Original Article

CONTROL OF *Conyza bonariensis* WITH GLYPHOSATE ASSOCIATED TO ADJUVANTS APPLIED WITH DIFFERENT SPRAY NOZZLES

CONTROLE DE Conyza bonariensis *COM GLYPHOSATE ASSOCIADO A ADJUVANTES APLICADOS COM DIFERENTES PONTAS DE PULVERIZAÇÃO*

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ABSTRACT: The study aimed to evaluate the control of *Conyza bonariensis* in delayed post-emergence stage using different spray nozzles and solutions with and without adjuvants; and to verify the occurrence of resistance to glyphosate, determining the dose-response curve. The experimental design was randomized in factorial (6 spray nozzles x 5 spray solution) in twenty repetitions. The treatments were represented by spray nozzles: XR 110015 XR 11002, TT 11002 AIXR 11002, AIC 11002 and AI 11002. Tested spray solution were: glyphosate; glyphosate + Nimbus®; glyphosate + Agral®; Glyphosate + LI-700® and without herbicide application. The use or not of adjuvants in the spray solution did not differ statistically from the solution containing the herbicide only. The spray nozzles AIXR 11002, TT 11002 and XR 11002 presented best results of control the horseweed from the evaluation of 07 days after application (DAP) and 28 DAP and such spray nozzles maintained the best average of control percentage. The higher dry matter reductions of horseweed were obtained by spray nozzles 110015 XR, TT 11002 and 11002 XR compared with the control. The population evaluated presents biotypes that resist to doses of 2.880 g.ha^{-1} a.e. of glyphosate. It is concluded that the evaluated biotypes presents tolerance to glyphosate, independent of your association or not with adjuvants.

KEYWORDS: Horseweed. Glyphosate. Resistance. Surfactante. Spray Systems.

INTRODUCTION

The herbicide resistance has become one of the main problems encountered in the agricultural sector in weed populations in Brazil. In commercial areas, the use of glyphosate for weed control has increased significantly since the allowance for genetically modified cultivars was granted, then, the intense frequency with which this product started being used caused the selection of biotypes of weeds such as the horseweed (*Conyza bonariensis*) which are tolerant/resistant to its active ingredient, resulting in the growth of the population in cultivation areas and the decrease in productivity in crops (TREZZI et al., 2011; CORREIA et al., 2010).

The allowance for planting genetically modified soybean tolerant to glyphosate started with the harvest of 2005/06 (ADEGAS et al., 2010). This new cultivation technique intensified the use of glyphosate on crops in post-emergent applications. This soybean tolerance to the glyphosate action mechanism is only possible because of the introduction of the gene (AroA) whose origin is in the genome *Agrobacterium* sp., lineage CP4, in its DNA, which encodes a variant of the enzyme EPSPs which causes the plant to be tolerant to the herbicide (PADGETTE et al. 1995).

The resistance shown by the biotypes of weeds is natural and hereditary, since within a population there are biotypes which genetically show a resistance that allows them to survive after their exposure to herbicide doses that are lethal to other individuals from the same species. Therefore, these biotypes resistance to these herbicides is a result of the gene present in resistant biotypes of the population, the herbicide not being a causative agent, but its intensive use acts as a selecting agent of resistant individuals which have low initial frequency within the population (MOREIRA et al., 2007).

The application techniques to be adopted in order to obtain a good uniformity of phytosanitary product applications, thus ensuring the solution deposition on the target, should be selected according to the weed morphological parameters which are present in the growth area, such as their height, shape and leaves size (GILO et al., 2016). Adding to this, the combination of adjuvants with herbicide solutions, especially glyphosate, has become a common practice that aims at increasing control efficiency and reducing the dose of the

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product, since the adjuvants act on the physicochemical features of the solution, increasing the surface wettability and reducing superficial tension and the contact angle of the drop, as well as enhancing the penetration capacity of the active ingredient through the plant cuticle (RODRIGUES-COSTA et al., 2011; MINGUELA; CUNHA, 2010).

Given the above, the study aimed to evaluate the control of *C. bonariensis* at the delayed post-emergence stage, using different nozzles and solutions with and without adjuvants; as well as to verify the occurrence of resistance to glyphosate, determining the dose-response curve.

