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Article

Sensitivity of Morningglory Plants and Their Progenies to Glufosinate Ammonium

Sensibilidade de Plantas de Corda-de-Viola e Suas Progênies ao Amônio-Glufosinate

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ABSTRACT - The sensitivity of weed species to herbicides is variable, and the behavior can be repeated or not in the next generation. The objective of this study was to evaluate the response of morningglory plants to different doses of glufosinate ammonium and the sensitivity of plant populations and their progenies to the herbicide. Three studies were conducted, all in a greenhouse and repeated in two periods. In the first study, two experiments were conducted to examine the dose-response curve, and the treatments were seven different doses of the herbicide glufosinate ammonium (0, 50, 100, 200, 400, 800, and 1,600 g a.i. ha^{-1}), with four replications each. In the second study, which examined the range in sensitivity of morningglory to glufosinate ammonium, 44 plants were sprayed with a dose of 200 g a.i. ha^{-1} of the herbicide. Finally, in the third study, the range in sensitivity of morningglory progenies to glufosinate ammonium was investigated; in this experiment, the progenies of seven of the previous plants were sprayed with 200 g a.i. ha^{-1} of herbicide. The ammonium contents in the tissues were measured, and percent injury were visually assessed. Ammonium content in morningglory leaves was increased more than seven times by glufosinate application and the maximum ammonium content was observed for the highest herbicide dose. Variability existed in the ammonium content among the individuals of the morningglory population; however, the behavior was not replicated in the same way in progenies. Plants survival after herbicide application allows the production of progenies with wide variability in their sensitivity to the product, regardless of the behavior verified in progenitor plants.

Keywords: ammonia, glutamine synthetase, *Ipomoea grandifolia*, weed.

RESUMO - A sensibilidade de espécies de plantas daninhas aos herbicidas é bastante variável, e o comportamento pode se repetir ou não na geração seguinte. Assim, o objetivo deste trabalho foi avaliar a resposta de plantas de corda-de-viola a diferentes doses de amônio-glufosinate e a sensibilidade de uma população das plantas e suas progênies ao herbicida. Foram realizados três estudos, todos implantados em casa de vegetação e repetidos em duas épocas. No primeiro, referente à curva de dose-resposta, realizaram-se dois experimentos, tendo como tratamentos sete doses do herbicida amônio-glufosinate (0, 50, 100, 200, 400, 800 e 1,600 g i.a. ha^{-1}), com quatro repetições cada; no segundo, de variação da sensibilidade de corda-de-viola ao amônio-glufosinate, 44 plantas foram pulverizadas com a dose de 200 g i.a ha^{-1} do herbicida; e, no terceiro, de variação da sensibilidade das progênies de corda-de-viola ao amônio-glufosinate, progênies de sete das plantas anteriores foram também pulverizadas com 200 g i.a ha^{-1} do herbicida. Realizaram-se avaliações visuais de percentual de injúria e teor de amônia nos tecidos foliares. Os teores de amônia em corda-de-viola aumentaram

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após a aplicação do amônio-glufosinate, e o valor máximo foi observado na maior dose do herbicida. Houve variabilidade quanto ao teor de amônia entre indivíduos de uma população de corda-de-viola, porém o comportamento não se repetiu da mesma maneira nas populações das progênies. A sobrevivência de plantas após a aplicação do herbicida pode gerar progênies com ampla variabilidade quanto à sensibilidade ao produto, independentemente do comportamento verificado nas plantas progenitoras.

Palavras-chave: amônia, glutamina sintetase, *Ipomoea grandifolia*, planta daninha.

INTRODUCTION

Glufosinate ammonium ([ammonium-DL-homoalanine-4-yl(methyl)] phosphinate) is an ammonium salt derived from phosphinothricin, a toxin that is isolated from two bacteria species, *Streptomyces viridochromogenes* and *S. hygroscopicus* (Duke and Lydon, 1987). With broad-spectrum mode of action, it controls a great variety of weeds in post-emergence (Duke, 1996), irreversibly inhibiting glutamine synthetase (GS) (EC 6.3.1.2), which is an enzyme that creates glutamine from glutamate and ammonium (Berlicki, 2008).

GS inhibition causes ammonium accumulation at toxic levels (Duke and Dayan, 2011), as well as photorespiration inhibition, due to reduced amino acid levels (Dayan and Duke, 2014), associated to chloroplast destruction, photosynthesis levels decrease and plant cell death (Fleck et al., 2001).

