

Conyza sumatrensis Intrapopulation Variation in Response to Glufosinate

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Abstract

We aimed to study the response of *Conyza sumatrensis* to different doses of glufosinate, intrapopulation variation in sensitivity to the herbicide, and the heritability of phenotypic response, and model the evolution of resistance. Three studies were conducted in the greenhouse with two repetitions. First, we tested doses of glufosinate (0, 50, 100, 200, 400, 800 g a.i. ha⁻¹) plus a non-treated check, with four replications. Second, we examined the range in sensitivity of 44 plants to 200 g a.i. ha⁻¹ glufosinate. Third, we evaluated the sensitivity of the progeny of six glufosinate-treated plants to 200 g a.i. ha⁻¹ glufosinate. Plant response was evaluated visually and the ammonia content in leaf tissues was measured. Glufosinate at 400 g a.i. ha⁻¹ caused the highest injury to *C. sumatrensis* plants. Ammonia accumulation occurred in response to glufosinate treatment, regardless of dose. Ammonia accumulation was correlated strongly with the level of visible plant injury; thus, it is a good indicator of herbicide efficacy. Sensitivity to glufosinate was highly variable within the population. Plants with high ammonia concentration (high injury) after treatment with glufosinate produced progenies that also had high ammonia concentrations after herbicide treatment. The variation in ammonia accumulation among siblings was high. Simulating the exclusion of plants that accumulated more ammonia produced a population that is expected to be less sensitive to glufosinate in the next generation. The stronger the selection pressures by a simulated treatment with glufosinate, the greater the reduction in ammonia accumulation in the progeny and expected sensitivity to glufosinate.

Keywords

Ammonia Accumulation, Differential Tolerance, Glutamine Synthetase,

1. Introduction

The genus *Conyza* includes plants that are highly competitive and with seed adaptation for long-distance dispersal. Thus, several *Conyza* species are formidable weeds. Among these, the most notorious are *Conyza canadensis* (L.) Cronq., *Conyza bonariensis* (L.) Cronq., and *Conyza sumatrensis* (Retz.) E. Walker [1], which are known as major invaders in different crops across continents [2] [3]. These species exhibit autogamous reproduction [4], and depending on the environmental conditions, they may assume an annual or biannual life cycle [5]. Each plant can produce 5000 to 200,000 seeds [6] [7]. *Conyza* species have high genetic diversity, which confers high adaptability to diverse environments [8], and the ability to evolve resistance to herbicides via a broad set of mechanisms [9].

Conyza sumatrensis is native to South America and has spread to the tropical and subtropical areas of all continents [2] [3]. Populations of this species have evolved resistance to herbicides under three mode-of-action groups, namely, photosystem I inhibitors [10] [11], glyphosate [12] and acetolactate synthase (ALS) inhibitors [11].

Of the three primary *Conyza* species, *C. sumatrensis* is the most susceptible to glyphosate [13]. Nevertheless, the control afforded by glyphosate is insufficient across the soybean areas of Parana state in Brazil [14]. The response of *C. sumatrensis* to glyphosate is variable and many plants recover from herbicide treatment. This situation favors selection for a resistant population. Studies on weed control alternatives are needed for the proper management of resistant weed biotypes [15]. The alternative herbicide must be more effective than glyphosate and economically viable [16].

In this context, the herbicide glufosinate may be a feasible alternative for controlling *Conyza* spp. Like glyphosate, glufosinate is a non-selective herbicide that is applied post-emergence [17]. While glyphosate inhibits 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), glufosinate inhibits glutamine synthetase (GS) [18], an enzyme that facilitates the rate-limiting step in nitrogen assimilation in plants [19]. Inhibition of GS results in the rapid accumulation of intracellular ammonium [20], and the concomitant depletion of glutamine and various other amino acids in the plant [21]. Excess ammonia destroys the cells and ruptures the chloroplast, thus blocking the electron transport chain [22] [23] and killing the plant faster than the starvation of amino acids.

Weedy species exhibit different levels of sensitivity to glufosinate [24]. Species in the genus *Conyza* may also exhibit differential sensitivity to this herbicide. Glyphosate- and glufosinate-treated fields show substantial variation in susceptibility to these herbicides, leaving enough chances for selection of tolerant indi-

viduals and accumulation of tolerance-endowing genes, which would lead to the evolution of an herbicide-resistant population.

This study was conducted to evaluate the response of *C. sumatrensis* plants to various glufosinate doses as well as evaluate variations in sensitivity to glufosinate among plants within a population and among the progeny. The hypotheses tested were: 1) the level of injury of *C. sumatrensis* in response to glufosinate correlates with ammonia accumulation in treated leaves; 2) *C. sumatrensis* plants within a population differ in ammonia accumulation after glufosinate treatment; and 3) the induction of ammonia accumulation by glufosinate is heritable; thus, progeny of survivors will be increasingly more tolerant to the herbicide.

2. Materials and Methods

2.1. Plant Materials and Experimental Conditions

Seeds of *C. sumatrensis* were collected from fields at the College of Agriculture of São Paulo State University (UNESP), Botucatu, São Paulo, Brazil (22°84'S, 48°43'W). These fields had not been treated with glufosinate previously. The herbicide treatments were applied at 60 DAE. All experiments were conducted in a greenhouse maintained at a temperature of 27°C ± 2°C under natural sunlight.

2.2. Experiment 1. Dose-Response of *C. sumatrensis* to Glufosinate

Seeds of *C. sumatrensis* were sown in 115-mL pots, filled with a substrate consisting of 70% sphagnum peat moss, 20% dried rice straw, 10% perlite, and supplemented with macro and micronutrients. The seedlings were thinned to one plant per pot 21 d after emergence (DAE). Glufosinate ammonium (Finale®, 200 g a.i. L⁻¹, Bayer CropScience Ltda, São Paulo, SP, Brazil) was applied using a motorized stationary sprayer in a closed room, with a spray bar fitted with four XR 11002 nozzle tips (Teejet, Jacto Máquinas Agrícolas SA, Pompéia, SP, Brazil) spaced 0.5 m apart. 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. The glufosinate doses were 0, 50, 100, 200, 400, 800 g a.i. ha⁻¹.

The treatments had four replicates and the experimental units (pots) were arranged in a completely randomized design. The experiment was conducted twice. All leaves were harvested 2 d after treatment (DAT) and ammonia was extracted immediately after. The samples were placed in falcon tubes containing 50 mL of water that was acidified with hydrochloric acid (pH 3.5) and placed in an ultrasonic bath, without warming, for 60 min in 42 kHz. The ammonia content of the solution was determined by spectrophotometry, in accordance with published methods [25] [26], using a Cintra 40 spectrophotometer (GBC Scientific Equipment Ltd.).

The level of injury was evaluated visually at 21 d after treatment using a scale

of visual assessments, of scores ranging from 0 to 100, in which “0” is related to the total absence of injury and “100” indicates death of plants [27].

2.3. Experiment 2. Intrapopulation Variation in Sensitivity to Glufosinate

Plants were established using the same procedure as in Experiment 1. At 60 DAE, 32 plants were sprayed with 200 g a.i. glufosinate ha⁻¹. Six plants without herbicide treatment were used as control. Data from Experiment 1 showed that the 200 g a.i. ha⁻¹ dose was sufficient to cause severe injury symptoms without killing the plants.