MATERIAL AND METHODS

The study was conducted in two trials in the years 2014 and 2015, at agricultural nursery, being evaluated the percentage of control of *Conyza bonariensis* and the determination of the dose-response curve. The trials were conducted in the agricultural nursery in galvanized steel structure (8,00 m x 18,00 m x 3,50 m), closing on 45 Monofilament screen black color 50% shading. Seeds of *C. bonariensis* were collected in areas of crops with a history of resistance in the municipality of Itaporã-MS (22°5’54,54” S and 54°47’51,83” W).

Trial 1 – Percentage of control of *Conyza bonariensis*

The experimental design was completely randomized in a factorial arrangement (6 spray nozzles x 5 spray solution) in 20 repetitions (Tables 1 and 2) distributed in five pots, each plant represented an experimental unit.

The treatments of the spray solutions consisted of 720 g.ha^{-1} of acid equivalent (a.e.) of the glyphosate herbicide, applied without adjuvants and with association of agricultural use adjuvants in concentration of 0.2% (v/v), in addition control without herbicide application (Table 1). The application of spray solutions occurred with the aid of a pressurized backpack sprayer by CO_2, calibrated to the working pressure (kPa), flow rate (L.min^{-1}), application volume (L.ha^{-1}) and displacement speed at 3.6 km.h^{-1} (Table 2).

Table 1. Different adjuvants in spray solution for the control of *Conyza bonariensis*.

Commercial product	Composition	Solution
-	-	Without application
Roundup Original®	Salt of isopropilamina of N	*Glyphosate
Nimbus®	Mineral oil	Glyphosate + Nimbus®
Agral®	Nonil fenoxi poli etanol	Glyphosate + Agral®
LI-700®	Lecitina and Propiônico Acid	Glyphosate + LI 700®

*v = volume; p.c. = commercial product.

Table 2. Spray nozzles and calibration used in the application of spray solution.

*Tipe of nozzles	Pressure (kPa)	Drop Size	Nozzle Flow (L.min^{-1})	Solution volume (L.ha^{-1})
XR 110015	180	Thin	0.46	153
XR 11002	100	Medium	0.46	153
TT 11002	100	Thick	0.46	153
AIXR 11002	100	Extremaly Thick	0.46	153
AIC 11002	200	Ultra Thick	0.65	217
AI 11002	200	Ultra Thick	0.65	217

* Spray nozzles jet Plano Teejet®. Drop size rating based on BCPC's specifications and in accordance to the Norm ASABE S572.1.

The application of solutions was performed at 64 days after sowing, when the plants were in the delayed post-emergence phenological stage, with about 30 cm. After spraying the plants returned to nursery. The pots were not irrigated for 24 hours to ensure the absorption of the herbicide, being thereafter irrigated daily. Environmental conditions during application in the trial were: temperature of 31.2°C, relative humidity of 70.75% and wind speed of 1.2 km.h^{-1}.

The percentage of control was obtained with visual evaluations following the the SBCPD method (1995), which is attributed notes to the according to symptoms in the plants at 07, 14, 21 and 28 days after treatment application. The variable effect of the herbicide on the plant was evaluated by determining the dry weight of shoot after being dried in forced air ventilation oven at 65 ± 2 ºC during 72 hours.

Trial 2 - Determination of dose-response curve

For analyzing the levels of tolerance/resistance of the weeds biotypes to glyphosate, the population's dose-response curve was developed, following the method described by Lacerda & Victoria Filho (2004). The experimental design used was completely randomized, with 6 doses in increasing scales of 0, 45, 90, 360, 720 and 2880 $g.ha^{-1}$ of acid equivalent (a.e) of glyphosate herbicide evaluated with a backpack sprayer pressurized by CO_2, equipped with nozzle XR 11002, calibrated at pressure 100 kPa, flow rate 0.46 $L.min^{-1}$ and solution volume 153 $L.ha^{-1}$The treatments were represented by groups of five pots, each pot contained four plants totaling 20 repetitions per treatment, each plant was considered an experimental unit.

The application of solutions occurred when the plants were in post-initial phenological stage with six defined leaves. The data on environmental conditions for implementation of the trial of determination of dose-response curve were monitored by the weather station installed in the unit, being measured at the application time temperature of 23.9 ºC, relative humidity of 91.2 % and wind speed of 2.4 km/h.

