



Article

DIESEL, F.^{1*}
TREZZI, M.M.¹
GALLON, M.¹
BALBINOT JR., A.A.²
PAGNONCELLI, F.B.¹

TOLERANCE TO GLYPHOSATE IN BROADLEAF BUTTONWEED AND WHITE-EYE BIOTYPES

Tolerância ao Glyphosate em Biótipos de Erva-Quente e Poaia-Branca

ABSTRACT - Infesting species of Rubiaceae family have great importance in soybean crops in Southwest of Paraná and North of Santa Catarina, especially due to the selection of tolerant populations caused by the pressure of selection exerted by intensive use of glyphosate. The study aimed to evaluate the levels of tolerance to glyphosate in biotypes of broadleaf buttonweed (*Borreria latifolia*) (BL) and white-eye (*Richardia brasiliensis*) (RB) by means of dose-response curves. Two experiments were conducted in a greenhouse, in completely randomized design (CRD), with four replications. Fourteen BL biotypes and eleven RB biotypes were evaluated, collected in soybean fields of Paraná and Santa Catarina. The doses of glyphosate were 0, 74, 163, 360, 792 and 1,742 g ha⁻¹, applied when the plants had 6 to 8 leaves. Visual control was evaluated at 14 and 28 days after application (DAA), as well as mass of green shoots and mass of dry shoots at 28 DAA. It was verified that there is response variability among the biotypes studied. BL biotypes 277, 283 and 300, and RB biotypes 283, 285 and Papanduva were not controlled with glyphosate at doses superior to those normally used in crops (720 g ha⁻¹), indicating selection by the recurrent use of glyphosate. The tolerance factors ranged from 1.1 to 4.1 and 2.8 to 8.1 for RB and BL, respectively. The greatest difficulties of control were reported in areas where RR technology had been adopted for more than six years.

Keywords: *Borreria latifolia*, herbicide, *Richardia brasiliensis*, selection.

RESUMO - Espécies infestantes da família Rubiaceae apresentam grande importância em lavouras de soja no sudoeste do PR e norte de SC, especialmente devido à seleção de populações tolerantes ocasionada pela pressão de seleção exercida pelo uso intensivo do herbicida glyphosate. Este trabalho objetivou avaliar os níveis de tolerância ao glyphosate em biótipos de erva-quente (*Borreria latifolia*) (BL) e poaia-branca (*Richardia brasiliensis*) (RB) por meio de curvas de dose-resposta. Dois experimentos foram conduzidos em casa de vegetação, em delineamento inteiramente casualizado (DIC) com quatro repetições. Foram avaliados 14 biótipos de BL e 11 biótipos de RB coletados em lavouras de soja do PR e SC. As doses de glyphosate foram de 0, 74, 163, 360, 792 e 1.742 g ha⁻¹, aplicadas quando as plantas estavam com 6 a 8 folhas. Avaliou-se o controle visual aos 14 e 28 dias após a aplicação (DAA), e a massa da parte aérea verde e seca, aos 28 DAA. Há variabilidade de resposta entre os biótipos estudados. Os biótipos de BL 277, 283 e 300 e os biótipos de RB 283, 285 e Papanduva não foram controlados com o glyphosate em doses superiores à usualmente utilizada nas lavouras (720 g ha⁻¹), evidenciando seleção pelo uso repetitivo do glyphosate. Os fatores de tolerância variaram de 1,1 a 4,1 e 2,8 a 8,1 para BL e RB, respectivamente. As maiores dificuldades de controle foram relatadas em áreas onde a tecnologia RR tinha sido adotada há mais de seis anos.

Palavras-chave: *Borreria latifolia*, herbicida, *Richardia brasiliensis*, seleção.

* Corresponding author:
<francielli_diesel@hotmail.com>

Received: March 20, 2017
Approved: April 12, 2017

Planta Daninha 2018; v36:e018175310

Copyright: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided that the original author and source are credited.



¹ Universidade Tecnológica Federal do Paraná, Paraná-PR, Brasil; ² Empresa Brasileira de Pesquisa Agropecuária, Embrapa Soja, Londrina-PR, Brasil.

INTRODUCTION

Rubiaceae is one of the greatest angiosperm families, with around 13,000 species worldwide, and 1,500 are found in Brazil (Delprete, 1999; Souza and Lorenzi, 2005). Among the species found in Brazil with high economic value, we can highlight coffee tree (*Coffea* sp.), *ipecacuanha* (*Psychotria ipecacuanha*), and gardenia (*Gardenia jasminoides*), for instance. On the other hand, many others are considered weeds (Kissmann and Groth, 2000), such as genera *Borreria* (*Spermacoce*) and *Richardia*, which are of great relevance in Southwest of Paraná, especially in soybean fields. As they interfere negatively with the crop yield, there is need to control these infesting species.

Chemical control is one of the main methods employed for weed control. However, the intensive and improper use of herbicides can provoke negative effects on the environment, such as the selection of weed species that are tolerant and resistant to the different existent herbicide mechanisms of action (Ferreira et al., 2009). The pressure of selection exerted by a given herbicide on the weed community can cause increased proportion of a species over the others because of tolerance to the herbicide (Christoffoleti et al., 2008).

Tolerance, also defined as natural resistance (Duke, 2011), is a feature of the species even before the application of the herbicide in a given area (Vargas et al., 2009). On the other hand, resistance is the ability of a plant to survive and reproduce after exposition to a dose of herbicide normally fatal for its wild biotype (Christoffoleti et al., 2008). Nevertheless, existence of resistance presupposes that there is a population of a weed biotype previously susceptible to the herbicide (Heap and Lebaron, 2001; Gazziero et al., 2009), what does not occur with populations tolerant to herbicides.

Glyphosate is the most applied herbicide worldwide due to low cost, great efficiency and broad adoption, by farmers, of genetically modified crops (GMCs) with resistance to this herbicide. The highest risk related to the adoption of GMCs in Brazil and South America is due to the selection of weeds with resistance and tolerance to herbicides (Cerdeira et al., 2010). In areas with frequent use of glyphosate, as in genetically modified soybean (RR) fields, coffee trees and orchards areas, there has been selection of species tolerant to this product.

Modifications in the composition of weed populations and seed banks have been registered in several countries because of the intensive use of glyphosate (Cerdeira et al., 2007; Cerdeira et al., 2010; Webster and Nichols, 2012). In Brazil, the intensified use of glyphosate causes the interspecific selection of some weed species, as wandering-jew (*Commelina* spp.), morning glory (*Ipomoea* spp.), white-eye (*R. brasiliensis* Gomes) and broadleaf buttonweed (*B. latifolia* (Aubl.) K. Schum). The reason for differential tolerance was elucidated in some species, as *Commelina benghalensis*, *Ipomoea grandifolia* and *Amaranthus hybridus* (Monquero et al., 2004), *Ipomoea nil*, *Tridax procumbens* and *Spermacoce latifolia* (Galon et al., 2013), although the knowledge of the mechanism of tolerance has not been yet elucidated for others.

