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Hairy fleabane (*Conyza bonarienis*) response to saflufenacil in association with different formulations of glyphosate subjected to simulated rainfall

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Abstract. Saflufenacil has been used to control glyphosate resistant weeds, including hairy fleabane. There are several glyphosate formulations, which are related to different salts and adjuvants. Differences between these formulations may result in variations in efficacy, especially in the face of unfavorable environmental conditions, such as the occurrence of unexpected rainfall after application. The objective of this study was to evaluate the control of hairy fleabane with saflufenacil in tank mix with different formulations of glyphosate subjected to simulated rainfall after application. The treatments evaluated were salts of glyphosate (isopropylamine, potassium and ammonium salt), different periods of simulated rainfall after herbicide application (30, 120 and 240 min, and no rainfall), and the presence or absence of saflufenacil and a non-ionic adjuvant. Absorption of saflufenacil by hairy fleabane is rapid, since simulated rainfall after 30 min after application did not result in loss of efficiency when the herbicide is applied with adjuvant. The association of isopropylamine and ammonium salts of glyphosate with saflufenacil increases the control of hairy fleabane and prevents the occurrence of regrowth when rainfall occurs at 30 min after the application. The occurrence of simulated rainfall after 240 min reduces the glyphosate efficiency by 30%, 15% and 60% for the isopropylamine, potassium and ammonium salt formulations, respectively. The addition of adjuvant improves the efficiency of glyphosate salt of potassium by 40%. The response of the mixture of glyphosate and saflufenacil is variable, mainly in situations of rainfall after application.

Key words: EPSPS, herbicide absorption, PPO, tank mix, weed resistance.

INTRODUCTION

Hairy fleabane [(*Conyza bonariensis* L. Cronquist (Asteraceae)] is one of the most important weeds in both spring and summer crops, such as soybean and corn. This specie is originated from South America, with annual or perennial life cycle and a great reproductive capacity. Each plant can produce approximately 600,000 seeds (Kaspary et al., 2017), that are easily dispersed by the wind. In addition to *C. bonariensis*, other important *Conyza* species, such as *C. canadensis*, *C. sumatrensis* and *C. albida*, are important weeds in several countries (Mylonas et al., 2014; Okada et al., 2014; Santos et al., 2014).

In addition to their great adaptive capacity to diverse environments and great capacity of reproduction, several populations of *Conyza* are resistant to glyphosate (Heap, 2020). Glyphosate is an inhibitor of the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSPS), which is responsible for the synthesis of the aromatic amino acids phenylalanine, tyrosine and tryptophan (Holländer & Amrhein, 1980). It is a non-selective, systemic herbicide used mainly in pre-sowing burndown or post-emergence of glyphosate resistant crops (GR), and also in integrated weed management programs (Vanaga et al., 2006; Duke & Powles, 2008). The glyphosate resistance in species of *Conyza* genus mainly involves non-target-site mechanisms, including reduction of translocation and sequestration of the herbicide in the vacuole, which reduce the herbicide concentration in the chloroplasts of meristem cells (Ge et al., 2011; Kleinman & Rubin, 2017; Moretti & Handson, 2017). However, mechanisms related to the target site have already been verified (Dinelli et al., 2008; Sammos & Gaines, 2014).

Although the occurrence of glyphosate resistant weeds is common, this herbicide remains the most widely used in the world (Viirlaid et al., 2015; Benbrook, 2016), since most of the weed community is mostly sensitive to this herbicide. In addition, glyphosate contributes to the control of resistant weeds, when associated with other herbicides, which, when applied alone, have lower efficiency in comparison with the application in mixture. However, some associations may cause antagonism, reducing herbicide efficiency. This response usually occurs when a contact herbicide, which causes oxidative stress, is associated with glyphosate (Eubank et al., 2013). This is due to the limited absorption and translocation of glyphosate, which is impaired by the rapid action of the contact herbicide (Werlang & Silva, 2002). Combinations of glyphosate with inhibitors of protoporphyrinogen oxidase (PPO), photosystems I and II, or glutamine synthetase (GS), usually result in antagonism (Starke & Oliver, 1998; Vidal et al., 2016).

Saflufenacil has been considered as an alternative for the management of hairy fleabane and other glyphosate-resistant or -tolerant weeds (Geier et al., 2009; Dennis et al., 2016). Although saflufenacil is a PPO inhibitor, its physic-chemical characteristics allow some mobility by xylem and phloem, allowing its association with glyphosate (Ashigh et al., 2010; Grossmann et al., 2010). Even though the hairy fleabane is a glyphosate resistant species, the addition of glyphosate to saflufenacil is necessary to prevent plant regrowth in later stages of development (Dalazen et al., 2015). However, while there are several studies that evaluate the effect of the glyphosate mixture with saflufenacil on the control of several weed species, including *Conyza* (Waggoner et al., 2011; Eubank et al., 2013; Dalazen et al., 2015), information about the effect of the different formulation of glyphosate in mixture with saflufenacil are restricted in the literature.

