, 100, 200, 400, 800, 1600 g a.i. ha^{-1}) were used as treatments.

In this study, the wandering jew seeds used were from a seed production area in the municipality of Engenheiro Coelho-SP (22°48'S, 47°20'W). After the previously described sowing and thinning procedures, the treatments were applied at 30 DAE when the plants had four to five expanded leaves. In the first experiment, ammonia content was assessed in plant tissues 2 d after treatment (DAT); in the second experiment, visual evaluations of injury levels were performed 0, 3, 7, 14, and 21 DAT. Both experiments were repeated at the end of the evaluations.

Study of wandering jew plant sensitivity to glufosinate ammonium

To assess variation in wandering jew plant sensitivity to glufosinate ammonium, the same seeding and thinning procedures as described previously were followed in this study using seeds from the same batch as those used in the previous experiment. At 30 DAE, when the plants had four to five expanded leaves, 26 plants were sprayed with a dose of 200 g a.i. ha^{-1} herbicide. Four control plants were not sprayed. At 2 DAT, leaf ammonia content in wandering jew plants was measured. The experiment was carried out in duplicate.

Study evaluations

In the studies examining sensitivity to glufosinate ammonium and dose-responses in wandering jew, evaluations were performed to assess the ammonia content in leaf tissues according to the following protocol.

Ammonia was extracted from fresh leaf tissue of wandering jew plants at 2 DAT, immediately after collection. Samples were placed in falcon tubes with 50 mL of water acidified with hydrochloric acid (pH 3.5) and placed in an ultrasound bath for 60 min. The ammonia content of the solution was assessed using a spectrophotometer (Cintra 40, GBC Scientific Equipment Ltd.) according to published methods (WENDLER; BARNISKE; WILD, 1990; DAYAN et al., 2015).

In the dose-response study, visual evaluations were performed at 0, 3, 7, 14, and 21 DAT to assess the injury levels using a visual scale of scores ranging from 0 to 100 in which "0" indicates the total absence of injuries and "100" represents total plant death (SBCPD, 1995).

Data analysis

The data from the ammonia accumulation analysis in the dose-response study were converted into mg ammonia kg fresh $mass^{-1}$ and subjected to analysis of variance. The means of the treatments were compared using the T-test ($p \leq 0.05$). The level of significance for comparisons between the control and the other treatments was determined using the T distribution.

The non-linear regression model of Mitscherlich (1909) was adjusted because there was a significant correlation:

$$Y = 100[1 - 10^{(-c(X+b))}]$$

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.”.

The Gompertz model (1825) was also adapted in the glufosinate ammonium sensitivity study:

$$Y = e^{[a - e^{(-b-c*x)}]}$$

Where a, b, and c correspond to the parameters of the equation. The maximum asymptote of the model is represented by the parameter “a,” 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 the parameter “c”. The non-accumulated frequency, which corresponds to the first derivative of the model according to the equation below, was chosen for improved visualization:

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

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

Analyses were performed using Statistical Analysis System software (SAS, portable version 9.2.1) and the graphics were designed using Sigmaplot version 12.0.

RESULTS AND DISCUSSION

Dose-response study in wandering jew plants

In this study, two experiments were carried out to analyze plant tissue ammonia content and two others to evaluate injury levels. The results of each replicate study were very similar, with analysis of variance showing no significant differences between the two experiments. As no significant differences were found between study times a new analysis was performed considering the data altogether.

The Mitscherlich model could be adjusted to the data on the level of injury as a function of glufosinate ammonium doses, as shown in Figure 1. The results showed that the percentage of injury increased with the dosage used at 21 DAT. Doses of 133 g a.i. ha^{-1} and 312 g a.i. ha^{-1} caused 50% and 80% injury in wandering jew plants, respectively, whereas doses of 800 g a.i. ha^{-1} or higher were sufficient to cause maximum injury.