The *Ipomoea grandifolia* species, commonly known as morningglory, is a farming weed, especially found in summer cultures. Both annual and herbaceous, it has seed propagation and, since it closes its cycle after culture ripening, it hampers harvesting, because its branches get entangled (Kissmann and Groth, 1999).

Species from the *Ipomoea* genus are among the most tolerant to glyphosate herbicide, showing tolerance variations among populations from the same species (Baldwin, 1995); tolerance is related to lower herbicide translocation in plants (Monquero et al., 2004). Therefore, Chachalis et al. (2001) suggest that, in transgenic glyphosate-resistant soya bean and cotton cultures, *Ipomoea* ssp. control must be performed with other herbicides, such as glufosinate ammonium.

Works conducted by Silva et al. (2016) demonstrated that *I. grandifolia* plants presented high intoxication levels after glufosinate application, causing glutamate and glutamine decrease and ammonium accumulation in plants treated from six hours after application.

Frequently, species from the same genus or family as weeds do not consistently respond to the application of the same herbicide, characterizing the differential susceptibility of the species (Mathis and Oliver, 1980; Gossett and Toler, 1999). Annual weeds are known to exhibit different sensitivities to treatments with glufosinate (Pline et al., 1999); thus, these differences possibly occur between plants of morningglory plants.

One of the factors that may contribute to the determination of different responses from weeds to herbicide application is genetic variability. So, populations presenting wide genetic diversity may turn into an obstacle for control, since plants may present variable responses to different herbicide types and/or concentrations, and also help selecting resistant genotypes (Allendorf and Luikart, 2007). Tolerance variability to an herbicide may exist among species and populations from the same weed species (Pazuch et al., 2013).

Because of differential responses to glufosinate ammonium, different concentrations of ammonium in the tissues and, consequently, different levels of visual symptoms of injury will possibly be observed between plants of the same populations of morningglory, with the opportunity for this diversity to be transmitted to the progenies. Thus, the objective of this study was to determine the response of morningglory plants to different doses of glufosinate ammonium and to identify the range in sensitivities of plants and their progenies to this herbicide.

MATERIAL AND METHODS

Three studies were conducted, and all were repeated at different times. The first study determined the dose-response curve, the second study determined the range in sensitivity of morningglory plants to glufosinate ammonium, and the third study determined the range in sensitivity of progenies of morningglory to glufosinate ammonium. All studies were conducted at the School of Agricultural Sciences of São Paulo State University, Botucatu campus, (São Paulo State, Brazil), in a greenhouse (23.48S, 48.78W) with a temperature of 27 ^{o}C ± 2 ^{o}C and natural sunlight.

In the studies, morningglory seeds were sown in pots with an approximate volume of 115 mL that were filled with substrate composed of 70% sphagnum peat, 20% roasted rice straw, 10% perlite, and macro- and micronutrients. At 10 days after emergence (DAE), thinning was performed, and only one plant per pot was kept. For the applications of the glufosinate ammonium (Finale®, 200 g a.i. L^{-1}, Bayer CropScience Ltd, São Paulo SP, Brazil), a stationary sprayer with a spray boom with four nozzles (XR 110.02, Teejet, Jacto Máquinas Agrícolas SA, Pompéia, SP, Brazil) spaced at 0.5 m was used in a closed room. The sprayer was positioned at a height of 0.5 m relative to the plants and had a spray volume corresponding to 200 L ha^{-1} under a constant pressure of 150 kPa pressurized by compressed air.

Study about response-dose in morningglory plants

Two dose–response experiments with four replications each were conducted in a completely randomized design. The first experiment sought to determine the ammonium content in the leaf tissue of the morningglory plants depending on the dose of glufosinate ammonium, and the second experiment sought to determine the level of injury to the plants, also as a function of the herbicide dose. In both experiments, seven doses of glufosinate ammonium herbicide (0, 50, 100, 200, 400, 800, and 1,600 g ai ha^{-1}) were used as treatments.

In this study, morningglory (*Ipomoea grandifolia)* seeds from the city Engenheiro Coelho, SP, Brazil (22^{o}48'S; 47^{o}20'W) were sown in pots according to the seeding and thinning procedures previously described. At 30 DAE, the treatments were applied. In the first experiment, at 2 d after application (DAA), the ammonium content in the plant tissue was determined; for the second experiment, which evaluated the level of injury, visual evaluations were performed at 0, 3, 7, 14, and 21 DAA. On completion of the evaluations, the experiments were repeated.