B. latifolia and *R. brasiliensis* have been documented in the literature as being tolerant to glyphosate (Sharma and Singh, 2001; Lacerda and Victoria Filho, 2004; Monquero et al., 2004; Cerdeira et al., 2010). Species or populations of the same weed, or even a single biotype, may present variability regarding tolerance to a given herbicide. In order to obtain control of around 90% of *Spermacoce latifolia* (sin. *B. latifolia*), the application of doses above 720 g a.e. (acid equivalent) ha⁻¹ is necessary (Lacerda and Victoria Filho, 2004). Applications of glyphosate (720 g a.e. ha⁻¹) to seedlings of *B. latifolia* with one-three and four-six leaves promoted control of 100% and 81%, respectively (Ramires et al., 2011).

Several studies conducted on *R. brasiliensis* have found that, regarding the species, there is also great variability of control by means of glyphosate. Use of glyphosate alone at a dose of 420 g ha⁻¹ resulted in 90% control at 14 DAA (Monquero et al., 2004). Vitorino et al. (2012) reported level of 99.5% control of white-eye at 28 DAA with the employment of glyphosate at a dose of 720 g ha⁻¹, and it was superior to the other alternative herbicides tested. On the other hand, use of glyphosate alone at a dose of 770 g ha⁻¹ presented low level of efficiency to control *R. brasiliensis*, only reaching 14% (Sharma and Singh, 2001).

In order to confirm the occurrence of resistance or differential tolerance, the performance of dose-response curves is recommended, and through them it is possible to know the dose needed

to control 50% population (C_{50}) as well as and the dose needed to reduce by 50% the population dry mass (GR_{50}) (Gazziero et al., 2009). These variables enable the calculation of either the resistance factor (RF) (Hall et al., 1998) or the tolerance factor (TF).

The hypothesis is that, due to selection for recurrent use of glyphosate, biotypes collected at different fields present differential tolerance to the herbicide. This study aimed at evaluating, through dose-response curves, the levels of tolerance to glyphosate of *B. latifolia* and *R. brasiliensis* biotypes.

MATERIAL AND METHODS

Two experiments were conducted between the months of January and April 2014. The species employed were *B. latifolia* and *R. brasiliensis*. The biotypes used in each experiment and their respective collection sites are listed in Table 1. The biotypes were collected in soybean fields with a history of glyphosate herbicide use and high infestation. *B. latifolia* and *R. brasiliensis* susceptible biotypes were obtained from the company Cosmos Agrícola Ltda., located in São Paulo/SP. *R. brasiliensis* biotype 271 was also considered susceptible for being collected at an area without history of use of glyphosate.

Concomitant with the collection of seeds of the species studied, questionnaires were applied to the owners of the areas so that to obtain information about the history of the management adopted by the farmers. After analysis of control levels, the biotypes highlighted in bold in the table were selected for presentation of the results since showed responses in contrast to the glyphosate (Table 1).

The experiments were conducted in a completely randomized design with four replicates in a 6 x 6 factorial, in which the first factor represented the number of biotypes of each species (*B. latifolia* and *R. brasiliensis*) (Table 1) and the second factor by six doses of glyphosate (0, 74, 163, 360, 792 and 1,742 g a.e. ha⁻¹).

Table 1 - Identification of the *B. latifolia* and *R. brasiliensis* biotypes used in the experiments and the respective collection sites

Specie	Identification of the biotype	Collection location
<i>Borreria latifolia</i>	263	São João/PR
	277	Renascença/PR
	280	Renascença/PR
	283	Pato Branco/PR
	284	Pato Branco/PR
	285	Itapejara D' oeste/PR
	287	Itapejara D' oeste/PR
	296	Rio Bonito do Iguaçu/PR
	300	Mariópolis/PR
	302	Pato Branco/PR
	Donato	Canoinhas/SC
	Major Oliveira	Major Oliveira/SC
	Papanduva	Papanduva/SC
	São Paulo*	São Paulo – Cosmos Agrícola
<i>Richardia brasiliensis</i>	271*	São João/PR
	277	Renascença/PR
	283	Pato Branco/PR
	285	Itapejara D' oeste/PR
	291	Nova Prata do Iguaçu/PR
	295	Rio Bonito do Iguaçu/PR
	Alvorada 2	Alvorada do Sul/PR
	Embrapa	Londrina/PR
	Major Oliveira	Major Oliveira/SC
	Papanduva	Papanduva/SC
	São Paulo – Cosmos*	São Paulo/SP

* Biotype considered sensitive to herbicide glyphosate. Bold: Biotypes with contrasting responses selected for presentation in Results and Discussion.

The seeds of *B. latifolia* were submitted to break of dormancy by heating at 60 °C for 30 min and subsequent immersion in 2% potassium nitrate for 3 h. The seeds of *R. brasiliensis*, in turn, do not need to overcome dormancy. The seeds were placed to germinate in gearbox-type boxes with double layer of germination paper moistened with distilled water, allocated in BOD-type germination chamber at 25 °C and photoperiod of 12 h. Around 15 days after germination, two seedlings of each species were transplanted into polyethylene pots with a capacity of 5 dm³, containing Distroferric Red Latosol collected at an area free of Rubiaceae species propagules. Thinning was performed 20 days after the transplantation, and only one plant per pot remained.

When the plants had six to eight completely expanded leaves, the treatments were applied with CO₂-pressurized backpack sprayer at 43 lbf pol⁻², with 1 m wide bar, 110.02-type fan nozzle tips, spaced 0.5 m apart, totaling a volume of 200 L ha⁻¹. Meteorological conditions in the beginning and end of the applications were as follows: air temperature (AT °C): 24 and 21.8; air relative humidity (RH %): 84 and 88.6.

At 14 and 28 days after application (DAA) of the treatments, visual control evaluations were conducted, based on the scale proposed by Frans et al. (1986), on which 0 represents null effect of herbicide symptoms on the plants and 100% represents their death. After the last evaluation (28 DAA), collection and weighing of the shoots of the plants were performed so that to obtain the mass of green shoots (GM). Subsequently, the plants were placed in an incubator at 60 °C until they reach constant mass, and the mass of dry shoots (DM) was obtained.

The visual control data were transformed into 100 - control, and GM and DM were expressed as percentage in relation to the control. The data obtained were submitted to the analysis of results variance by F test at 5% error probability through WinStat software (Machado and Conceição, 2005). The relation between quantitative factor levels and response variables was adjusted by non-linear regression, by means of SigmaPlot Version 10.0 software. The values of C₅₀ (dose required to achieve 50% control) and GR₅₀ (dose required to reduce DM by 50%) were calculated with help of the adjusted model. Thus, through the adjusted equations, C₈₀ or GR₈₀ were also calculated, using an Excel® spreadsheet.