After application, the pots remained inside the environment without irrigation during 24 hours to ensure herbicide absorption, and thereafter were irrigated daily. The percentage of control was obtained with visual evaluations following SBCPD method (1995), which was attributed notes according to the symptoms in plants at 07, 14 and 21 days after treatment application. At 21 days, we performed the determination of green biomass with the help of a balance with precision of 0.0001g. Then, they were placed in a paper bag and taken to forced air ventilation oven at 65 ± 2 ºC during 72 hours, obtaining dry biomass.

The average data obtained from control assessments were submitted to analysis of variance by F test and Tukey test at 5% probability of error, by the software program Statistical Analysis System (SAS – versão 9.02). For determining the dose-response curve was used the mathematical log-logistic model proposed by SEEFELDT (1995):

$$Y= C + (D-C) / (1 + Exp\{b[log(X)-log(RC_{50})]\})$$

Wherein: Y – plant responde; X – herbicide dose; D - upper limit curve; C – lower limit curve; b - curve declivity; RC_{50} - dose required to reduce 50% of the weed growth compared to the control.

RESULTS AND DISCUSSION

Significant differences were observed at 1% of error probability when using different spray nozzles for the control of *C. bonariensis*. The evaluation of the spray solutions containing glyphosate herbicide associated or not with adjuvants showed significant results when compared with the control, however, statistically speaking, there were no significant differences amongst the products (Table 3).

Table 3. Percentage of control of *C. bonariensis* at 7, 14, 21 and 28 days after application according to the nozzles and products used.

Spray Nozzles	**D7**	**D14**	**D21**	**D28**
AI 11002	15.65 c	34.45 c	42.41 c	48.16 d
AIC 11002	17.24 bc	36.25 bc	44.76 c	51.94 cd
AIXR 11002	20.28 a	40.56 bc	50.69 bc	58.15 bcd
TT 11002	19.01 ab	44.88 ab	54.74 ab	63.16 ab
XR 110015	12.03 d	35.08 c	50.40 bc	58.85 bc
XR 11002	20.00 a	50.33 a	61.00 a	70.71 a
Spray solution	**D7**	**D14**	**D21**	**D28**
Withou application	0.00 b	0.00 b	0.00 b	0.00 b
Glyphosate + Agral	16.81 a	37.88 a	48.57 a	56.44 a
Glyphosate + LI 700	17.94 a	44.48 a	51.94 a	60.58 a
Glyphosate + Nimbus	17.72 a	40.29 a	51.76 a	58.22 a
Glyphosate	17.00 a	38.41 a	50.42 a	58.77 a

	F calculated			
Nozzles	76.62**	149.47**	35.01**	36.55**
Solution	314.36**	532.14**	203.35**	205.77**
Nozzles x Solution	1.08^{NS}	0.09^{NS}	4.23^{NS}	2.75^{NS}
Mean	16.67	38.65	48.64	56.16
CV (%)	25.42	41.7	34.8	31.94

D 7 = 7 days D 14 = 14 days; D 21= 21 days; D 28= 28 days. Means followed by the same letters in the same column do not differ by Tukey test at 5% probability. ^{NS} = not significant; ** = significant at 0.01; CV = coefficient of variation.