Glyphosate is commercially available in various formulations, which vary in acid equivalent concentrations, adjuvants and salt type. The addition of a salt to the glyphosate molecule is necessary because of the low solubility of this compound. Glyphosate in its acid form has solubility of 1.54% at 25 °C, whereas formulations with addition of different salts increase the water solubility to more than 90% (Shaner, 2014). The main salts used are isopropylamine, ammonium and potassium, and the efficiency of these formulations varies according to the weed species (Molin & Hirase, 2004; Li et al., 2005; Mueller et al., 2006; Mahoney et al., 2014).

The several formulations of glyphosate have distinct absorption and translocation patterns, which may influence the control efficiency under adverse conditions. The occurrence of rainfall after herbicide application can compromise the weed control, making reapplications necessary in some situations (Pacanoski & Mehmeti, 2019). In addition, the use of adjuvant and variations in formulation may alter the weed control response (Monquero & Silva, 2007; Souza et al., 2014). For glyphosate, periods between one and six hours are required, depending on the factors mentioned previously (Pedrinho Júnior et al., 2002). For PPO inhibiting herbicides, such as saflufenacil, absorption is faster, requiring one to two hours of rain free periods in order to maintain the herbicide efficacy (Grossmann et al., 2010).

In this way, the objectives of this study were: i) to evaluate the effect of different formulations of glyphosate in association with saflufenacil on hairy fleabane control; ii) to determine the effect of simulated rainfall after the application of glyphosate and saflufenacil on hairy fleabane control; iii) to evaluate the effect of adjuvant to the mixture of glyphosate and saflufenacil on hairy fleabane control.

MATERIAL AND METHODS

Plant Material. A hairy fleabane seeds were collected in RS state, Brazil (27° 66’ 37” S; 53° 44’ 29” W), in a soybean production area with a glyphosate resistant hairy fleabane population, with a resistance factor (RF) of 3.98 (Dalazen et al., 2019). It was decided to use resistant plants, because in Brazil the vast majority of the populations of hairy fleabane are resistant to glyphosate. F2 generation seeds were used in this experiment. Although dormancy in hairy fleabane seeds is not common, to improve germination seeds were immersed in water and kept at temperature of 6 °C, during four days. After, seeds suspended in water were placed in the surface of trays, containing organic substrate, which was sieved and moistened close to the field capacity. The trays were kept at a temperature of 24 °C and a 12/12 hours photoperiod (day/night) until the stage of two leaves. The seedlings (three-leaf stage) were transplanted individually to 250 mL punctured pots, containing substrate produced from the mixture of haplic gleisoil and organic compound (10:1), in addition to NPK fertilizer 5-20-20 at 2.5 g kg^{-1} substrate. The plants were kept in a greenhouse until they reached 15 cm height, at which moment they were submitted to treatments. The environmental conditions inside the greenhouse were 25 °C ± 3 °C temperature and 14/10 hours photoperiod (day/night). The pots were kept in trays with a 0.5 cm layer of water, to keep the substrate moist.

Treatments and Experimental Design. The experiment was repeated twice, during the spring of 2014 and 2015 years, under the same conditions. The experimental design was completely randomized in a factorial scheme, with four replications. The factor A was the different formulations of glyphosate: isopropylamine salt (Roundup Original®, 360 g ae L^{-1}, Monsanto Brazil, São Paulo); potassium salt (Roundup Transorb R®, 480 g ae L^{-1}, Monsanto Brazil, São Paulo); and ammonium salt (Roundup WG®, 720 g ae kg^{-1}, Monsanto Brazil, São Paulo). All the formulations were applied at the dose of 720 g ae glyphosate ha^{-1}. The factor B was formed by 20-mm-rainfall applied at different periods after application of the herbicides (30, 120 and 240 minutes, and no rainfall). The factor C was the presence or absence of saflufenacil (Heat®, 700 g ai kg^{-1}, Basf, São Paulo, Brazil) at 35 g ia ha^{-1}. And factor D was the presence or absence of non-ionic adjuvant at 0.5% v/v (Dash®, mixture of methyl esters, aromatic hydrocarbon, unsaturated fatty acid and 933 g L^{-1} surfactant, Basf, São Paulo, Brazil).