At the time of glufosinate application, the meristematic region and the youngest leaf of each plant were covered with a plastic bag. The bags were removed after the spray solution had dried. At 2 DAT, all leaves that were sprayed were harvested and the ammonia content was analyzed as described in Experiment 1.

The protected leaves were left on the plants so that the plants could recover from herbicide treatment and produce seeds. The surviving plants were individually transplanted into pots containing 1 L of potting medium. At the onset of reproductive stage, all the flower buds were covered with paper bags firmly attached to the main rachis of inflorescences to prevent cross pollination. The seeds were collected, and the plants were classified into ascending order according to the respective levels of ammonia accumulation in the leaf tissues of the mother plant. The experiment was conducted two times.

2.4. Experiment 3. Sensitivity of Glufosinate-Treated *Conyza sumatrensis* Progenies to Glufosinate

The F1 seeds obtained from Experiment 2 were used in this experiment. Six treated plants from Experiment 2 with large amount of seed, representing different levels of ammonia accumulation, were selected to assess the differential response of F1 plants to glufosinate and the corresponding ammonia accumulation. Two of the selected parent plants had 100 mg·kg⁻¹ and 500 mg·kg⁻¹ ammonia in treated leaves, respectively. The rest had ammonia concentrations between this range. One-half of the seeds produced by each parent plant were sown according to the same procedure in previous experiments. Seedlings were thinned to one plant per pot at 10 DAE. The F1 plants (Table 1) were treated with glufosinate, scored for injury, and the ammonia in treated leaves analyzed following the same procedures described previously.

2.5. Resistance Selection Modeling

Frequency distribution models were fitted to the ammonia concentrations in F1 plants, considering three scenarios: 1) all F1 families from six treated parents; 2) four F1 families after excluding those from two parents showing highest concentrations of ammonia; and 3) the F1 family from the parent plant exhibiting the lowest ammonia concentration after glufosinate treatment. Plants with high

Table 1. Number of *Conyza sumatrensis* plants used to study the sensitivity of the progeny to glufosinate ammonium in the first and second experiments and in each class of the original plants.

Classification	Progeny			Total
	1 st generation ¹	2 nd experiment	3 rd experiment	
Low ammonia (<100 mg.kg ⁻¹)	1	20	6	26
Intermediate ammonia (100 to 500 mg.kg ⁻¹)	3	57	25	82
High ammonia (>500 mg.kg ⁻¹)	2	26	22	48

¹Number of plants from first study.

ammonia concentrations after glufosinate treatment are expected to be sensitive; plants with low ammonia concentrations are expected to be tolerant.

2.6. Data Analysis

Analysis of the two repetitions of each experiment showed that the effects of repetition and of the treatment x repetition interactions were not significant, thus allowing the combined analysis across repetitions.

The ammonia data from the dose-response experiment were converted into mg of ammonia kg⁻¹ of fresh leaf weight and subjected to an analysis of variance. The two experiments conducted to analyze the ammonia levels in leaf tissues produced similar results. Thus, data from both experiments were pooled for the final analysis. Treatment means were compared using a t-test ($p \leq 0.05$). The level of significance was determined for the contrasts between the control and the treated plants using a t-distribution. To assess the effects of the treatments, a correlation analysis was performed in relation to the ammonia level and the injury to the plants. Because the correlation was significant, a modified Mitscherlich [28] non-linear regression model was fitted as follows:

$$\% \text{ of Injury} = a \left[1 - 10^{(-c(X+b))} \right] \quad (1)$$

$$\text{Ammonia accumulation} = a' \left[1 - 10^{(-c'(X+b'))} \right] \quad (2)$$

wherein a , a' , b , b' , c and c' correspond to the equation parameters. The lateral displacement of the curves correspond to parameters b and b' . The concavity of the curves correspond to parameters c and c' . The parameters a and a' correspond to the horizontal asymptotes of the models or to the maximum values expected for the dependent variable.

From Equations (1)-(3) was developed, expressing the % of injury as a function of ammonia accumulation.

$$\% \text{ of Injury} = a \left[1 - 10^{c \left(b' - b + \left(\frac{1}{c'} \right) \log \left(1 - \frac{\text{Ammonia accumulation}}{a'} \right) \right)} \right] \quad (3)$$

For the studies on glufosinate sensitivity among the F1 progeny, the data were fitted with the Gompertz model [29] according to Velini [30], using Equation (4):

$$Y = e^{\left[a - e^{(-b-cx)} \right]} \quad (4)$$

where in a , b and c correspond to the equation's parameters. The maximum asymptote of the model is represented by expression " e^a ", and the shift of the curve along the x -axis and the slope or concavity of the curve in relation to the cumulative frequency are represented by parameters " b " and " c ", respectively. For better visualization, the non-cumulative frequency was presented, which corresponds to the first derivative of the model in accordance with the following Equation (5) [30]:

$$Y = c \times e^{\left(a - b - cX - e^{(-b-cx)} \right)} \quad (5)$$

The fit of the data to the Gompertz model was evaluated using the determination coefficients (R^2) of the equations. Also based on the Gompertz model, the position (mode, mean, and median) and dispersion (coefficient of variation) measures of the data set were determined. For the mode, the equation parameters were used, with the following formula [30] (Equation (6)):

$$Y = \frac{-b}{c} \quad (6)$$

To calculate the median value, the following Equation (7) was used:

$$Y = -\frac{\ln(a - 3.912) + b}{c} \quad (7)$$

The analyses were performed in SAS (Statistical Analysis System, SAS Institute, version 9.1.3, Carry, North Carolina, USA), and the graphs were prepared using SigmaPlot (Systat Software, version 12.0, San Jose).

3. Results

3.1. Response of *Conyza sumatrensis* to Glufosinate

The two experiments conducted to analyze the ammonia level in leaf tissues showed similar results as shown by the analysis of variance. Thus, a new analysis of variance was conducted, in both experiments were jointly considered (**Table 2**). The joint analysis was also adopted for the two experiments conducted to evaluate efficacy of glufosinate as a consequence of the herbicide dose.

The two runs of the experiment was analyzed jointly to evaluate the efficacy of glufosinate across different doses, and the Mitscherlich non-linear regression model had a good fit to the original data (**Table 2**). All the contrasts between the control and glufosinate treatments were significant at $p < 0.01$.

All glufosinate treatments tested caused visible injury and increased ammonia accumulation in leaves. The highest ammonia concentration (approximately 724 mg·kg⁻¹) was observed at the highest dose (800 g a.i. ha⁻¹). Injury increased with glufosinate dose from 50 to 400 g a.i. ha⁻¹ at 21 DAT. Injury was maximized at doses equal to or greater than 400 g a.i. ha⁻¹ (**Figure 1(A)**). The predicted doses required for 50% and 80% plant injury were 52.5 and 122.3 g a.i. ha⁻¹, respectively (**Figure 1(B)**).