Study about morningglory sensitivity to glufosinate ammonium

To determine the range in sensitivity of morningglory plants to glufosinate ammonium, the same seeding and thinning procedure in the pots was performed, using the same batch of seeds from the previous experiment. At 30 DAE, 44 plants were sprayed with a dose of 200 g ha^{-1} of herbicide, with six plants remaining untreated. The dose of 200 g ha^{-1} is enough to cause severe intoxication symptoms and ammonium accumulation in morningglory leaf tissues.

At the time of application, the meristematic region and the youngest leaf of each plant were protected with a plastic bag so that they would not receive the herbicide, thus keeping the plants alive. After thorough drying of the spray solution on the leaves, the protective bag was removed. At 2 DAA, an analysis of the ammonium content on the leaves exposed to the application was conducted. The protected leaves were kept on the plants, enabling them to recover from herbicide treatment and to produce seeds used to assess the sensitivity of morningglory progenies to glufosinate ammonium.

The plants were individually transplanted to pots containing 1 L of substrate. When the plants had reached the reproductive stage, all the flower buds were covered with paper bags tightly fastened to floral peduncles to prevent cross-pollination and guarantee that only self-pollination would take place. The seeds were collected and identified, and the plants sorted in ascending order of the ammonium content accumulated in the leaf tissue. This experiment was repeated.

Study about the sensitivity of morningglory progenies to glufosinate ammonium

To determine the behavior of the progenies of the plants previously analyzed, seven plants were selected after self-fertilization: two with a high concentration of ammonium, four with an intermediate concentration of ammonium, and one with a low concentration of ammonium. Among the 88 plants initially evaluated, only the seven picked plants produced large numbers of seeds enabling the evaluation of the sensitivity of the progenies to glufosinate ammonium. The evaluation of the progenies was repeated twice using half of the seeds produced by each plant.

Ten days after germination, thinning and maintenance of one plant per pot were conducted; the progeny plant numbers are presented in Table 1. At 30 DAE, a dose of 200 g ha^{-1} of herbicide was applied, and an analysis of the ammonium content in the tissue of the progeny plants was conducted. After this analysis, the experiment was repeated with the remaining half of the seeds following the previously described procedures.

Table 1 - Number of morningglory plants used in the study about progenies sensitivity to glufosinate ammonium, in the first and second experiments, in each class of original plants identified in the study about morningglory sensitivity to glufosinate ammonium

Classification	1st generation	Progenies		Total
		1st experiment	2nd experiment	
Low concentration	2	5	2	7
Medium concentration	3	6	5	11
High concentration	2	4	4	8

Evaluations of the studies

In the studies about response-dose, morningglory sensitivity to glufosinate ammonium, and progenies sensitivity to glufosinate ammonium, evaluations to determine ammonium contents in tissues were performed using the following protocol.

Ammonium was extracted from morningglory fresh leaf tissue, on day 2 DAA, immediately after leaf collection. The samples were placed in falcon tubes, containing 50 mL of water acidified with hydrochloric acid (pH 3.5), and placed in an ultrasonic for 60 minutes. The ammonium content of the solution was determined by spectrophotometry, according to published methods (Wendler et al., 1990; Dayan et al., 2015), using a spectrophotometer (Cintra 40, GBC Scientific Equipment Ltd).

In the study about response-dose, in order to evaluate injury levels, visual evaluations were performed on day 0, 3, 7, 14 and 21 DAA, through visual scale with grades ranging from 0 to 100, where 0 was associated with the complete absence of injuries and 100 was associated with the complete death of the plants (SBCPD 1995).

Data analysis

Data obtained in the experiments from the study about response-dose, in ammonium accumulation analysis, were converted to ammonium mg fresh $weight^{-1}$ kg and submitted to analysis of variance; treatment averages were compared with the help of a t test ($p \leq 0.05$). Significance level was determined for the contrasts between the sample treatment and the other ones, using t distribution.

In order to verify treatment effect, correlation analysis of ammonium contents and plant injury was performed. Since there was significant correlation, the adapted Mitscherlich non-linear regression model was adjusted:

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

where b and c correspond to the equation parameters. Side curve shift corresponds to the b parameter, and curve concavity to the c parameter.