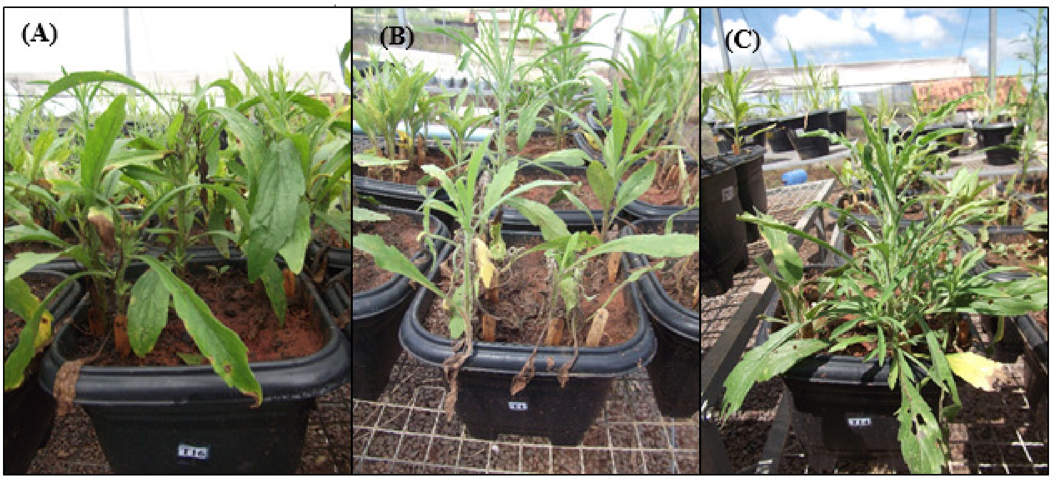

Figure 1. Symptoms of the treatments: (A) nozzle AIXR 11002, Glyphosate + LI 700; (B) nozzzle XR 110015, Glyphosate; (C) nozzle AIXR 11002, Glyphosate + Agral® in plants of *C. bonariensis*.

By assessing the spray nozzles (Table 3), we found that the nozzles AIXR 11002, TT 11002 and XR 11002 showed the best control results from the evaluation at 7 days after application (DAA). At 28 DAA, the nozzle XR 11002 stood out, having the highest average control, statistically higher than those obtained by the models XR 110015, AI 11002, AIC 11002 and AIXR 11002, not differing significantly from the model TT 11002. Possibly this result is related to the glyphosate concentration difference shown in the solution volume applied with induction and without induction nozzles (Table 2), where models AIC 11002 and AI 11002 require solution volume higher than the models XR 110015, XR 11002, TT 11002 and AIXR 11002 to meet the glyphosate application recommendations. Another factor is the difference in uniformity of the drop spectrum presented by the spray nozzle models, where XR 11015, XR 11002 and TT 11002 form more uniform drops which are classified according to their manufacturers as thin, medium and thick (Table 2). The fact that these drops have a higher glyphosate active ingredient concentration and better uniformity of spray solution deposition resulted in plants with higher control average since there was a greater absorption of the active ingredient by the plant.

Costa et al. (2008), achieved good results of control when studying the effect of spray nozzles in desiccation of *Brachiaria brizantha* cv. Marandu, using the models AI 11002 and XR 11002. The authors highlight the good uniformity of the spray solution deposition promoted by the model XR 11002, a result of its increased production of medium and thin drops. On the other hand, the AI 11002 model showed the smallest drop deposition on target, but due to its runoff and increased interception of the drops by the leaves and stems, this model had the highest average of distribution uniformity. Souza et al. (2007) mentions in their study that irregularities in deposits caused by nozzles of thick and extremely thick drops may require a higher applied dose in order to ensure satisfactory levels of control, especially if the herbicide used is of contact.

The use or not of adjuvants in solution (Table 3) did not differ statistically from the solution containing only the herbicide. Possibly this was due to the tolerance/resistance to glyphosate that the biotype tested presents (Figure 2). Symptoms of chlorosis in meristematic regions followed by foliar necrosis observed in Figure 1 reflect the performance of the herbicide active ingredient on meristematic regions and on young leaves of the plant. This fact is similar to the description of the glyphosate action mode carried out by Cardinali (2009), in which it acts by blocking the shikimic acid cycle due to the competition of its molecule for the same site of action, inhibiting the action of 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPs). Thus, plants which are susceptible to the glyphosate action mechanism die because of the lack of aromatic amino acids phenylalanine, tryptophan and tyrosine, generating shikimic acid accumulation as a consequence. On the other hand, there is no shikimic acid accumulation in plants which are resistant.