Ammonia concentration at 2 DAT and visible plant injury at 21 DAT increased

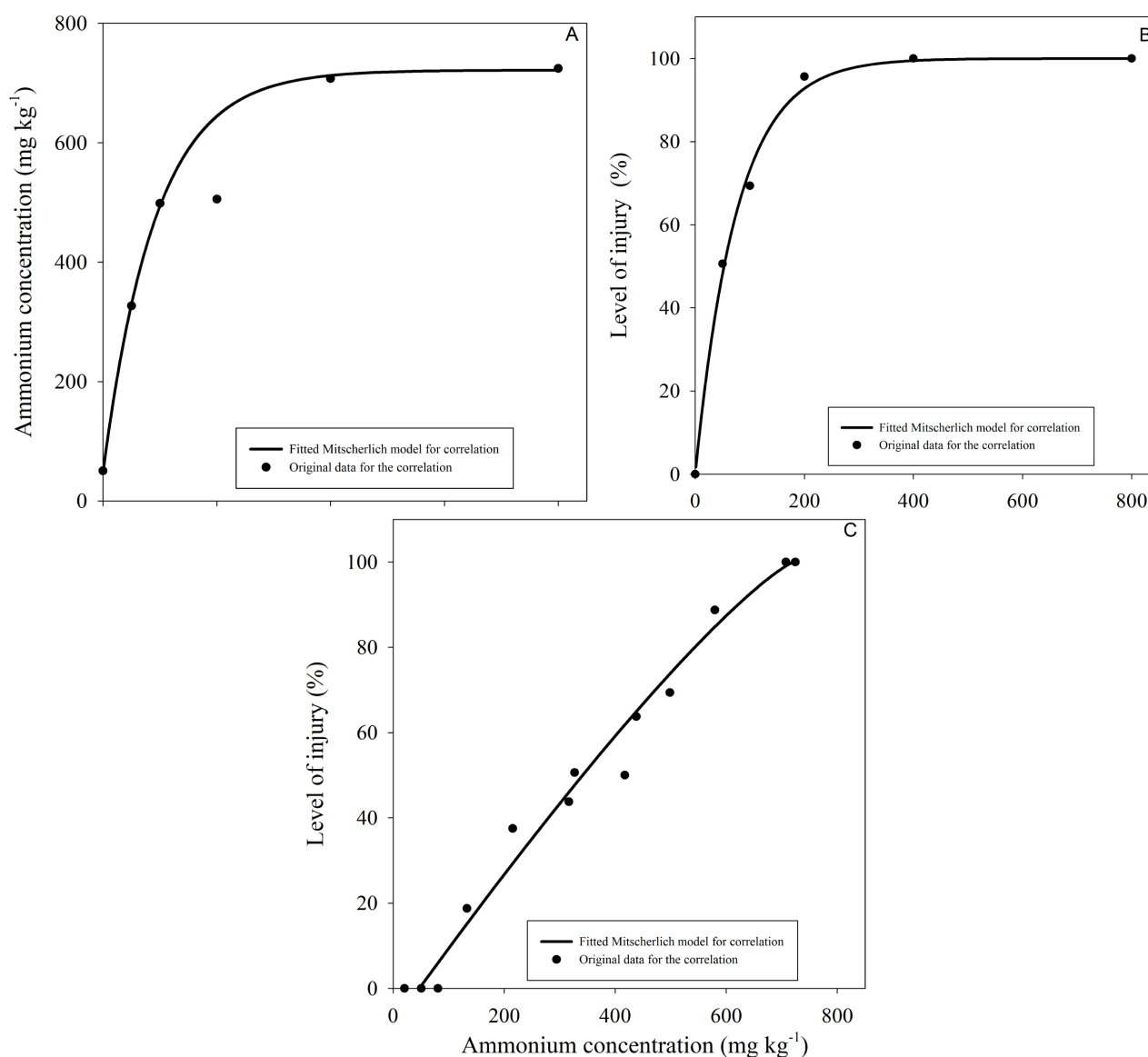


Figure 1. (A) Correlation of ammonium concentration (mg·kg⁻¹) in leaves of *Conyza sumatrensis* and dose of glufosinate ammonium (g a.i. ha⁻¹) applied. Equation: $Y = 798.90 \left[1 - 10^{(-0.00389(X+7.7839))} \right]$; (B) Correlation of the injury level (%) on *Conyza sumatrensis* and dose of glufosinate ammonium applied (g a.i. ha⁻¹). Equation: $Y = 100 \left[1 - 10^{(-0.0057(X+0.2931))} \right]$; (C) Correlation of the injury level (%) and ammonia level per fresh leaf weight (mg·kg⁻¹). Equation: $Y = 100 \left[1 - 10^{(0.0057(7.7839 - 0.2957 + (\frac{1}{0.00389}) \log(1 - \frac{X}{798.90}))} \right]$.

with glufosinate dose up to about 200 g a.i. ha⁻¹ (**Figure 1(A)** and **Figure 1(B)**). Thus, the level of ammonia accumulated in the leaves was strongly and positively correlated with the level of observable plant injury (**Figure 1(C)**). The Mitscherlich non-linear regression model had a good fit to the original data (**Table 2**). The constants of the model have biological relevance where: “*e*” is the horizontal asymptote, corresponding to the maximum injury or the maximum expected ammonia concentration; “*c*” is the responsivity of the dependent variable to glufosinate dose; and “*b*” is the lateral displacement of the curve or the ab-

scissa when the dependent variable has a value equal to zero.

3.2. Intrapopulation Variation in Sensitivity to Glufosinate

Gompertz non-linear regression model was fitted to the data with ammonium concentration and accumulated frequencies as independent and dependent variables, respectively. The parameter estimates for the fitted models and estimates of the mean, median, and modal ammonium concentrations in the progenies of three groups of plants based on ammonia accumulation are shown in **Table 3**.

Table 2. Estimates of regression parameters for the fitted Mitscherlich models describing the relationship of injury level and glufosinate herbicide dose, and the relationship of injury level and ammonia accumulation in *Conyza sumatrensis* after glufosinate treatment.

Particulars	Ammonium concentration (mg·kg ⁻¹) × dose of glufosinate (g a.i. ha ⁻¹)	Injury level (%) × dose of glufosinate (g a.i. ha ⁻¹)	Injury level (%) × ammonium level (mg·kg ⁻¹)
Regression parameters	<i>a</i>	-	100.000
	<i>b</i>	-	0.296
	<i>c</i>	-	0.006
	<i>a'</i>	798.900	-
	<i>b'</i>	7.783	-
	<i>c'</i>	0.004	-
R ²	0.939	0.999	0.980
F regression	57.260	9785.030	269.310

Table 3. Estimates of parameters for the fitted Gompertz models with ammonium content and cumulative frequency as independent and dependent variables, respectively. Estimates of statistics of ammonia concentration in the progeny of the three groups of plants with different levels of ammonia accumulation are also presented.

Particulars	Ammonia concentration in first generation of survivors ² (mg·kg ⁻¹ leaf tissue)	Ammonia concentration in progeny of survivors (mg·kg ⁻¹ leaf tissue)		
		Low ammonia	Intermediate	High ammonia
Regression parameters ¹	<i>a</i>	4.605	4.605	4.605
	<i>b</i>	-2.201	-1.761	-2.053
	<i>c</i>	0.012	0.007	0.005
Mean	250	316	408	565
Median	213	284	358	553
Mode	183	235	304	485
R ²	0.997	0.997	0.995	0.989
F regression	4943	4051	2723	15909
CV (%)	58.33	47.13	36.61	35.40

¹Parameter estimates from Gompertz model $F = e^{\left[\frac{a-d^{b+c}}{b+c}\right]}$. *b*, displacement of the curve along the *x*-axis; *c*, concavity of the curve; and expression e^d , the maximum asymptote of the model. ²Ammonia concentration after treatment with 200 g a.i. ha⁻¹ glufosinate.