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

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

where a, b and c correspond to the equation parameters. The maximum asymptote of the model is represented by the e^a expression; the curve shift along the x axis is represented by the b parameter; and the curve inclination or concavity in relation to accumulated frequency is represented by the c parameter (Velini, 1995). For a better view, it was decided to present non-accumulated frequency, which corresponds to the first derivation of the model, according to the equation:

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

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

In the study about progenies sensitivity to glufosinate ammonium, data from the first experiment and duplicate were grouped; the analyses were performed establishing confidence interval by t test ($p \leq 0.05$), for the averages of progenies from each class identified in the previous study. In order to determine the confidence interval, the following equation was used, according to (Carbonari et al., 2011):

$$IC = \frac{t * DP}{\sqrt{n}}$$

where t corresponds to the fixed t value ($p \leq 0.05$); DP corresponds to data standard deviation; and n corresponds to the number of replications.

The analyses were performed with the help of the Statistical Analysis System program (SAS, portable version 9.2.1); graphs were elaborated by Sigmaplot version 12.0.

RESULTS AND DISCUSSION

Study about response-dose in morningglory plants

For this study, two experiments were performed in order to analyze ammonium content, and two more to evaluate injuries, which turned up to be quite similar. Results obtained in the analysis of variance demonstrated that there were no significant differences between them; thus, a new analysis was performed, combined with the experiments, demonstrated in Table 2.

With this new analysis, contrasts between the treatments with glufosinate ammonium herbicide application and the sample indicated that there was a difference between them. Plants that did not receive application and the ones that received 50 and 100 g a.i. ha^{-1} glufosinate doses presented significantly lower ammonium contents in tissues ($p \leq 0.05$), compared to the other ones. Despite presenting higher content with the application of these two lower doses, they did not differ from the sample; there was higher accumulation in doses from 200 g a.i. ha^{-1}, thus indicating that the accumulation occurred in the other treatments is related to the performed application.

Correlating injury percentage and ammonium content in leaf tissues, it was possible to adjust the Mitscherlich non-linear regression model. It was possible to verify that plants with ammonium contents close to or higher than 400 mg kg fresh $weight^{-1}$ presented 100% injury levels on day 21 DAA (Figure 1).

The three higher doses used in the study, 400, 800 and 1,600 g a.i. ha^{-1}, provided higher ammonium contents in tissues; there was no significant difference among them, which

Table 2 - Analysis of variation of the experiment results of the study about response-dose in morningglory plants for ammonium contents in plant tissues, according to glufosinate ammonium herbicide doses applied on morningglory plants

Doses (g a.i. ha^{-1})	Ammonium content (mg ammonium kg fresh $weight^{-1}$)	F	Value p
0	22.07	-	1
50	67.60	0.28	0.601
100	178.89	3.29	0.077
200	384.74	17.60	<0.001
400	659.41	54.36	<0.001
800	617.08	47.38	<0.001
1600	721.08	65.39	<0.001
F treatments		23.05	<0.001
F experiments		3.85	0.056
F (treatment x experiment)		1.40	0.235
DMS (t p≤0.05)	174.45		
VC (%)	45.65		