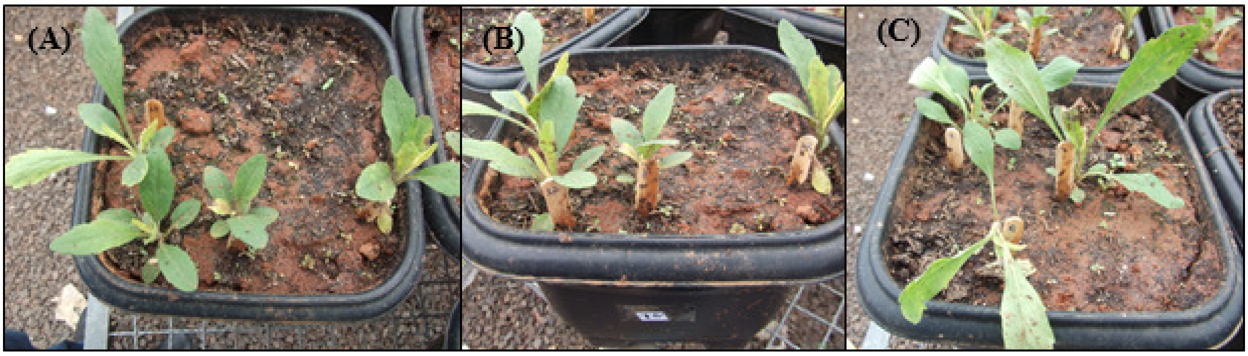

Figure 2. Symptoms (A); (B); (C) of the treatment with 2,880 g.ha^{-1} a.e. of glyphosate in determining the dose-response curve of *C. bonariensis*.

The spray nozzles proved to be efficient to reduce the green and dry biomass of *C. bonariensis* (Table 4). There were no statistical differences with or without the addition of adjuvants to solution, the same result occurred with the interaction between spray nozzles and the products used in the composition of the solution. Biomass reduction effect was expressed by means of lower values, it is then understood that the growth and development of the weed were harmed by the herbicide's active ingredient action in its metabolism.

Table 4. Green mass (GM) and dry mass (DS) according to nozzles used in the control of *Conyza bonariensis* with glyphosate.

Spray Nozzles	**GM (g plant^{-1})**	**DM (g plant^{-1})**
XR 11002	1.8593 d	0.5466 c
TT 11002	1.9359 cd	0.5800 c
XR 110015	2.4108 cd	0.6224 c
AIXR 11002	2.8023 bcd	0.7178 bc
AIC 11002	3.8000 abc	0.8621 bc
AI 11002	5.3166 a	1.2778 a
Without application	4.6230 ab	1.0445 ab
Spray Solution	**GM (g plant^{-1})**	**DM (g plant^{-1})**
Glyphosate + Nimbus	2.7218^{NS}	0.7244^{NS}
Glyphosate	2.8561	0.7029
Glyphosate + Agral	3.0477	0.7740
Glyphosate + LI 700	3.4548	0.8694
Without application	4.6230	1.0445
	F calculated	
Nozzles	10.83**	10.62**
Solution	1.85^{NS}	1.74^{NS}
Nozzles x Solution	0.57^{NS}	0.59^{NS}
Mean	3.08	0.78
CV (%)	110.54	90.43

Means followed by the same letters in the same column do not differ by Tukey test at 5% probability. ^{NS} = not significant; ** = significant at 0.01; CV = coefficient of variation.

When evaluating the nozzles used, the model 11002 XR once again excelled in reducing green mass, its mean being statistically higher when compared to those obtained with the use of the nozzles AI 11002, AIC 11002 and Control. When assessing the dry mass, the nozzles with air induction AI 11002, AIC 11002 and AIXR 11002 did not differ statically from Control. The higher reductions of dry mass were obtained by XR 110015, TT 11002 and XR 11002 when compared to Control, but they were not statistically different from each other. The means obtained with the use of the nozzle AI 11002 were higher than those of Control due to the presence of biotypes resistant to glyphosate in the treatment with solutions, as biotypes were not controlled, they developed and elevated the means of the green and dry biomass (Table 4). According to Bauer et al. (2006), the nozzle type used affects the deposition of the drops on the plants. This fact is dependent on a good coverage uniformity and drop size provided by the spray nozzles. Thus, it is possible to observe in the results that nozzles forming thin to thick drops (Table 2) led to higher green and dry mass reductions. Thus, we observed that the choice of the spray nozzle for controlling *C. bonariensis* achieved significant results, but the success in the control is not only due to the usage of the application technology, but also due to using different management actions in areas where the problem is, such as the use of herbicides with different action mechanisms, sequential application and crop rotation.

For the parameters obtained by the equation of the dose-response curve (Table 5 and Figure 2), the results of the analyzes of variance showed that there was a significant ($p>0.01$), by the mathematical model chosen for the glyphosate doses used to check the control and growth percentage, through reducing the green and dry mass. Analyzing the parameters of the evaluations carried out at 7, 14 and 21 days, we notice that the dose required to reduce 50% of the weed growth in relation to Control (RC 50) and the curve declivity (b) were the same. This allows us to infer that after the glyphosate active ingredient absorption by the plant, its operations in the plant metabolism may be observed at 07 days after its application (DAA), and in the subsequent evaluations at 14 and 21 DAA the symptoms become more visible. This fact can be understood by the coefficient of variation (CV) obtained in the evaluations, in which, as the assessment intervals became more distant from the application date, the accuracy of visual assessment through the scale increased. Thus, in order to determine the dose-response curve, it is suggested that the assessment be carried out at 28 DAA, as it has proved to be more adequate for verifying the findings researched in this study.