Ammonia accumulation is a good indicator of the expected injury level in *C. sumatrensis* plants treated with glufosinate. The higher the ammonia accumulation the greater is the sensitivity to glufosinate (**Figure 1(C)**). Using the model in **Figure 1(C)**, the respective ammonia concentrations corresponded to predicted injury levels of 36.44%, 30.13%, and 24.72%.

C. sumatrensis plants from the same population accumulated between 50 and 740 mg ammonia kg⁻¹ fresh leaf weight after treatment with 200 g a.i. ha⁻¹ glufosinate (**Figure 2**). The plants were classified arbitrarily into low (<100 mg·kg⁻¹), intermediate (100 to 500 mg·kg⁻¹), and high (>500 mg·kg⁻¹) ammonia concentration categories. One, three, and two plants, respectively, produced enough seeds in each category for analysis of progeny.

The frequency of plants in the first generation that accumulated <200 mg·kg⁻¹ ammonia in leaf tissues was low (**Figure 2**). Cumulative frequency increased with ammonia concentration up to about 400 mg·kg⁻¹ and tapered off thereafter. Since the level of injury is positively correlated with ammonia accumulation, we can deduce that tolerant plants were rare in the first generation. Conversely, few plants were extremely sensitive to glufosinate.

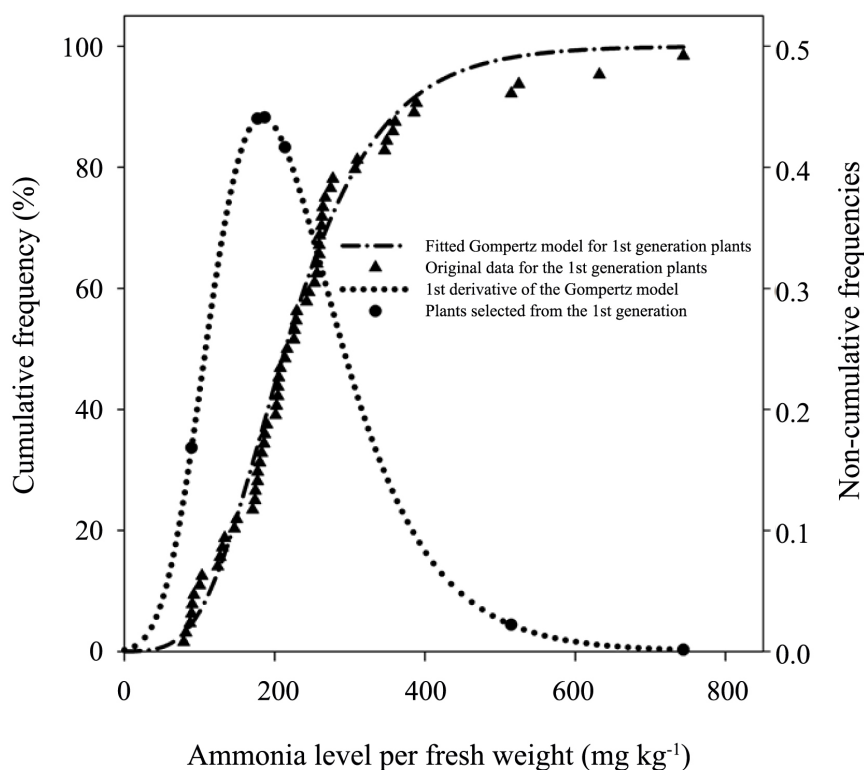


Figure 2. Cumulative and non-cumulative frequencies of ammonia concentrations in *Conyza sumatrensis* plants from the first generation of survivors in response to glufosinate ammonium. The non-cumulative frequency distribution corresponds to the first derivative of the Gompertz model for the dispersion of ammonia accumulation levels in the selected population. Cumulative frequency: $Y = e^{\left[4.60517 - e^{-(2.2010) - 0.012X}\right]}$; noncumulative frequency: $Y = 0.012e^{\left(4.60517 - (-2.2010) - 0.012X - e^{-(2.2010) - 0.012X}\right)}$.

The majority of plants had ammonia concentrations between 200 and 400 mg·kg⁻¹. The non-cumulative frequencies of ammonia levels in the leaf tissues of plants from the first generation had an asymmetric distribution with different values of the mean, median, and mode, and those of the mean and median are higher than the modal value (Table 3 and Figure 2). The majority of first generation plants produced about 200 mg·kg⁻¹ ammonia in leaf tissues and very few (two plants) were highly sensitive.

3.3. Sensitivity of *Conyza sumatrensis* Progenies to Glufosinate

The progeny populations also exhibited high amplitudes of ammonia concentration in leaf tissues (Figure 3). In the first-generation plants, the ammonia levels per fresh weight of leaf tissue ranged between 0 and 740 mg·kg⁻¹ (Figure 2); in the progeny, the levels varied from 0 to 940 mg·kg⁻¹ (Figure 3(A)). The frequency distribution of ammonia accumulation from the low-ammonia-accumulating parent was skewed farthest to the left, relative to those of the two other parental categories (Figure 3(B)). Thus, progeny of the low-ammonia-accumulating parent had the highest proportion of plants that accumulated the least ammonia after glufosinate treatment. The high-ammonia-accumulating parents (also the most susceptible) had progenies with the majority of plants also accumulating the highest level of ammonia. Thus, the susceptible parents produced predominantly susceptible offspring and tolerant parents produced predominantly tolerant offspring.

The ammonia accumulation data of the various progeny populations were fitted with three different models, each simulating a specific selection condition. The first model was fitted to all six progeny populations with a total of 156 plants (Table 4). The second model excluded the susceptible plants; thus, the progenies of the two high-ammonia-accumulating plants (which were expected to be most sensitive to glufosinate) were excluded. This subset included 108 plants with intermediate to high expected tolerance to glufosinate. The exclusion of the progenies from plants with ammonia concentrations over 500 mg·kg⁻¹ aimed at simulating a low selection pressure by glufosinate, which can eliminate only the highly susceptible plants.

Table 4. Estimates of regression parameters for the fitted Mitscherlich models describing the relationship of injury level and glufosinate herbicide dose, and the relationship of injury level and ammonia accumulation in *Conyza sumatrensis* after glufosinate treatment.

Model		All groups	Without the susceptibles	Tolerant plants only
Parameters ¹	<i>a</i>	4.60517	4.60517	4.60517
	<i>b</i>	-1.7120	-1.8883	-1.6271
	<i>c</i>	0.00525	0.00673	0.00724
R ²		0.9991	0.9993	0.9974
F regression		93511.5	72912.6	4759.9

¹Parameter estimates from Gompertz model $Y = e^{\left[\frac{a-e^{(-b \cdot x)}}{c}\right]}$. *a*, the displacement of the curve along the *x*-axis; *b*, the displacement of the curve along the *x*-axis; *c*, concavity of the curve; and expression *e^a*, the maximum asymptote of the model.