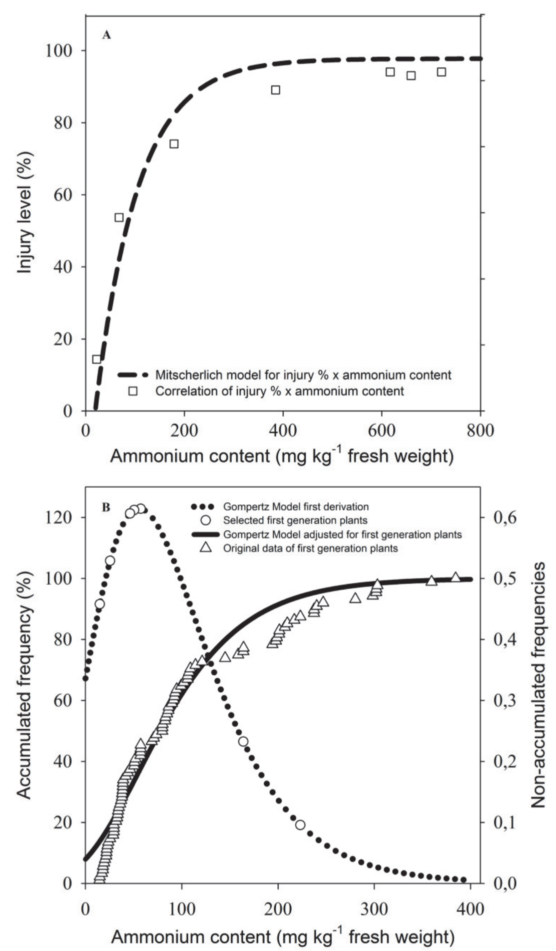

Figure 1 - (A) Correlation of injury level x ammonium content in tissues in the study about response-dose ($Y = 100[1 - 10^{(0{,}00503(X+(-19.2412)))}]$, $R^2 = 0.9833$), and (B) accumulated frequency of first generation plants and non-accumulated frequency, which corresponds to the first derivation of the Gompertz model for the dispersion of ammonium content in plants of the study about morningglory sensitivity to glufosinate ammonium.

demonstrated that there was no linearity in the correlation between applied dose and ammonium in tissues. After glufosinate ammonium herbicide application, the glutamine synthetase enzyme is inhibited and, with that, it stops turning ammonium and glutamate substrates into glutamine. Thus, ammonium accumulation in tissues occurs; it may be measured (Wendler et al., 1990) and used as an indicator of the herbicide performance (Wild et al., 1987; Carbonari et al., 2016). Researches demonstrated that ammonium contents in plants are related to herbicide toxicity (Petersen and Hurle, 2001; Britto and Kronzucker, 2002).

Checking the results of injury level evaluations in this study, it was possible to identify that the application of a 200 g a.i. ha^{-1} glufosinate ammonium dose caused injuries to the plants, but did not lead them to death. Thus, this dose was used during the study about sensitivity of morningglory plants to glufosinate ammonium herbicide, in order to keep plants alive and enable the production of seeds and the performance of the third study, with progenies of the selected plants.

Study about morningglory sensitivity to glufosinate ammonium

In order to verify the behavior of different individuals from a morningglory population regarding glufosinate ammonium application, a study about herbicide sensitivity was conducted. Gompertz non-linear equation model was adjusted with the obtained data;

parameter estimates and measures of position and dispersion are presented on Table 3.

Non-accumulated frequencies of first generation plants presented asymmetrical distribution. Measures of position, mean, median and mode are different among them, and mean and median values are higher than mode values; therefore, this asymmetrical distribution is positive (Table 3). Thus, it is possible to identify that there is higher variability among higher ammonium contents, compared to lower ones, in the frequency distribution curve; curves tend to quickly go up and then go down in a slower way (Araldi et al., 2013). This behavior is verified in Figure 1B, with the first derivation of Gompertz model. According to the adjusted model (Figure 1A), the variability of population to glufosinate application can be verified, considering the diversity in the contents of ammonium obtained in the evaluated plants, which vary from 0 to 400 mg ammonium kg fresh weight^{-1}.

Table 3 - Estimate of parameters, measures of position and dispersion of Gompertz model adjusted for data obtained in the analyses of ammonium contents in morningglory first generation plants

Model		Values
Parameter estimate[(1)]	b	-0.9292
	c	0.0167
Mean		104.97
Median		77.59
Mode		55.64
R^2		0.9901
VC (%)		86.21

[(1)] Parameters estimated by Gompertz model $F = e^{[a - e^{(-b.c*x)}]}$, where the expression e^a is the model maximum asymptote ($e^{4.60517}$); "b" is the curve shift along the x axis; and "c" is the curve concavity.

These plants were classified in ascending order, according to the ammonium content found in their leaf tissues, and thus allowing the separation into three plant groups: high concentration, low concentration and medium concentration. Information about genetic or environmental variability of plant sensitivity to glufosinate ammonium are quite restricted, but in a study conducted with *Convolvulus arvensis*, it was verified that a single population contained five biotypes with different susceptibility levels to glyphosate herbicide, presenting three visual injury levels, varying from about 30 to 100%, using a 0 to 100% scale (Degennaro and Weller 1984).

The application of an herbicide, even a non-selective one like glufosinate ammonium, may cause different sensitivity responses in weeds, mainly due to their wide genetic variability. Causes may be explained by different factors. Species that are more sensitive to glufosinate absorb and translocate the herbicide more than the less sensitive ones (Pline et al., 1999; Skora-Neto et al., 2000), even if glufosinate ammonium is a contact product, with low translocation; some species present metabolic degradation, reducing sensitivity to glufosinate (Mersey et al., 1990).