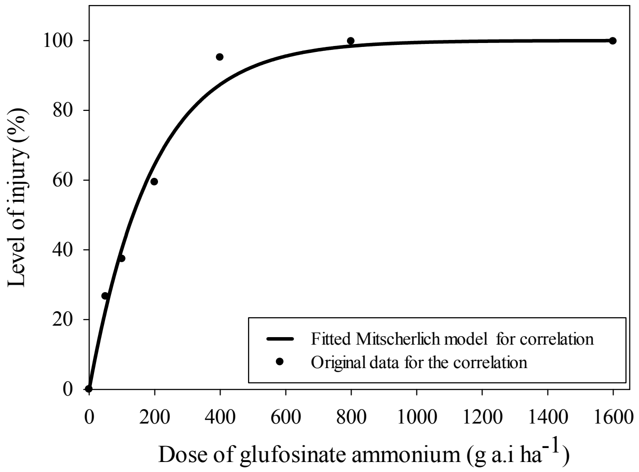

Figure 1. Correlation of the level of injury with the glufosinate ammonium herbicide dose applied to wandering jew plants in a dose-response study at 21 days after treatment.
$Y = 100[1 - 10^{(-0.00225(X-0))}]$, R^2=0.9968.

Differences between the treatments were observed when analyzing the doses of herbicide in relation to the level of ammonia accumulation in leaf tissues. Untreated plants and those treated with doses of 50 g a.i. ha^{-1} glufosinate had significantly lower tissue levels of ammonia ($p \leq 0.05$) than plants treated with larger doses (Table 1). Although the ammonia content did increase with the application of this dose, it did not differ significantly from that of the control. Significantly higher levels of ammonia accumulation were observed with doses of 100 g a.i. ha^{-1} or more, indicating that ammonia accumulation is related to treatment dosage used.

Table 1. Ammonia accumulation in the tissue of wandering jew plants exposed to different glufosinate ammonium doses in a dose-response study.

Doses g a.i. ha^{-1}	Ammonia content mg ammonia kg fresh $mass^{-1}$	F	p-value
0	13.77	-	1
50	49.35	1.05	0.312
100	85.13	4.22	0.046
200	186.68	24.76	<0.001
400	169.82	20.16	<0.001
800	162.13	18.23	<0.001
1600	221.53	35.74	<0.001
F treatments		12.06	<0.001
F experiments		0.10	0.757
F (treatment x experiments)		1.46	0.217
LSD[1] (t $p \leq 0.05$)	63.81		
CV[2] (%)	49.83		

[1]LSD: Least significant difference; [2]CV: Coefficient of variation.

Correlating the percentage of injury with the ammonia content in the foliar tissues of wandering jew plants enabled the adjustment of the data to the non-linear regression mitscherlich model, with plants with ammonia levels close to, or greater than, 250 mg kg fresh $mass^{-1}$ being those with the highest levels of injury at 21 DAT (Figure 2).

While the doses used in the study caused an increase in ammonia levels, no linearity in the correlation between the dose applied and the tissue ammonia content was observed. Application of glufosinate ammonium results in the inhibition of glutamine synthetase and a resulting increase in ammonia accumulation in plant tissues (WENDLER; BARNISKE; WILD, 1990). This accumulation can be measured and used as a performance indicator of the herbicide (WILD; SAUER; RÜHLE, 1987).

Study of wandering jew plant sensitivity to glufosinate ammonium

The sensitivity of wandering jew plants to the herbicide was examined by assessing the performance of several individuals in a population of wandering jew plants after the application of glufosinate ammonium. The non-linear Gompertz equation model was adjusted using the data obtained. The parameter estimates, position, and dispersion measurements are outlined in Table 2.

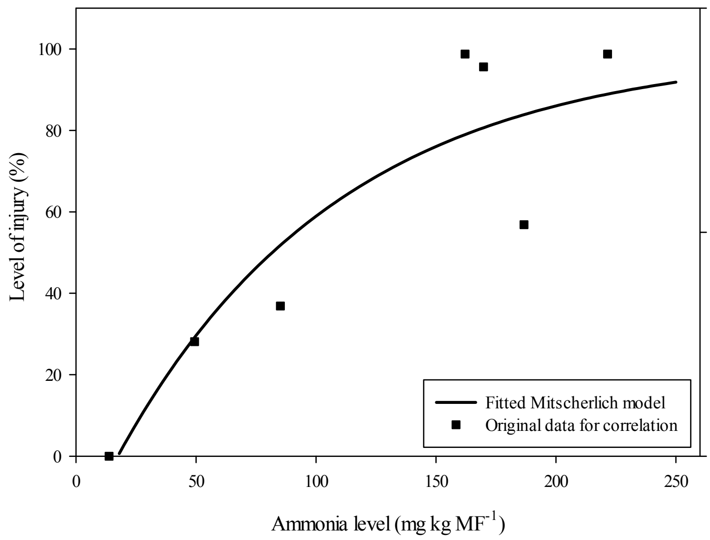

Figure 2. Correlation of injury levels at 21 days after treatment with tissue ammonia levels in wandering jew plants tested for a dose-dependent response to glufosinate ammonium.