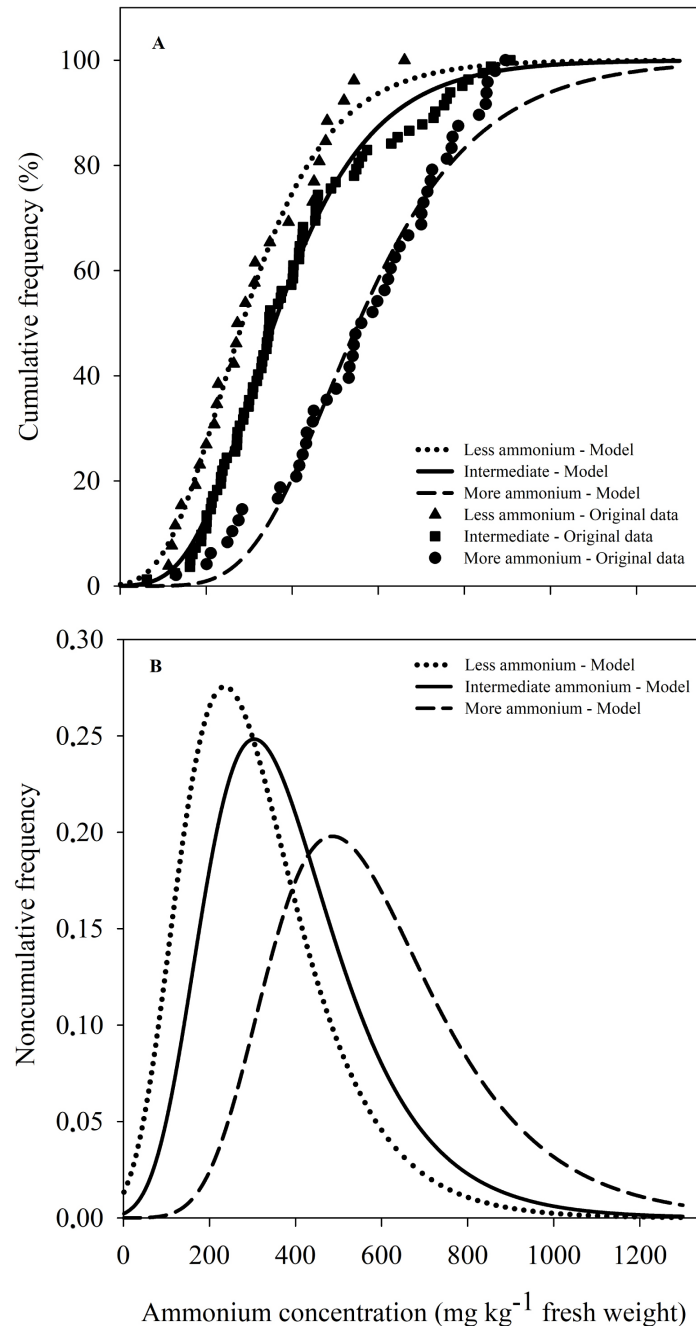


Figure 3. (A) Cumulative frequency (%) of ammonia concentrations in the progeny of three sensitivity categories of *Conyza sumatrensis* plants fitted with the Gompertz model and its original data; and (B) Non-cumulative frequencies corresponding to the first derivative of the Gompertz model for the ammonia level in the. (A) Low ammonium:

$$Y = e^{[4.60517 - e^{-(1.761) - 0.0075 X}]}; \text{ Intermediate ammonium: } Y = e^{[4.60517 - e^{-(2.0528) - 0.00675 X}]}; \text{ High ammonium: } Y = e^{[4.60517 - e^{-(2.6107) - 0.00538 X}]}$$

$$Y = e^{[4.60517 - e^{-(2.6107) - 0.00538 X}]}; \text{ (B) Low ammonium:}$$

$$Y = 0.0075 e^{[4.60517 - (1.7618) - 0.0075 X - e^{-(1.7618) - 0.0075 X}]}; \text{ Intermediate ammonium:}$$

$$Y = 0.00675 e^{[4.60517 - (2.0528) - 0.00675 X - e^{-(2.0528) - 0.00675 X}]}; \text{ High ammonium:}$$

$$Y = 0.00538 e^{[4.60517 - (2.6107) - 0.00538 X - e^{-(2.6107) - 0.00538 X}]}$$

The estimates of parameters for the fitted models are shown in **Table 4**. The third model, already shown in **Figure 4**, included only the data for the progeny