The tolerance factor (TF) was calculated through the quotients of C₅₀ or GR₅₀ of each biotype by C₅₀ or GR₅₀ of the biotype most susceptible to the herbicide. This factor represents a comparative index of the biotype with greatest tolerance in relation to the biotype with lowest tolerance (Hall et al., 1998). There was also analysis of Pearson correlation between control data and DM data by means of Genes software (Cruz, 2006).

RESULTS AND DISCUSSION

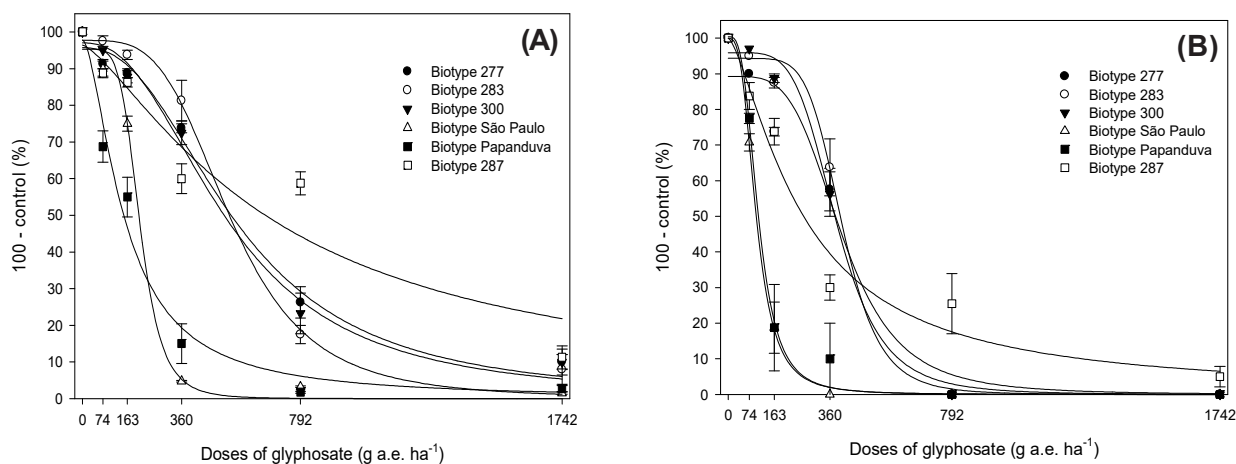
All variables analyzed for *B. latifolia* and *R. brasiliensis* presented significance of biotypes factor x doses at 5% probability.

Response of *B. latifolia* biotypes

There was high response variability of *B. latifolia* biotypes to glyphosate (Figure 1A and 1B). At 28 days after application (DAA), there was a considerable increase in the control of *B. latifolia* biotypes (Figure 1B), compared to 14 DAA (Figure 1A).

According as the herbicide doses were increased, it was possible to distinguish biotypes regarding tolerance. Both the evaluation performed at 14 days after application (DAA) and the one performed at 28 DAA presented superior control efficacy on biotypes São Paulo and Papanduva, that is, they showed greater susceptibility to glyphosate, while for biotypes 277, 287 and 283, the control efficacy was lower and, therefore, there was greater tolerance to the herbicide (Figure 1A and B). The dose of 360 g ha⁻¹ of glyphosate was strongly selective, both at 14 and 28 DAA, as it caused very large control differences between biotypes. For this dose, the control level for biotype São Paulo was around 100%, while the control levels for biotypes 283, 277 and 300 were between 15% and 26%.

The aforementioned results show variability of tolerance to glyphosate among *B. latifolia* biotypes collected at different locations of Paraná and North of Santa Catarina.



Vertical bars represent the standard error of the mean of each treatment.

Figure 1 - Control (100 - control) of *Borreria latifolia* biotypes at 14 DAA (A) and 28 DAA (B) in response to doses of glyphosate.

Analysis of the literature indicates that the control of *B. latifolia* is variable, depending mainly on the dose of glyphosate employed and on the development stage of the plants (Monquero et al., 2004; Lacerda; Victoria Filho, 2004; Cerdeira et al., 2010; Galon et al., 2013). A study developed by Lacerda and Victoria Filho (2004) evidenced need of doses superior to 540 g ha⁻¹ to obtain control near to 90% for *B. latifolia*, evidencing that this species shows intermediate susceptibility to glyphosate doses. The use of 720 g ha⁻¹ of glyphosate in seedlings of *B. latifolia* at one-three and four-six leaves development stage resulted in control levels of 100% and 81% at 35 DAA, respectively (Ramires et al. 2011), demonstrating the importance of the development stage to achieve good levels of control.

Not touching to C₅₀ values adjusted by SigmaPlot at 14 DAA (Table 2), the highest values were reached by biotypes 277, 283, 287 and 300, totaling 560.87; 540.45; 702.31 and 527.80 g ha⁻¹, respectively, while the biotype considered susceptible presented only 208.79 g ha⁻¹. In relation to C₈₀, the same biotypes stood out; however, biotype 287 presented C₈₀ value higher than the highest dose tested in this study, differing from the others. Biotypes Papanduva and São Paulo presented the lowest values of C₅₀ and C₈₀, in this order, with values of 153 and 350 g ha⁻¹ for Papanduva and 208 and 269 g ha⁻¹ for São Paulo, respectively.

Biotypes 277, 283, 287 and 300 showed the highest C₅₀ values adjusted by SigmaPlot at 28 DAA (Table 3), they being 403.73; 405.16; 267.09 and 384.80 g ha⁻¹, respectively, while the susceptible biotype showed C₅₀ of only 99.41 g ha⁻¹, much lower than the others. As shown by C₅₀, the great tolerance of biotype 287 stands out, requiring 709 g ha⁻¹ to reach 80% control, with need of a dose usually used in the fields (720 g ha⁻¹). However, biotypes São Paulo and Papanduva required approximately 160 g ha⁻¹ to reach 80% control. The tolerance factors (TF) varied between

Table 2 - Parameters of the adjusted equations, C₅₀ and C₈₀ for the control of *Borreria latifolia* biotypes at 14 DAA, in response to doses of glyphosate

Biotype	Parameter ⁽¹⁾				C ₈₀ **
	A	B	X0(C ₅₀)*	R ²	
277	95.69	2.38	560.87	0.99	981
283	97.68	3.76	540.45	0.99	775
300	97.82	2.48	527.80	0.99	912
São Paulo ⁽²⁾	95.32	5.20	208.79	0.99	269
Papanduva	97.84	1.65	153.89	0.98	350
287	96.40	1.35	702.31	0.92	>1742

⁽¹⁾ Logistic equation of three parameters. ⁽²⁾ Biotype considered sensitive. A = maximum asymptote; B = slope of the curve; C₅₀ = dose providing 50% control; C₈₀ = dose providing 80% control. * Values adjusted by Sigmaplot program. ** Estimated index through the Excel® spreadsheet.