Table 5. Analyzes of variance of the regression model and parameters obtained from the dose-response curve equation according to the percentage of *Conyza bonariensis* at 7, 14 and 21 days and green and dry mass.

Parameters	07 days	14 days	21 days	Green mass	Dry mass
D	10.14	32.75	39.43	1.66	0.33
C	42.60	64.00	71.75	0.61	0.14
Upper limit	6.62	28.08	34.61	1.41	0.28
Lower limit	50.46	74.45	82.54	1.21	0.25
RC 50	2,190.00	2,190.00	2,190.00	2,190.00	2,190.00
b	1,200.00	1,200.00	1,200.00	1,200.00	1,200.00
R^2	0.40	0.56	0.66	0.37	0.36
F calculated	15.43**	28.66**	44.60**	13.19**	12.57**
CV (%)	108.86	47.03	35.94	75.62	64.41

^{NS} = not significant; ** = significant at 0.01; CV = coefficient of variation.

When evaluating *C. bonariensis* regarding its sensitivity to the glyphosate active ingredient (Table 5), using the RC50 parameter found by the dose response curve equation at 21 days as reference, the obtained value was 2,190 g.ha^{-1} a.e. of herbicide to provide 50% of population control. RC50 parameters for the variables of green and dry mass were identical to the ones of control percentage; however, the R2 model adjust values were low. This result occurred because of the absence of vegetal material from plants susceptible to herbicide, which decomposes rapidly with the influence of the region's climatic conditions where the study was carried out. The value obtained in this study was higher than the one found by Lacerda & Victoria Filho (2004), who found that the highest RC50 was obtained by *Commelina benghalensis* (1,440 g.ha^{-1} a.i.) when evaluating the dose-response curve to glyphosate in different weed species. This difference shows that the amount of the dose to obtain RC50 for *C. bonariensis* is 1.52 times greater than the one used by the authors, which allows us to infer that plants of *C. bonariensis* from the farming region of Itaporã-MS show tolerance to the glyphosate active ingredient.

WSSA (1998) apud Domingos (2014) defines the resistance of plants to herbicides as "the inherited ability of a plant to survive and reproduce after exposure to a dose of herbicide which is normally lethal to the wild variant". On the other hand, the herbicide tolerance is defined as "the ability inherited from one species to survive and reproduce after herbicide application. This implies that there was no genetic selection or manipulation to cause the plant to become tolerant to herbicides; it is naturally tolerant". This fact corroborates the results obtained in this study, since the glyphosate acted on the metabolism and the physiology of the plant, the symptoms were obeserved in meristematic regions and young leaves (Figure 2).

Dose-response curves of *C. bonariensis* with the application of glyphosate doses at 07, 14 and 21 DAA were similar in all control percentage assessments because RC50 and b values obtained from the regression parameters were the same (Figure 3). The population evaluated had biotypes which were susceptible, tolerant and resistant. Susceptible biotypes were fully controlled. On tolerant biotypes the apical tissues were affected, which stimulated the sprouting in lateral buds, increasing the dispersion of seeds generated from the new stems. On the other hand, the resistant biotypes have not suffered damage to growth and development, maintaining the main stem. The percentage of control was proportional to dose, being more expressive at 21 DAA, at dose of 2,880 g.ha^{-1} a.e. However, due to the tolerance/resistance of the weeds to glyphosate, the percentage of control did not exceed 61% of the population.