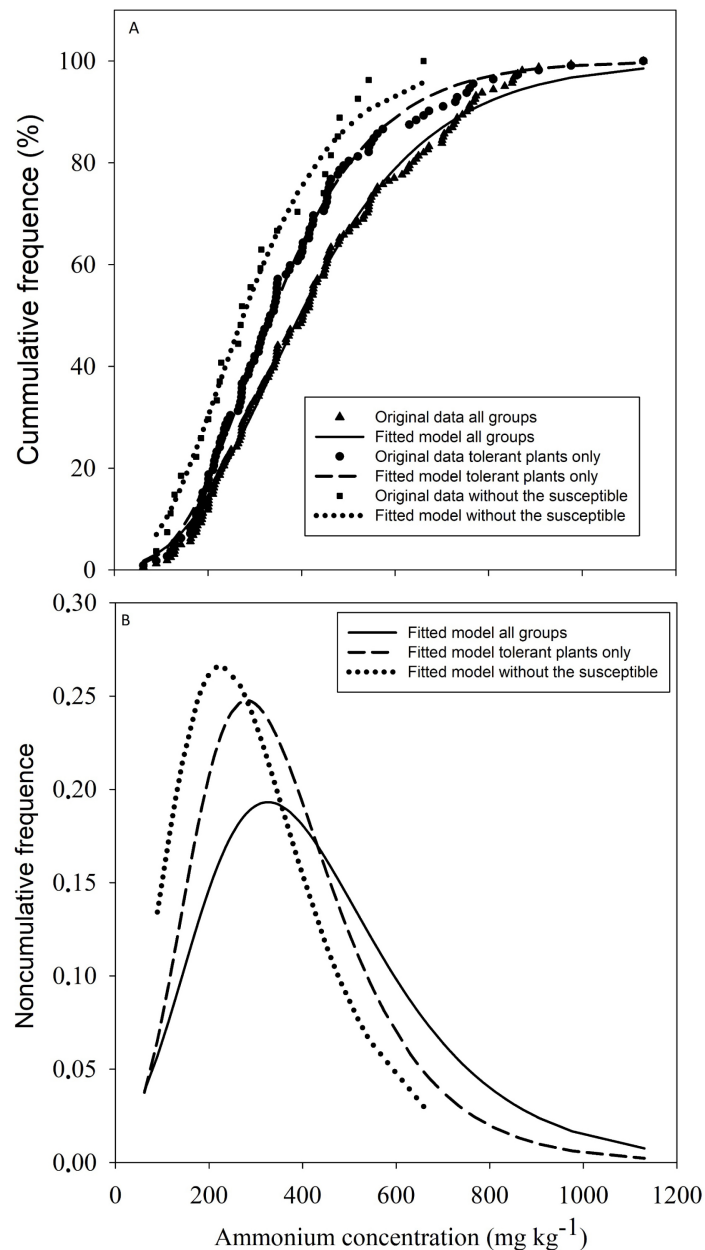


Figure 4. (A) Cumulative frequency (%) of ammonia accumulation data, fitted with Gompertz model; and (B) Non-cumulative frequencies corresponding to the first derivative of the Gompertz model in the progeny of *Conyza sumatrensis* plants. Equations for (A): all progeny plants, $Y = e^{\left[4.60517 - e^{(-1.7120) - 0.00525 X}\right]}$; excluding expected sensitive plants, $Y = e^{\left[4.60517 - e^{(-1.8883) - 0.00673 X}\right]}$; expected tolerant plants only, $Y = e^{\left[4.60517 - e^{(-1.6271) - 0.00724 X}\right]}$. Equations for (B): all progeny plants, $Y = 0.00525 e^{\left[4.60517 - (-1.7120) - 0.00525 X - e^{(-1.7120) - 0.00525 X}\right]}$; excluding expected sensitive plants, $Y = 0.00673 e^{\left[4.60517 - (-1.8883) - 0.00673 X - e^{(-1.8883) - 0.00673 X}\right]}$; expected tolerant plants only, $Y = 0.00724 e^{\left[4.60517 - (-1.6271) - 0.00724 X - e^{(-1.6271) - 0.00724 X}\right]}$.

of the plant accumulating the lowest amounts of ammonia. The third model was fitted to a subset of 26 plants. The third model corresponds to the frequency distribution of plants with high expected tolerance to glufosinate. The exclusion of progenies from the five plants showing ammonia concentrations above 100 mg·kg⁻¹ aimed at simulating a high selection pressure by glufosinate, which can eliminate the most sensitive plants and those with intermediate tolerance to glufosinate.

4. Discussion

The correlation between ammonia levels in the leaf tissue and the observed injury from glufosinate treatment was high. This behaviour is consistent with the knowledge about the mode of action of glufosinate. The inhibition of glutamine synthetase activity by glufosinate leads to the rapid accumulation of ammonia because of the impediment of nitrogen assimilation into amino acids, starting with the immediate depletion of glutamine [31]. This biochemical response to the inhibition of glutamine synthetase allows for a non-destructive assessment of plants for relative sensitivity to glufosinate. Thus, the ammonia accumulation assay is an effective tool in monitoring the change in population response to glufosinate after each cycle of selection.

Although glufosinate is a nonselective herbicide, some weed species are less sensitive to glufosinate than others. Ridley and McNally [32] obtained differences in LD₅₀-values, for the seven species, *Avena fatua*, *Cassia obtusifolia*, *Elymus repens*, *Galium aparine*, *Brassica napus*, *Setaria viridis* and *Xanthium spinosum*, 2 weeks after spraying. The most tolerant species was *C. obtusifolia*, with a LD₅₀ of 8.5 kg·ha⁻¹, while the most susceptible species was young *S. viridis*, with LD₅₀ less than 0.125 kg·ha⁻¹. The levels of efficacy vary widely across species, locations, and time. In São Paulo, Brazil, *Alternanthera tenella* is controlled 83% with 500 g a.i. ha⁻¹ of glufosinate [33]. *Parthenium hysterophorus* in Gainesville, Florida, USA was controlled 90% with 281 g a.i. ha⁻¹ [34] 3 weeks after application. In Stoneville, Mississippi, USA, Reddy *et al.* [35] reported similar (89% to 95%) control of *Parthenium hysterophorus* with a higher dose (410 g a.i. ha⁻¹) of glufosinate, in field studies conducted during 2005 and 2006.

A population of a relatively tolerant species poses a high risk of having escaped, or survivors, after an application of glufosinate. Various factors contribute to this including variability in plant size at the time of application, variable density (and therefore coverage) of weeds in the field, the time of day when glufosinate was applied, application volume (as it affects coverage), or the environmental conditions around the time of application.

Glufosinate is substantially effective on *C. bonariensis*, providing > 80% control regardless of the stage of plant development at the time of application [36]. The same researchers obtained > 95% control of *C. bonariensis* that were less than 10 cm tall. One environmental factor that affects glufosinate activity strongly is relative humidity. Glufosinate translocated better and was more effective on

Amaranthus species (*A. palmeri*, *A. retroflexus*, and *A. tuberculatus*) grown at 90% relative humidity than those grown at 35% relative humidity [37]. Relative humidity has a stronger effect on glufosinate activity than temperature.

As glufosinate use increases with increased adoption of glufosinate-resistant crops, the risk of selecting for resistance to this alternative chemical tool will also increase. Our data support this hypothesis. *C. sumatrensis* is a hexaploid, which endows high genetic variability within this species, making it ideal for resistance selection. Indeed, the amplitudes of ammonia concentrations in leaf tissues were large, showing high plant-to-plant variability in glufosinate response of the field population. Further, plants showing low, intermediate, or high ammonia concentrations produced progenies with low, intermediate, or high average ammonia concentrations as shown in **Figure 3(A)** and **Figure 3(B)**. This indicates that the level of tolerance is heritable. Highly sensitive plants are easily eliminated from the population by inadvertent suboptimal doses of glufosinate for reasons mentioned previously. Therefore, once highly sensitive plants are eliminated from the population, the remaining plants will harbor a higher level of tolerance to glufosinate. Iteration of this selection process in succeeding seasons will eventually lead to the evolution of a glufosinate-resistant population. Our data indicates that this selection process could be short, at least with *C. sumatrensis*.

In our simulation, increasing the selection pressure by glufosinate treatments in the first generation would result in progeny accumulating less ammonium after glufosinate treatment. In other words, the progeny of selected plants will be more tolerant to glufosinate than the original population. The higher the simulated selection pressure, the greater is the expected elevation in tolerance of the second generation. However, the amplitude of ammonia concentrations in *C. sumatrensis* leaves after glufosinate application is large, even among progenies of selfed plants, suggesting a high level of heterogeneity and heterozygosity of tolerance expression, characteristic of species with high ploidy. This also reflects the complex genetic control of achieving tolerance, or accumulating tolerance genes to glufosinate, which is modified further by environmental conditions.

However, granting that the heritability of tolerance trait is low and the variability of tolerance among plants is high, eliminating highly sensitive individuals from the population still would result in elevated tolerance in the next generation. The stronger the selection pressure by a simulated treatment with glufosinate, the higher is the reduction in ammonia accumulation and expected sensitivity to glufosinate.

This evolutionary pattern across generations in response to a persistent selector has been documented in other herbicide-species combination. Among the earliest research on this involved 13 *Kochia* (*Kochia scoparia* (L.) Schrad.) selections with 2,4-D herbicide, which had been selfed for four generations. The resultant selected lines differed in visible injury and seed production in response to 2,4-D [38]. The most susceptible line was injured at 0.35 kg a.i. ha⁻¹, and the most tolerant did not exhibit symptoms until treated with 0.70 kg a.i. ha⁻¹. A similar study *Chenopodium album* treated with a dose of glyphosate (840 g a.e.

ha⁻¹) [39]. After six years of selection, they obtained a progeny with 45% survival from the same treatment, compared to only 33% survival among the non-selected, original field population. The selection for glyphosate-resistant *Lolium rigidum* with a sublethal dose of glyphosate took less time [40]. In this case, after three generations of selection, the estimated LD₅₀ doubled compared to the parental population and up to 33% of the glyphosate-selected progeny survived treatment with a recommended field dose of glyphosate. In like manner, exposed *L. rigidum* to six consecutive cycles of recurrent selection with pyroxasulfone at 60 g·ha⁻¹ to 240 g·ha⁻¹ [41].