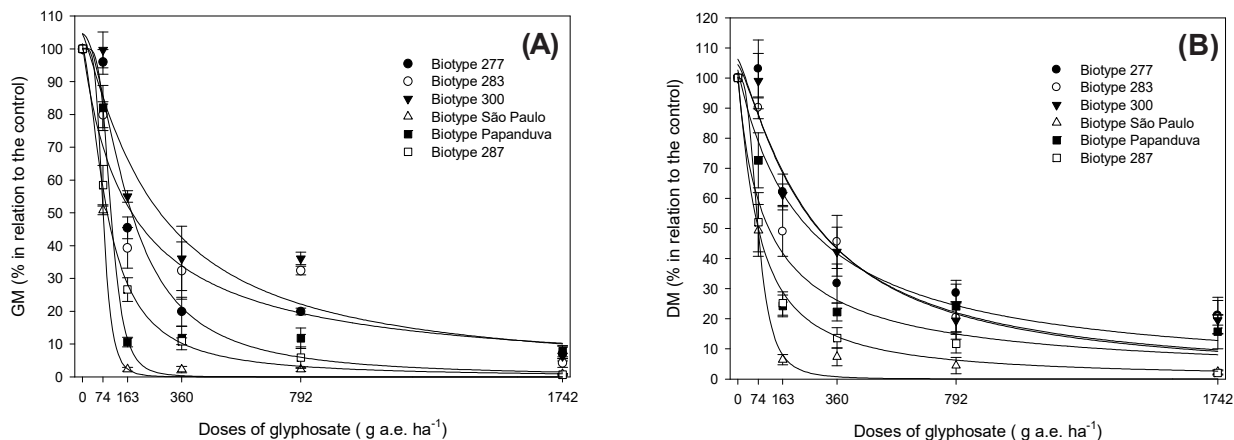
Table 3 - Parameters of the adjusted equations, C_{50} , C_{80} , tolerance factor (FT) and correlation (control x DM) for the control of *Borreria latifolia* biotypes at 28 DAA, in response to doses of glyphosate

Biotype	Parameter ⁽¹⁾				C_{80} **	FT	Control x DM
	A	B	$X0(C_{50})$ **	R^2			
277	89.27	4.02	403.73	0.96	380	4.1	0.88*
283	94.34	6.13	405.16	0.99	397	4.1	0.88*
300	95.93	4.76	384.80	0.99	378	3.9	0.92**
São Paulo ⁽²⁾	99.92	3.03	99.41	0.99	99	1.0	0.94**
Papanduva	100.43	3.19	106.76	0.99	107	1.1	0.97**
287	100.05	1.42	267.09	0.97	267	2.7	0.87*
General							0.91**

⁽¹⁾ Logistic equation of three parameters. ⁽²⁾ Biotype considered sensitive. A = maximum asymptote; B = slope of the curve; C_{50} = dose providing 50% control; C_{80} = dose providing 80% control; * Values adjusted by Sigmaplot program. ** Estimated index through the Excel® spreadsheet.

1.0, for biotype São Paulo, and 4.1, for biotypes 277 and 283 (Table 3), indicating that these biotypes required a dose four times greater than the susceptible biotype (São Paulo) to achieve 50% control.

The results of GM and DM reinforce the data presented for visual control, evidencing high variability of response to glyphosate among the *B. latifolia* biotypes tested. It is possible to verify there is a high reduction of GM (Figure 2A) and DM (Figure 2B) with increased doses of glyphosate. Biotypes 277, 283 and 300 stood out due to the smallest reductions of DM, of 79%, 79% and 81%, in this order, with use of the highest dose of glyphosate. Nevertheless, it is verified that biotype São Paulo showed approximately 90% DM reduction with only 163 g ha⁻¹, making its greater susceptibility to the herbicide evident.



Vertical bars represent the standard error of the mean of each treatment.

Figure 2 - Reduction of the mass of the green shoots (GM) (A) and mass of the dry shoots (DM) (B) of *Borreria latifolia* biotypes at 28 DAA, in response to doses of glyphosate.

Regarding GR_{50} values, it was verified that biotypes 277, 283 and 300 required the highest doses to reach 50% control, of 264.52; 246.59 and 256.78 g ha⁻¹, respectively. However, biotypes São Paulo, Papanduva and 287 presented GR_{50} inferior to 100 g ha⁻¹.

GR_{80} values, estimated by Excel Program, varied between 115 and 1,033 g ha⁻¹ (Table 4). Biotypes São Paulo and 287 were the most susceptible to the herbicide, presenting the lowest values of GR_{50} and GR_{80} . Biotypes 277, 300, 283 and Papanduva required doses greater than 800 g ha⁻¹ to reduce by 80% DM (GR_{80}), superior to that usually used in commercial fields. It is worth mentioning that biotype Papanduva, although presenting a low GR_{50} (99.26 g ha⁻¹), required

Table 4 - Parameters of the adjusted equations, GR_{50} , GR_{80} and tolerance factor (FT) for DM of the *Borreria latifolia* biotypes at 28 DAA, in response to doses of glyphosate

Biotype	Parameter ⁽¹⁾				GR_{80}^{**}	FT
	A	B	$X0(GR_{50})^*$	R^2		
277	106.28	1.22	264.52	0.91	876	3.7
283	102.58	0.99	246.59	0.93	1033	3.4
300	109.78	1.30	256.78	0.94	815	3.6
São Paulo ⁽²⁾	102.04	3.03	72.21	0.99	115	1.0
Papanduva	101.34	0.62	99.26	0.91	954	1.4
287	100.22	1.14	73.62	0.99	249	1.0

⁽¹⁾ Logistic equation of three parameters. ⁽²⁾ Biotype considered sensitive. A = maximum asymptote; B = slope of the curve; GR_{50} = dose providing 50% reduction of DM; GR_{80} = dose providing 80% DM reduction. * Values adjusted by Sigmaplot program. ** Estimated index through the Excel® spreadsheet.

954 g ha⁻¹ of glyphosate to reach GR_{80} , i.e., a 900% dose increase was required to achieve this reduction.

The tolerance factors (TF) varied between 1.0, for biotype São Paulo and 287, and 4, for biotypes 277, 283 and 300 (Table 4), indicating these biotypes required a dose four times greater than the susceptible biotype (São Paulo) for a 50% DM reduction.

No biotype had TF inferior to 1, that is, the biotype that was considered susceptible, acquired from a company that sells weed seeds, was effectively the most susceptible to glyphosate among those studied.

The correlation between the TFs calculated by visual control at 28 DAA and DM was 0.88 (Table 3). The TFs calculated by means of DM were slightly inferior to those calculated by the control variable, but the TFs obtained by both variables allowed the identification of the most tolerant biotypes.

The correlations between the visual control and DM for *B. latifolia* biotypes in the evaluation at 28 DAA were high and significant (Table 3). The values of the correlation coefficients for control x DM presented significance at 1% and 5%, ranging from 0.87 to 0.97, with a general correlation coefficient of 0.91.