Seven plants from the evaluated population were selected, being highlighted in the Gompertz model on Figure 1B. The two plants presenting lower ammonium contents, on the left of the curve, are in the region of the lowest injury percentage, adjusted for the correlation between injury percentage and ammonium content; the two plants with higher ammonium contents, on the right side of the curve, are in the higher injury percentage region; and three plants with intermediate ammonium contents and injury percentage are located near the curve peak. Thus, a selection of plants was obtained, representing the behavior of the analyzed population; they were led to produce seeds for the study about progenies sensitivity (third study), related to the sensitivity of morningglory progenies to glufosinate ammonium.

Study about morningglory progenies sensitivity to glufosinate ammonium

The seven morningglory plants that were selected in the previous study produced seeds by self-fertilization only. Despite the large amount of seeds to perform the experiment and its duplicate, the germination rate was low, thus generating a number of plants lower than expected. Low germination was probably related to dormancy, a common characteristics in morningglory seeds, since experiments were performed in a short period of time.

Progenies populations did not reproduce the sharp differences identified among first generation plants. Verifying the bar overlap in confidence intervals of the averages in progenies from plants of the three selected classes, significant differences were not identified among them (Figure 2).

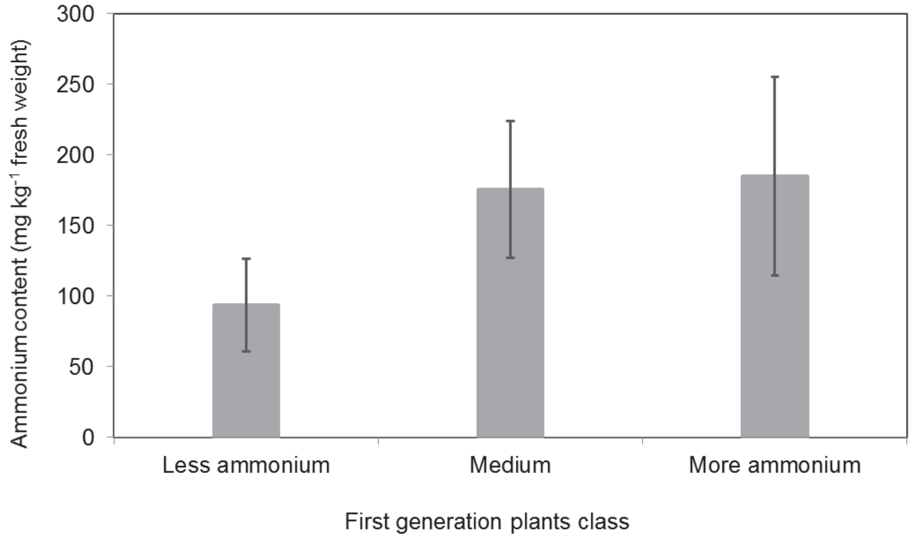

Figure 2 - Means comparison of ammonium contents in morningglory progenies in the three sensitivity classes identified in the first generation, after glufosinate ammonium herbicide application. Bars corresponding to the confidence interval (IC).

As observed in the previous study, where the first derivation of the Gompertz model presented positive asymmetry, and demonstrated higher variability among higher ammonium contents, progenies generated by plants that were classified as having more ammonium also presented higher variation, verified by IC = 70.28.

Differences verified in the first generation may be related to plant phenotypes, to individual leaf morphological characteristics (allowing higher or lower input of glufosinate herbicide into plants), and even to the different deposited herbicide quantities.

Nevertheless, there is a possibility that this behavior may be related to the population genetic diversity. The genetic diversity existing in a population is the result of the natural evolution process of species, which mainly derives from Mendelian variance, interspecies hybridization and polyploid (Winkler et al., 2002). The way in which these processes contribute to herbicide resistance is not clear and, more specifically, nor is it clear how they may contribute to the development of glufosinate ammonium resistance in weed populations that are sensitive to this herbicide.

Morningglory control by glufosinate ammonium may be correlated to ammonium contents in plant tissues, which increases according to herbicide application, but not in a linear way, according to the dose. There was variability in relation to ammonium contents among individuals from the studied morningglory populations and, consequently, in relation to plant control.

Bearing in mind the worrying possibilities related to the selection of plants that survive after herbicide application, studies demonstrated that plants that are initially less sensitive do not produce progenies with this single characteristic or with less sensitivity than the first generation; however, the opposite is also true. Thus, as for first generation, the survival of morningglory individuals after glufosinate ammonium application does not have the ability of generating plants with reduced sensitivity to the herbicide, this characteristic not being inherited by progeny.

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