After six cycles of selection, 54% of the progeny survived the recommended dose of pyroxasulfone (100 g·ha⁻¹) while the original population had only 5% survival. With *Amaranthus palmeri*, it took just three cycles of selection with dicamba to increase the population LD₅₀ from 111 g a.e. ha⁻¹ for parental individuals to 309 g a.e. ha⁻¹ for the third generation [42]. This selected population also had elevated tolerance to 2,4-D relative to the original field-collected population. This scenario indicates that the new technology involving crops with resistance to dicamba or 2,4-D should be used with utmost integration with other tools to avoid creating yet another weed resistance problem. The modes of action of these herbicides are starkly different: 2,4-D is an auxin mimic, glyphosate inhibits EPSPS, pyroxasulfone inhibits the synthesis of very-long-chain fatty acids, and dicamba is a hormone-type herbicide. Therefore, the outcome of repeated exposure of weed species to sublethal doses of herbicides, regardless of the mode of action, is resistance. It is just a matter of time.

Overall, our research indicates that *C. sumatrensis* can evolve resistance to glufosinate after perhaps three or four generations of selection depending on the inherent variability in tolerance within the population, coupled with other factors that reduce the efficacy of glufosinate. How soon this will occur depends on how quickly the susceptible individuals are eliminated from the population and how much of the survivors are able to produce seed.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

- [1] Vargas, L., Silva, D.R.O., Agostinetto, D., Matallo, M.B., Santos, F.M., Almeida, S.D.B., Chavarria, G. and Silva, D.F.P. (2014) Glyphosate Influence on the Physiological Parameters of *Conyza bonariensis* Biotypes. *Planta Daninha*, **32**, 151-159. <https://doi.org/10.1590/S0100-83582014000100017>
- [2] Thebaud, C. and Abbott, R.J. (1995) Characterization of Invasive *Conyza* Species (Asteraceae) in Europe: Quantitative Trait and Isozyme Analysis. *American Journal Botany*, **82**, 360-368. <https://doi.org/10.1002/j.1537-2197.1995.tb12640.x>
- [3] Pruski, J.F. and Sancho, G. (2006) *Conyza sumatrensis* var. *Leiotheca* (Compositae: Astereae), a New Combination for a Common Neotropical Weed. *Novon: A Journal for Botanical Nomenclature*, **16**, 96-101. [https://doi.org/10.3417/1055-3177\(2006\)16\[96:CSVLCA\]2.0.CO;2](https://doi.org/10.3417/1055-3177(2006)16[96:CSVLCA]2.0.CO;2)

- [4] Cruden, R.W. (1976) Pollen-Ovule Ratios: A Conservative Indicator of Breeding Systems in Flowering Plants. *Evolution*, **31**, 32-46. <https://doi.org/10.2307/2407542>
- [5] Regehr, D.L. and Bazzaz, F.A. (1979) The Population Dynamics of *Erigeron Canadensis*, a Successional Winter Annual. *Journal of Ecology*, **67**, 923-933. <https://doi.org/10.2307/2259221>
- [6] Bhowmik, P.C. and Bekech, M.M. (1993) Horseweed (*Conyza canadensis*) Seed Production, Emergence and Distribution in No-Tillage and Conventional-Tillage Corn (*Zea mays*). *Agronomy*, **1**, 67-71.
- [7] Holm, L.G., Plucknett, D.L., Pancho, J.V. and Herberger, J.P. (1977) The World's Worst Weeds: Distribution and Biology. University Press of Hawaii, Honolulu, HI, 610 p.
- [8] Circunvis, B.C., Renesto, E., Mangolin, C.A., Machado, M.F.P.S. and Takasusuki, M.C.C.R. (2014) Genetic Characterization of *Conyza* sp. Samples in the State of Paraná. *Planta Daninha*, **32**, 173-179. <https://doi.org/10.1590/S0100-83582014000100019>
- [9] Heap, I. (2014) Global Perspective of Herbicide-Resistant Weeds. *Pest Management Science*, **70**, 1306-1315. <https://doi.org/10.1002/ps.3696>
- [10] Hanioka, Y. (1989) Paraquat-Resistant Biotype of *Erigeron Sumatrensis* Retz in Mulberry Fields in Saitama Prefecture. *Weed Research*, **34**, 210-214. <https://doi.org/10.3719/weed.34.210>
- [11] Heap, I. (2018) The International Survey of Herbicide Resistant Weeds. <http://www.weedscience.org>
- [12] Santos, G. (2012) Multiple Resistance to Glyphosate and Chlorimuron-Ethyl in *Conyza sumatrensis*. Thesis, Universidade Estadual de Maringá, Maringá-PR, Brazil.
- [13] González-Torralva, F., Cruz-Hipolito, H., Bastida, F., Mulleder, N., Smeda, R.J. and Prado, R. (2010) Differential Susceptibility to Glyphosate among the *Conyza* Weed Species in Spain. *Journal of Agricultural and Food Chemistry*, **58**, 4361-4366. <https://doi.org/10.1021/jf904227p>
- [14] Santos, G., Oliveira, R.S., Constantin, J., Francischini, A.C., Machado, M.F.P.S., Mangolin, C.A. and Nakajima, J.N. (2014) *Conyza sumatrensis*: A New Weed Species Resistant to Glyphosate in the Americas. *Weed Biology and Management*, **14**, 1-9. <https://doi.org/10.1111/wbm.12037>
- [15] López-Ovejero, R.F., Christoffoleti, P.J. and Vargas, L. (2004) Resistance of Weeds to Herbicides. In: Vargas, L. and Roman, E.S., Eds., *Weed Management and Control Manual*, Embrapa Uva e Vinho, Bento Gonçalves, RS, Brasil, 185-214.
- [16] Peterson, D.E. (1999) The Impact of Herbicide-Resistant Weeds on Kansas Agriculture. *Weed Technology*, **13**, 632-635. <https://doi.org/10.1017/S0890037X00046315>
- [17] Duke, S.O. (1996) Herbicide-Resistant Crops ± Agricultural, Environmental, Economic, Regulatory, and Technical Aspects. CRC/Lewis Publishers, Boca Raton, FL.
- [18] Velini, E.D., Trindade, M.L.B., Alves, E., Catâneo, A.C., Marino, C.L., Maia, I.G., *et al.* (2005) Eucalyptus ESTs Corresponding to the Enzyme Glutamine Synthetase and the Protein D1, Sites of Action of Herbicides that Cause Oxidative Stress. *Genetics and Molecular Biology*, **28**, 555-561. <https://doi.org/10.1590/S1415-47572005000400010>
- [19] Ray, T. (1989) Herbicides as Inhibitors of Amino Acid Biosynthesis. In: Böger, P. and Sandman, G., Eds., *Target Sites of Herbicide Action*, CRC Press, Boca Raton, FL, 106-107.