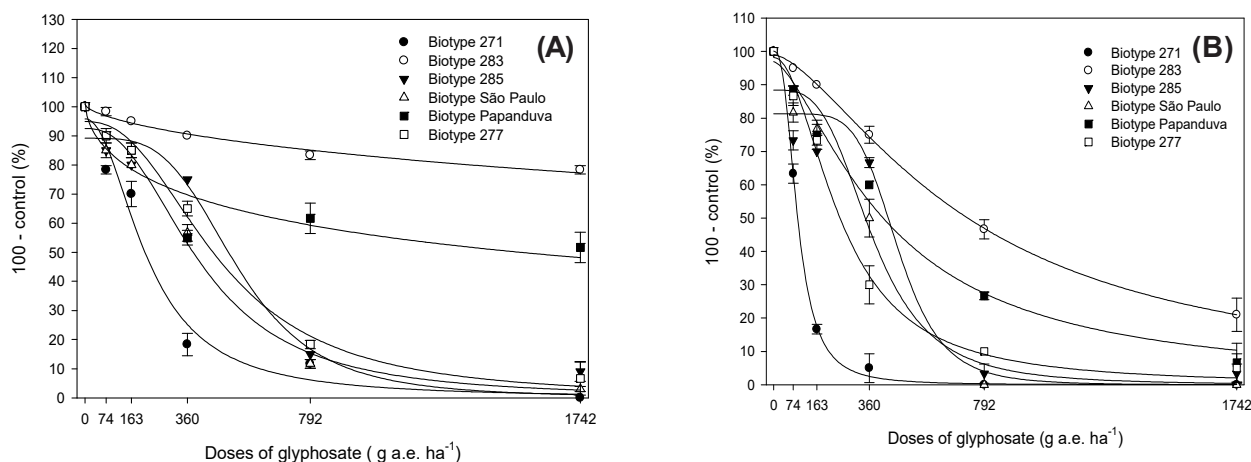
Response of *R. brasiliensis* biotypes

R. brasiliensis biotypes presented contrasting responses of control by glyphosate in the evaluation performed at 14 and 28 DAA (Figures 3A and 3B). A more pronounced control was observed at 28 DAA (Figure 3A), compared to 14 DAA (Figure 3B), making it possible to classify the biotypes regarding tolerance to glyphosate. Biotype 283 had unsatisfactory control levels at 14 and 28 DAA (Figure 3A and 3B), but biotypes São Paulo, 271 and 277 were satisfactorily controlled according as the doses were increased, evidencing their higher susceptibility to glyphosate.

It is important to emphasize that biotypes 271 and São Paulo were collected at areas with no glyphosate application history and, therefore, the higher tolerance in the other biotypes is probably associated with the selection pressure exerted by the herbicide.

The literature shows a great result variability of control of *R. brasiliensis* through glyphosate, because while many studies indicate the difficulty of controlling this weed with this herbicide, some indicate satisfactory levels of control at low doses. The greater tolerance presented by *R. brasiliensis* biotype 283 in this study is similar to that demonstrated in other studies with the same species, such as the study developed by Sharma and Singh (2001), where they observed that use of glyphosate alone at a dose of 577 g ha⁻¹ for control of *R. brasiliensis* presented low efficiency, reaching a maximum of 14% (Sharma and Singh, 2001).

However, 99.5% control of this weed at 28 DAA with 540 g ha⁻¹ of glyphosate, superior to other alternative herbicides tested, was verified by Vitorino et al. (2012). When studying the species



Vertical bars represent the standard error of the mean of each treatment.

Figure 3 - Control (100 - control) of the biotypes of *Richardia brasiliensis* at 14 DAA (A) and 28 DAA (B), in response to doses of glyphosate.

Richardia scabra, belonging to the same genus as *R. brasiliensis*, Reddy and Singh (1992) obtained control levels higher than 94%, with 375 and 750 g ha⁻¹ of glyphosate.

The contrasting results may be related to differences between biotypes, and also to the development stage of the plant at the time of application. The application of glyphosate alone at the dose of 420 g ha⁻¹ resulted in 60% control of *R. brasiliensis* plants at initial development stage at 7 DAA, and 90% at 14 DAA, but the dose of 2160 g ha⁻¹ of glyphosate did not efficiently control this weed in adult phase (Monquero et al., 2004).

C_{50} values adjusted by SigmaPlot at 14 DAA for biotypes Papanduva and 283 were greater than 1000 g ha⁻¹ (Table 5). However, the C_{50} value of the susceptible biotype was of only 219.7 g ha⁻¹.

In relation to C_{80} , the same biotypes stood out, presenting C_{80} value higher than the highest dose tested in this study. Biotypes 283 and Papanduva are included in the most tolerant group (C_{80} above 1000). The susceptible biotype showed C_{80} of 415 g ha⁻¹.

In the evaluation performed at 28 DAA, the control levels of the biotypes were increased, which resulted in C_{50} values adjusted by SigmaPlot lower than in the evaluation at 14 DAA. Biotypes 283, 285 and Papanduva showed the highest C_{50} values, totaling 735.56; 468.71 and 429.80 g ha⁻¹, respectively, while the susceptible biotype showed C_{50} of 90.56 g ha⁻¹ (Table 6). Biotipo 283 presented C_{80} higher than the highest dose used in the experiment (1742 g ha⁻¹), while biotipo 271, considered susceptible, required only 153 g ha⁻¹ to cause the same effect (Table 6).

Table 5 - Parameters of the adjusted equations, C_{50} and C_{80} for the control of the biotypes of *Richardia brasiliensis* at 14 DAA, in response to doses of glyphosate

Biotype	Parameter ⁽¹⁾				C_{80} **
	A	B	X0(C_{50})*	R ²	
271 ⁽²⁾	95.80	2.09	219.70	0.97	415
283	100.82	0.63	1403.93	0.97	>1742
285	89.13	3.78	538.29	0.96	747
São Paulo	92.51	2.42	406.48	0.98	692
Papanduva	101.67	0.56	1443.28	0.86	>1742
277	94.88	2.39	470.86	0.99	818

⁽¹⁾ Logistic equation of three parameters. ⁽²⁾ Biotype considered sensitive. A = maximum asymptote; B = slope of the curve; C_{50} = dose providing 50% control; C_{80} = dose providing 80% control. * Values adjusted by Sigmaplot program. ** Estimated index through the Excel® spreadsheet.