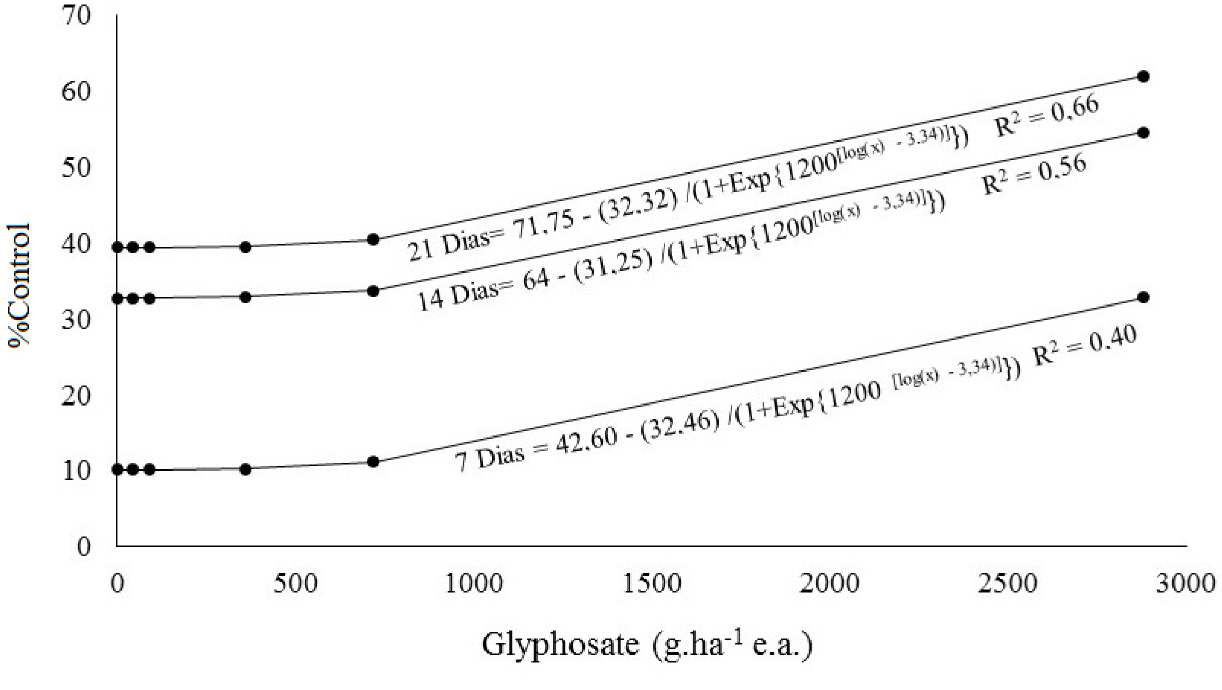

Figure 3. Dose-response curves for *Conyza bonariensis* treated with glyphosate.

CONCLUSIONS

The nozzles XR 11002 and TT 11002 showed the best results per control percentage of *Conyza bonariensis*.

The use or not of adjuvants in solution containing glyphosate did not show significant results that justify its use in controlling the population assessed, because the plants of *Conyza bonariensis* showed tolerance / resistance to glyphosate, regardless of the spray nozzle model and the association or not of adjuvants to the solution.

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RESUMO: O estudo teve como objetivo avaliar o controle de *Conyza bonariensis* no estádio pós-emergência tardia, empregando diferentes pontas e caldas com e sem adjuvantes; e verificar a ocorrência de resistência ao glyphosate, determinando a curva dose resposta. O delineamento experimental utilizado foi o inteiramente casualizado em esquema fatorial (6 pontas de pulverização x 5 caldas de pulverização) em vinte repetições. Os tratamentos foram representados pelas pontas de pulverização: XR 110015, XR 11002, TT 11002, AIXR 11002, AIC 11002 e AI 11002. As caldas testadas foram: glyphosate; glyphosate + Nimbus®; glyphosate + Agral®; glyphosate + LI-700® e sem aplicação do herbicida. O uso ou não de adjuvantes na calda não diferiram estatisticamente da calda contendo somente o herbicida. As pontas AIXR 11002, TT 11002 e XR 11002 apresentaram melhores resultados de controle da buva a partir de 07 dias após a aplicação (DAP) e aos 28 DAP e tais pontas mantiveram as melhores médias de porcentagem de controle nas demais avaliações. As maiores reduções de massa seca de buva foram obtidas pelas pontas XR 110015, TT 11002 e XR 11002 quando comparada com a testemunha. A população avaliada apresenta biótipos que resistem a doses de 2.880 g.ha^{-1} de e.a. do glyphosate. Conclui-se que os biótipos avaliados apresentam tolerância ao glyphosate, independente de sua associação ou não com os adjuvantes.

PALAVRAS-CHAVE: Buva. Aditivos. Resistência. Surfactante. Tecnologia de Aplicação.

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