- [20] Carbonari, C.A., Latorre, D.E., Gomes, G.L.G.C., Velini, E.D., Owens, D.K., Pan, Z. and Dayan, F.E. (2016) Resistance to Glufosinate Is Proportional to Phosphinothricin Acetyltransferase Expression and Activity in LibertyLink® and WideStrike® Cotton. *Planta*, **243**, 925-933. <https://doi.org/10.1007/s00425-015-2457-3>
- [21] Tachibana, K., Watanabe, T., Sekizawa, Y. and Takematsu, T. (1986) Accumulation of Ammonia in Plants Treated with Bialaphos. *Journal of Pesticide Science*, **11**, 33-38.
- [22] Tan, S., Evans, R. and Singh, B. (2006) Herbicidal Inhibitors of Amino Acid Biosynthesis and Herbicide-Tolerant Crops. *Amino Acids*, **30**, 195-204. <https://doi.org/10.1007/s00726-005-0254-1>
- [23] Dayan, F.E. and Zaccaro, M.L.M. (2012) Chlorophyll Fluorescence as a Marker for Herbicide Mechanisms of Action. *Pesticide Biochemistry Physiology*, **102**, 189-197. <https://doi.org/10.1016/j.pestbp.2012.01.005>
- [24] Pline, W.A., Wu, J. and Hatzios, K.K. (1999) Absorption, Translocation, and Metabolism of Glufosinate in Five Weed Species as Influenced by Ammonium Sulfate and Pelargonic Acid. *Weed Science*, **47**, 636-643. <https://doi.org/10.1017/S0043174500091268>
- [25] Dayan, F.E., Owens, D.K., Corniani, N., Silva, F.M.L., Watson, S.B., Howell, J.L. and Shaner, D.L. (2015) Biochemical Markers and Enzyme Assays for Herbicide Mode of Action and Resistance Studies. *Weed Science*, **63**, 23-63. <https://doi.org/10.1614/WS-D-13-00063.1>
- [26] Wendler, C., Barniske, M. and Wild, A. (1990) Effect of Phosphinothricin (Glufosinate) on Photosynthesis and Photorespiration of C₃ and C₄ Plants. *Photosynthesis Research*, **24**, 55-61. <https://doi.org/10.1007/BF00032644>
- [27] Gazziero, D.L.P. (1995) Procedures for Installation, Evaluation and Analysis of Experiments with Herbicides Brazilian Weed Science Society (SBCPD). Londrina-PR, Volume 1, 1-42.
- [28] Mitscherlich, E.A. (1909) Das gesetz des minimums und das gesetz des abnehmenden bodenertrages. *Landwirtsch Jahrbuch*, **38**, 537-552.
- [29] Gompertz, B. (1825) On the Nature of the Function Expressive of the Law of Human Mortality, and on a New Mode of Determining the Value of Life Contingencies Philosophical. *Transactions of the Royal Society B: Biological Sciences*, **115**, 513-583. <https://doi.org/10.1098/rstl.1825.0026>
- [30] Velini, E.D. (1995) Studies and Development of Experimental and Sample Methods Adapted to Matology. Thesis, São Paulo State University, Jaboticabal-SP, Brazil.
- [31] Avila-Garcia, W.V. and Mallory-Smith, C. (2011) Glyphosate-Resistant Italian Ryegrass (*Lolium perene*) Populations Also Exhibit Resistance to Glufosinate. *Weed Science*, **59**, 305-309. <https://doi.org/10.1614/WS-D-11-00012.1>
- [32] Ridley, S.M. and McNally, S.F. (1985) Effects of Phosphinothricin on the Isoenzymes of Glutamine Synthetase Isolated from Plant Species Which Exhibit Varying Degrees of Susceptibility to the Herbicide. *Plant Science*, **39**, 31-36. [https://doi.org/10.1016/0168-9452\(85\)90188-8](https://doi.org/10.1016/0168-9452(85)90188-8)
- [33] Franzoni, M.M. (2018) Aspects of Ammonium Glufosinate as the Main Control Tool for Weed Management in Soybean. University of São Paulo, Piracicaba, 51 p.
- [34] Fernandez, J., Odero, D., Macdonald, G., Ferrell, J. and Gettys, L. (2015) Confirmation, Characterization, and Management of Glyphosate-Resistant Ragweed Parthenium (*Parthenium hysterophorus* L.) in the Everglades Agricultural Area of South Florida. *Weed Technology*, **29**, 233-242. <https://doi.org/10.1614/WT-D-14-00113.1>
- [35] Reddy, K.N., Bryson, C.T. and Burke, I.C. (2007) Ragweed Parthenium (*Parthenium*

- hysterophorus*) Control with Preemergence and Postemergence Herbicides. *Weed Technology*, **21**, 982-986. <https://doi.org/10.1614/WT-07-053.1>
- [36] Oliveira Neto, A.M., Guerra, N., Dan, H.A., Braz, G.B.P., Jumes, T.M.C., Santos, G., *et al.* (2010) *Conyza bonariensis* Management with Glyphosate + 2,4-D and Glufosinate According to Development Stage. *Brazilian Herbicide Journal*, **9**, 73-80. <https://doi.org/10.7824/rbh.v9i3.87>
- [37] Coetzer, E., Al-Khatib, K. and Loughin, T.M. (2001) Glufosinate Efficacy, Absorption, and Translocation in Amaranthus Species as Affected by Relative Humidity and Temperature. *Weed Science*, **49**, 8-13. [https://doi.org/10.1614/0043-1745\(2001\)049\[0008:GEAATI\]2.0.CO;2](https://doi.org/10.1614/0043-1745(2001)049[0008:GEAATI]2.0.CO;2)
- [38] Bell, A.R., Nalewaja, J.D. and Schooler, A.B. (1972) Response of Kochia Selections to 2,4-D, Dicamba, and Picloram. *Weed Science*, **20**, 458-462. <https://doi.org/10.1017/S0043174500036134>
- [39] Kniss, A.R., Miller, S.D., Westra, P.H. and Wilson, R.G. (2007) Glyphosate Susceptibility in Common Lambsquarters (*Chenopodium album*) Is Influenced by Parental Exposure. *Weed Science*, **55**, 572-577. <https://doi.org/10.1614/WS-07-002.1>
- [40] Busi, R. and Powles, S.B. (2009) Evolution of Glyphosate Resistance in a *Lolium rigidum* Population by Glyphosate Selection at Sublethal Doses. *Heredity*, **103**, 318-325. <https://doi.org/10.1038/hdy.2009.64>
- [41] Busi, R., Porri, A., Gaines, T.A. and Powles, S.B. (2018) Pyroxasulfone Resistance in *Lolium rigidum* Is Metabolism-Based. *Pesticide Biochemistry and Physiology*, **148**, 74-80. <https://doi.org/10.1016/j.pestbp.2018.03.017>
- [42] Tehranchian, P., Norsworthy, J., Powles, S., Bararpour, M.T., Bagavathiannan, M.V., Barber, T. and Scott, R.C. (2017) Recurrent Sublethal-Dose Selection for Reduced Susceptibility of Palmer Amaranth (*Amaranthus palmeri*) to Dicamba. *Weed Science*, **65**, 206-212. <https://doi.org/10.1017/wsc.2016.27>