Table 6 - Parameters of the adjusted equations, C_{50} , C_{80} , tolerance factor (FT) and correlation (control x DM) for the control of the biotypes of *Richardia brasiliensis* at 28 DAA, in response to doses of glyphosate

Biotype	Parameter ⁽¹⁾				C_{80}^{**}	FT	Control X DM
	A	B	$X0(C_{50})^*$	R^2			
271 ²	100.07	2.64	90.56	0.99	153	1.00	0.86*
283	98.86	1.57	735.56	0.99	>1742	8.12	0.92**
285	81.29	5.62	468.71	0.93	572	5.18	0.49 ^{ns}
São Paulo	93.21	2.29	288.88	0.98	533	3.18	0.71 ^{ns}
Papanduva	96.94	1.52	429.80	0.98	1043	4.75	0.65 ^{ns}
277	98.21	2.00	254.57	0.99	503	2.81	0.86*
General							0.75**

⁽¹⁾ Logistic equation of three parameters. ⁽²⁾ Biotype considered sensitive. A = maximum asymptote; B = slope of the curve; C_{50} = dose providing 50% control; C_{80} = dose providing 80% control. * Values adjusted by Sigmaplot program. ** Estimated index through the Excel® spreadsheet.

There was an expressive reduction of DM with increased glyphosate doses (Figure 4), reinforcing the data presented for visual control, and it was also possible to demonstrate a high variability of glyphosate response among the *R. brasiliensis* biotypes evaluated.

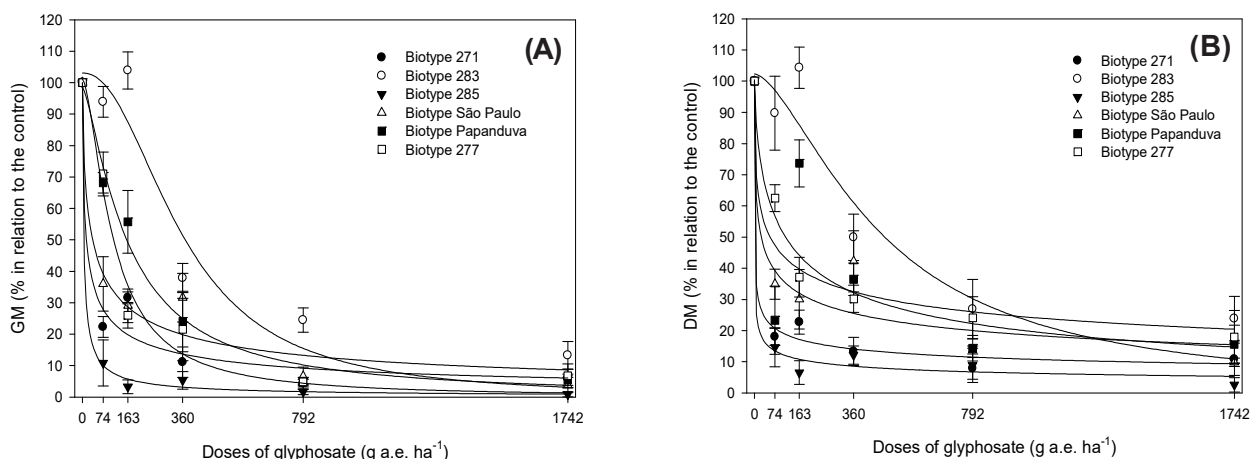
Biotype 283 presented the lowest reduction of DM with the employment of 792 g ha⁻¹, totaling 73%, while biotype 271 (susceptible) presented a 92% reduction. When the highest dose (1742 g ha⁻¹) was employed, biotype 283 presented the lowest DM reduction, i.e., 76%, while the susceptible biotype showed a 90% reduction.

GR_{50} values, estimated by Sigma Plot, varied between 0.23 (biotype 285) and 459.77 g ha⁻¹ (biotype 283). The values estimated by Excel for GR_{80} varied between 17 and > 1742 g ha⁻¹ (Table 7).

Biotype Papanduva showed a GR_{80} superior to the highest dose used, that is, it was not able to reduce 80% biotype DM. For comparison purposes, biotype 271, considered susceptible, presented GR_{80} of only 87 g ha⁻¹.

In *R. brasiliensis* species, the correlation coefficients between the visual control and DM for the biotypes in the evaluation at 28 DAA were smaller, and only three were significant (Table 6), varying between 0.49 and 0.92. The general correlation, considering all biotypes, was 0.75.

The aforementioned results evidenced the high variability of response to glyphosate between *B. latifolia* and *R. brasiliensis* biotypes. This variability can be attributed to the glyphosate selection process to which weed populations are submitted. From the late 1990s, the intensification of glyphosate use in soybean fields in South of Brazil increased with the widespread and rapid



Vertical bars represent the standard error of the mean of each treatment.

Figure 4 - Reduction of the mass of the green shoots (GM) (A) and mass of the dry shoots (DM) (B) of biotypes of *Richardia brasiliensis* at 28 DAA, in response to doses of glyphosate.

Table 7 - Parameters of the adjusted equations, GR_{50} , GR_{80} and tolerance factor (FT) for DM of the biotypes of *Richardia brasiliensis* at 28 DAA, in response to doses of glyphosate

Biotype	Parameters ⁽¹⁾				Biotype	FT
	A	Biotype	X0(GR_{50})*	R ²		
271 ⁽²⁾	99.99	0.29	0.79	0.99	87	1
283	102.31	1.59	459.77	0.89	1116	581.99
285	99.99	0.32	0.23	0.99	17	0.29
São Paulo	99.84	0.39	24.73	0.93	794	31.30
Papanduva	99.39	0.39	58.44	0.70	>1742	73.97
277	100.46	0.64	111.04	0.98	979	140.56

⁽¹⁾ Logistic equation of three parameters. ⁽²⁾ Biotype considered sensitive. A = maximum asymptote; B = slope of the curve; GR_{50} = dose providing 50% reduction of DM; GR_{80} = dose providing 80% DM reduction. * Values adjusted by Sigmaplot program. ** Estimated index through the Excel® spreadsheet.

adoption by farmers of genetically modified crops (GMC), which are resistant to glyphosate. The selection of weeds with resistance and/or tolerance to herbicide is considered the process of highest risky in the adoption of GMC in Brazil and South America (Cerqueira et al., 2010).

Since the introduction of genetically modified cultivars resistant to glyphosate in soybean cultivation, there has been selection of species tolerant to this herbicide, as day flower (*Commelina* spp.), morning glory (*Ipomoea* spp.), white-eye (*R. brasiliensis*), and broadleaf buttonweed (*B. latifolia*), among others. This phenomenon has occurred in other parts of the world, in species as *Ambrosia artemisiifolia* and *Sesbania exaltata*, for instance. In Brazil, there is also a great increase in the area infested with glyphosate-resistant biotypes, mainly in species of *Conyza* and *Digitaria insularis* (Correia et al., 2010; Lamego et al., 2013).

Weeds are biological organisms evolving in response to environmental changes (disturbance and stress), which determine changes in species composition in a given area (Radosevich et al., 2007). In addition, the recurrent use of the same herbicide or herbicides with the same mechanism of action and spectrum of weed control for several years causes changes in the infesting flora, since there is intensification of the process of selection of resistant weeds, tolerant to the sorting agent (Monquero et al., 2004).