$Y = 100[1 - 10^{(-0.00468\,(X-17.3996))}]$. R^2=0.9481.

Table 2. Estimates of the parameters, position, and dispersion measures of Gompertz model adjusted to the ammonia content data from first-generation wandering jew plants.

Model		Values
Parameter estimates	b	-1.3425
	c	0.0272
Mean		85.94
Median		62.83
Mode		49.36
R^2		0.996
CV[1] (%)		93.43

[1]CV: Coefficient of variation.

Variations within the population in terms of the response to the application of glufosinate could be assessed according to the adjusted model (Figure 3) using the diversity of ammonia content levels measured in the study plants these ranged from 0 to 250 mg ammonia kg fresh mass^{-1}.

The non-cumulative frequencies had an asymmetric distribution. The position, mean, median, and mode data measurements were different from each other, and the mean and median values were higher than the modal value. This positive asymmetric distribution (Table 2) enables the identification of greater variability between the higher levels of ammonia versus the lower levels in the frequency distribution curve, with the curves tending to rise rapidly and fall more slowly (ARALDI et al., 2013). This performance is also shown in Figure 3, with the first derivative of the Gompertz model.

The application of an herbicide, even a non-selective one such as glufosinate ammonium, may cause different sensitivity responses in weeds, mainly because of their wide genetic variability. The different sensitivities might be explained by differences in herbicide translocation, uptake, and metabolism (EVERMAN et al., 2009; MERSEY et al., 1990; PLINE; WU; HATZIOS, 1999; SKORA-NETO; COBLE; CORBIN, 2000). The genetic diversity in a population results from the natural process of evolution that is mainly caused by mendelian variation, interspecific hybridization, and polyploidy (WINKLER; VIDAL; BARBOSA NETO, 2002).

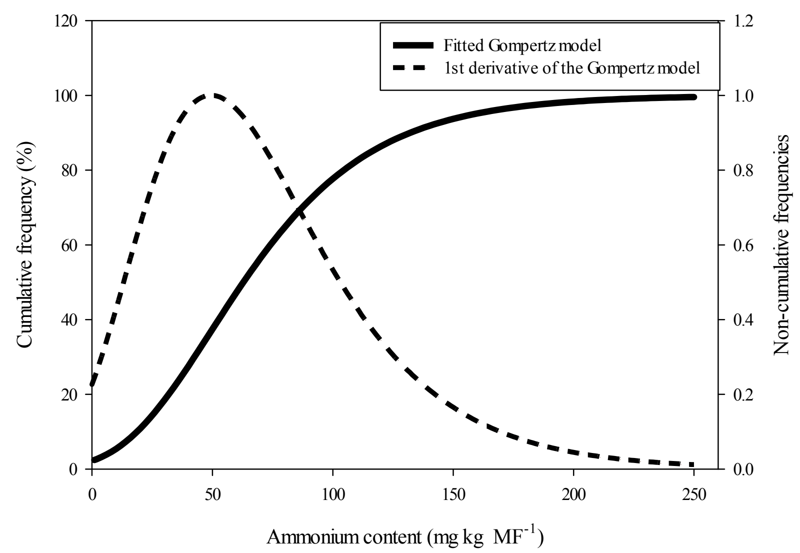

Figure 3. Gompertz model (accumulated frequency) and the first derivative of the Gompertz model (non-cumulative frequencies) for the ammonia content of leaf tissues of wandering jew plants after the application of glufosinate ammonium herbicide.

CONCLUSIONS

Wandering jew plants were satisfactorily controlled using the glufosinate ammonium herbicide. The studied wandering jew populations showed variability in the accumulation of ammonia in leaf tissues that may be correlated with the differential sensitivity of plants to glufosinate ammonium.

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