In the literature, the distinctive responses among different studies evaluating the response of *B. latifolia* and *R. brasiliensis* can be attributed to several factors. Firstly, the genetic variability inherent to the biotypes. Secondly, the development conditions of the plants before and after the application of the herbicide, such as solar radiation, temperature, water availability, and relative humidity. Thirdly, the physiological stage of weeds at the time of application, as younger plants present lower defense to the action of the herbicides (Chauhan and Abughho, 2012). Dias et al. (2013) verified that day flower plants become four times more tolerant to glyphosate every ten phenological development units on the Biologische Bundesanstalt, Bundessortenamt and Chemical industry (BBCH) scale, adapted from Hess et al., 1997. In other words, chemical control measures at early stages of day flower growth reduce the dose of glyphosate and, consequently, its cost of application, with decrease in the selection pressure on biotypes tolerant to this herbicide, and reduction of damages to the agroecosystem.

Application of glyphosate with a maximum of three pairs of leaves resulted in a control level of 87.5% with 810 g ha⁻¹ in *Borreria densiflora*, of the same genus of *B. latifolia*, and preliminary observations of post-emergence applications showed that the weed becomes tolerant when it presents the phenology of 4-5 pairs of leaves, thus causing the failure of control by herbicides (Martins and Christoffoleti, 2014). Takano et al. (2013) observed that glyphosate alone resulted in 99.5% control of plants at the phenological stage above 10 leaves in *B. latifolia*, whereas *R. brasiliensis* presented only 82.5% control for the same conditions mentioned above.

There are at least six general mechanisms that may explain weed tolerance to herbicides: reduced absorption and translocation; increased metabolism of the herbicide for substances with less phytotoxic activity; compartmentalization of the herbicide molecule; lack of herbicide affinity with the specific site of action, and over expression of the target enzyme (Galon et al.,

2009; Monquero et al., 2004; Sandberg et al., 1980). A study developed by Galon et al. (2013) showed that *B. latifolia* had smaller glyphosate translocation than *B. pilosa* (a species considered susceptible to glyphosate), and approximately 89% sprayed product remained on the treated leaf 72 hours after application, and only 2% herbicide reached the roots, which may indicate that this is one of the mechanisms that provide greater tolerance to the herbicide. In *Ipomoea nil*, the same authors observed that glyphosate translocation occurred efficiently, and the tolerance mechanism could be associated with the metabolism or exudation of the product due to the large amount of herbicide that reached the species roots (Galon et al. 2013).

The mechanisms of tolerance of *C. benghalensis* to glyphosate are the absorption and differential metabolism of the herbicide when they become AMPA (aminomethylphosphonic acid), the main metabolite of glyphosate in some plants naturally tolerant to this herbicide), and *I. grandifolia* tolerance occurs due to the herbicide small translocation (Monquero et al., 2004).

In this research, several biotypes were used for the two species studied, which were sprayed at the same stage of development (six to eight completely expanded leaves), under similar ambient conditions. It is therefore evident that the control differences are related to the genetic variability inherent to the different populations, and may have caused situations of lower absorption, translocation and/or greater metabolism of the herbicide.

With the application of questionnaires to the owners that had analyzed biotypes on the management of the areas, it was possible to identify reasons that contributed to the differential tolerance to glyphosate.

Regarding the questionnaires answered, 67% reported a high degree of difficulty to control Rubiaceae family weeds, and 22% reported a medium degree of difficulty to control them, i.e., 89% analyzed areas presented infestations with Rubiaceae species as one of the main problems of control (Table 8). Another relevant point observed was the glyphosate doses used to manage the areas, ranging from 720 to 1620 g ha⁻¹, often without satisfactory control of Rubiaceae species present (Table 8). In areas with greater difficulty in controlling the Rubiaceae species, farmers were using doses above 1080 g ha⁻¹, level superior to those recommended for desiccation applications in the region. It was also evidenced that the areas where the samples were collected had different periods from the beginning of the adoption of RR technology in soybean fields, and that the period from the beginning of adoption was directly proportional to the doses used and to the difficulty of controlling the Rubiaceae species using glyphosate (Table 8), that is, the greatest control difficulties were reported in areas that RR technology had been adopted for more than six years.

Table 8 - Characteristics of collection points of tolerant and glyphosate-sensitive biotypes

Biotype	Difficulty to control	Doses of glyphosate used	Growing time of RR cultivars (years)	Tolerance factor*
<i>B. latifolia</i>				
277	High	4.0 – 4.5 L ha ⁻¹	8	4.1
283	High	4.0 – 4.5 L ha ⁻¹	9	4.1
300	High	3.5 – 4.0 L ha ⁻¹	7	3.9
São Paulo	–	–	–	–
Papanduva	Average	2.5 L ha ⁻¹ / sequential	2	1.1
287	Average	2.5 – 3.0 L ha ⁻¹	3	2.7
<i>R. brasiliensis</i>				
271	–	–	–	–
283	High	4.0 – 4.5 L ha ⁻¹	9	8.12
285	High	4.0 L ha ⁻¹	8	5.18
São Paulo	–	–	–	–
Papanduva	High	3.5 – 4.0 L ha ⁻¹ / sequential	6	4.75
277	Low	2.0 L ha ⁻¹	3	2.81

* FT = GR₅₀ biotype/GR₅₀ biotype S.

With the results of this study, it was possible to prove that *R. brasiliensis* biotype 271, collected at an area with no glyphosate application history, was the most susceptible among the biotypes studied, contributing to the affirmation that weed cropping and plant management systems in fields have influence on the selection of more tolerant biotypes.

Increasing the selection pressure of the herbicide on a population promotes survival of the most tolerant individuals and leads to the mortality of the most susceptible, which increases the frequency of tolerance genes in the surviving population, leading to the gradual increase in C_{50} value, similarly to what occurs with populations in which the herbicide resistance character is dominated by multiple genes (Neve et al., 2003). Thus, it is believed that the populations with the highest C_{50} values, detected in this experiment, are located at a more advanced stage of the glyphosate tolerance evolution process.

In recent times, the incidence of tolerant species and resistant populations in Brazil assumed quite large proportions, and levels of tolerance to worrisome herbicides have already been reached by several weeds throughout the country (Galon et al., 2013). Many pieces of research in the literature have studied the mechanisms of resistance to glyphosate in weed populations; however, little focus is given on the mechanisms of tolerance to this herbicide in weeds. It is very important to investigate the differences between biotypes of tolerant species collected in different situations of selection pressure, thus allowing the identification of changes in populations after increasing the selection pressure imposed by the herbicides.

The existence of populations with greater tolerance indicates need to conduct joint actions, whether by the farmers, through conscious use of herbicides, or by the companies, by providing a greater diversity of herbicides with differentiated mechanisms of action, as well as the interest of the academic community and research bodies in deepening the knowledge of biology and physiological mechanisms that confer this character to the weeds present in the cropping systems.

In general, variability of glyphosate herbicide response was observed among *B. latifolia* and *R. brasiliensis* biotypes collected at different locations in Paraná and Santa Catarina. Some biotypes of both species studied were not controlled with doses of glyphosate above those usually used in the crops, evidencing selection by recurrent use of glyphosate. The results showed a positive relation between the difficulty of controlling some biotypes of both species studied and the period of use of glyphosate in agricultural areas.

REFERENCES

- Cerdeira A.L. et al. Review of potential environmental impacts of transgenic glyphosate resistant soybean in Brazil. **J Environ Sci Health (B)**. 2007;42:539-49.
- Cerdeira A.L. et al. Agricultural impacts of glyphosate-resistant soybean cultivation in South America. **J Agric Food Chem**. 2010;59:5799-807.
- Chauhan B.S., Abugho S.B. Threelobe Morningglory (*Ipomoea triloba*) germination and response to herbicides. **Weed Sci**. 2012;60:199-204.
- Christofolletti P.J. et al. Glyphosate sustainability in South America cropping systems. **Pest Manage Sci**. 2008;64:422-7.
- Correia N.M. et al. Controle de plantas daninhas na cultura de soja resistente ao glyphosate. **Bragantia**. 2010;69:319-27.
- Cruz C.D. **Programa Genes: Biometria**. Viçosa, MG: Universidade Federal de Viçosa, 2006. 382p.
- Delprete P. The status of monographic and floistic studies of Neotropical Rubiaceae, with emphasis on the Flora of The Guianas. **Flora Guianas Newsl**. 1999;12:11-13.
- Dias A.C.R. et al. Fenologia da trapoeraba como indicador para tolerância a herbicida glyphosate. **Planta Daninha**. 2013;31:185-91.
- Duke S. Glyphosate degradation in glyphosate-resistant and -susceptible crops and weeds. **J Agric Food Chem**. 2011;59:5835-41.

- Ferreira E.A.F. et al. Manejo de plantas daninhas tolerantes ou resistentes ao glyphosate no Brasil. In: Velini E.D. et al. **Glyphosate**. Botucatu: Fepaf, 2009. p.357-400.
- Frans R.R. et al. Experimental design and techniques for measuring and analyzing plant responses to weed control practices. In: Camper N.D. **Research methods in weed science**. Champaign: Southern Weed Science Society, 1986. p.37-38.
- Galon L. et al. Tolerância de culturas e plantas daninhas a herbicidas. In: Agostinetto D., Vargas L. editores. **Resistência de plantas daninhas a herbicidas no Brasil**. Passo Fundo: BERthier, 2009.
- Galon L. et al. Glyphosate translocation in herbicide tolerant plants. **Planta Daninha**. 2013;31:193-201.
- Gazziero D.L.P. et al. Critérios para relatos oficiais estatísticos de biótipos de plantas daninhas resistentes a herbicidas. In: Agostinetto D., Vargas, L. editores. **Resistência de plantas daninhas a herbicidas no Brasil**. Passo Fundo: Berthier, 2009. p.91-101.
- Hall L.M. et al. Resistance to acetolactate synthase inhibitors and quinclorac in a biotype of false clover (*Gallium spurium*). **Weed Sci**. 1998;46:390-6.
- Heap I., Le Baron H. Introduction and overview of resistance. In: Powles S.B., Shaner D.L. editores. **Herbicide resistance and world grains**. Boca Raton: CRC Press, 2001. p.1-22.
- Hess M. et al. Use of the extended BBCH scale – general for the descriptions of the growth stages of mono- and dicotyledonous weed species. **Weed Res**. 1997;37:433-41.
- Kissmann K.G., Groth D. **Plantas infestantes e nocivas**. 2ª. ed. São Paulo: BASF, 2000.
- Lacerda A.L.S., Victoria Filho R. Curvas dose-resposta em espécies de plantas daninhas com o uso do herbicida glyphosate. **Bragantia**. 2004;63:73-9.
- Lamego F.P. et al. Manejo de *Conyza bonariensis* resistente ao glyphosate: coberturas de inverno e herbicidas em pré-semeadura da soja. **Planta Daninha**. 2013;31:433-42.
- Machado A.A., Conceição A.R. **WinStat: sistema de análise estatística para Windows. Versão Beta** [Software]. Pelotas: UFPEL, 2005.
- Martins B.A.B., Christoffoleti P.J. Herbicide efficacy on *Borreria densiflora* control in pre- and post-emergence conditions. **Planta Daninha**. 2014;32:817-25.
- Monquero P.A. et al. Absorção, translocação e metabolismo do glyphosate por plantas tolerantes e susceptíveis a este herbicida. **Planta Daninha**. 2004;22:445-51.
- Neve P. et al. Simulating evolution of glyphosate resistance in *Lolium rigidum* II: past, present and future of glyphosate use in Australian cropping. **Weed Res**. 2003;43:418-27.
- Radosevich S.R. et al. **Ecology of weeds and invasive plants**: Relationship to agriculture and natural resource management. 3rd ed. Hoboken: John Wiley & Sons, 2007.
- Ramires A.C. et al. Glyphosate associado a outros herbicidas no controle de *Commelina benghalensis* e *Spermacoce latifolia*. **Semina**. 2011;32:883-96.
- Reddy K.N., Singh M. Organossilicone adjuvants increased the efficacy of glyphosate for control of weeds in citrus (*Citrus* spp.). **Hortic Sci**. 1992;27:1003-5.
- Sandberg C.L. et al. Absorption, translocation and metabolism of ¹⁴C-glyphosate in several weed species. **Weed Res**. 1980;20:195-200.
- Sharma S.D., Singh M. Surfactants increase toxicity of glyphosate and 2,4-D to Brazil pusley. **HortScience**. 2001;36:726-8.
- Souza V.C., Lorenzi H. **Botânica sistemática**: guia ilustrado para identificação das famílias de angiospermas da flora brasileira, baseado em APG II. Nova Odessa: Instituto Plantarum, 2005.
- Takano H.K. et al. Efeito da adição do 2,4-D ao glyphosate para o controle de espécies de plantas daninhas de difícil controle. **Rev Bras Herbic**. 2013;12:1-13.

Vitorino H.S. et al. Eficiência de herbicidas no controle de plantas daninhas latifoliadas em mamona. **Arq Inst Biol.** 2012;79:127-31.

Vargas L. et al. Resistência de plantas daninhas a herbicidas. In: Agostinotto D., Vargas L. **Resistência de plantas daninhas a herbicidas no Brasil.** Passo Fundo: Berthier. 2009. p.9-36.

Webster T.M., Nichlos R.L. Changes in the prevalence of weed species in the major agronomic crops of the Southern United States: 1994/1995 to 2008/2009. **Weed Sci.** 2012;60:145-57.