The spraying of herbicides was carried out by an automated spray chamber (Greenhouse Spray Chamber, model Generation III, Devries Manufacturing, Hollandale, MN), pressurized with compressed air at 32 psi, with a Teejet® 8002E nozzle, at velocity of 1.16 m s^{-1}, resulting in a spray volume of 150 L ha^{-1}.

Data Analysis. The evaluations of control were performed at 7, 14, 21 and 28 days after the application of the treatments (DAT) using Frans et al. (1986) scale, in which zero means no control and 100 means total control of the plants. At 28 DAT, the aerial part was harvested to determinate the shoot dry mass (SDM). Plants were maintained in a dryer at 60°C until reaching a constant mass. Data were analyzed for normality (Shapiro-Wilk test) and then subjected to ANOVA by the *F test* ($P < 0.05$) using the softwere Assistat (version 7.7) (Silva & Azevedo, 2006). Percentage control data has been transformed to arcsen (x+1). There was no significant difference between both experiments and data were pooled.

RESULTS AND DISCUSSION

ANOVA indicates the occurrence of interaction among the variation factors (Table 1). A unique pattern of effect was not identified, and the focus of the analysis was related with the target objectives.

Table 1. Summary of variance analysis table (mean squares) for control of *Conyza bonariensis* at 7, 14, 21 and 28 days after the application of treatments (DAT) and shoot dry mass (SDM)

Mean squares						
VF [a]	DF[b]	7 DAT [c]	14 DAT	21 DAT	28 DAT	SDM [d]
Rainfall (R)	3	534 ***	26.71 **	54.65 **	59.33 **	2.15 **
Formulation (F)	3	7718 ***	163.84 **	122.35 **	108.65 **	3.67 **
Saflufenacil (S)	1	227112 ***	1640.87 **	2187.30 **	2503.34 **	77.09
Adjuvant (A)	1	8453 ***	2.93 **	7.31 **	11.62 **	0.80 **
R × F	9	724 ***	3.80 **	8.07 **	8.57 **	0.78 **
R × S	3	817 ***	24.51 **	53.17 **	52.37 **	1.12 **
F × S	3	3701 ***	0.07 ^{ns}	0.62 ^{ns}	0.36 ^{ns}	0.23 ^{ns}
R × A	3	1311 ***	166.55 **	121.77 **	102.82 **	6.41 **
F × A	3	1146 ***	0.38 ^{ns}	3.63 **	8.27 **	2.27 **
S × A	1	1575 ***	0.22 ^{ns}	0.61 ^{ns}	0.68 ^{ns}	0.01 ^{ns}
R × F × S	9	314 ***	3.27 **	7.15 **	7.16 **	0.81 **
R × F × A	9	223 ***	0.34 ^{ns}	0.65 ^{ns}	0.59 ^{ns}	0.36 **
R × S × A	3	138 ***	0.19 ^{ns}	0.36 ^{ns}	0.87 ^{ns}	0.37 **
F × S × A	3	2594 ***	2.12 **	5.32 **	11.93 **	1.92 **
F × R × S × A	9	735 ***	0.31 ^{ns}	0.81 *	1.15 *	0.57 **
Treatments	63	39.91 **	45.50 **	54.46 **	58.83 **	2.46 **
Residue	192	0.22	0.24	0.35	0.57	0.10
Mean		48.80	61.07	55.44	52.00	1.31
CV (%) [e]		7.27	6.83	8.56	11.27	20.20

** significant at the 1% probability level ($p < 0.01$); * significant at the 5% probability level ($p < 0.05$); ^{ns} not significant ($p > 0.05$).

[a] VF: variation factor; [b] DF: degrees of freedom; [c] DAT: days after application of treatments; [d] SDM: shoot dry mass; [e] CV (%): coefficient of variation.

Hairy fleabane control at 7 DAT demonstrates the rapid effect of saflufenacil (Fig. 1). Even with the occurrence of rain 30 min after the application, hairy fleabane control was higher than 70%. The addition of adjuvant improved saflufenacil efficiency by 12 to 20%, except when rainfall occurred at 30 min after application (Fig. 1, A). The addition of glyphosate in both isopropylamine (IPA) and potassium (K) salts to saflufenacil increased the control by about 10% (Fig. 1, B, C). However, the addition of the ammonium (NH_4) salt of glyphosate resulted in a control reduction of approximately 15%, when the simulated rainfall occurred at 30 and 120 min after application (Fig. 1, D). For the treatments with absence of saflufenacil, the application of glyphosate K salt resulted in better hairy fleabane control (Fig. 1, C), with greater effect as the interval between the application and the occurrence of simulated rainfall increased. The addition of adjuvant was positive only in this glyphosate formulation, with an increase of 10 to 20% in relation to the application without adjuvant.

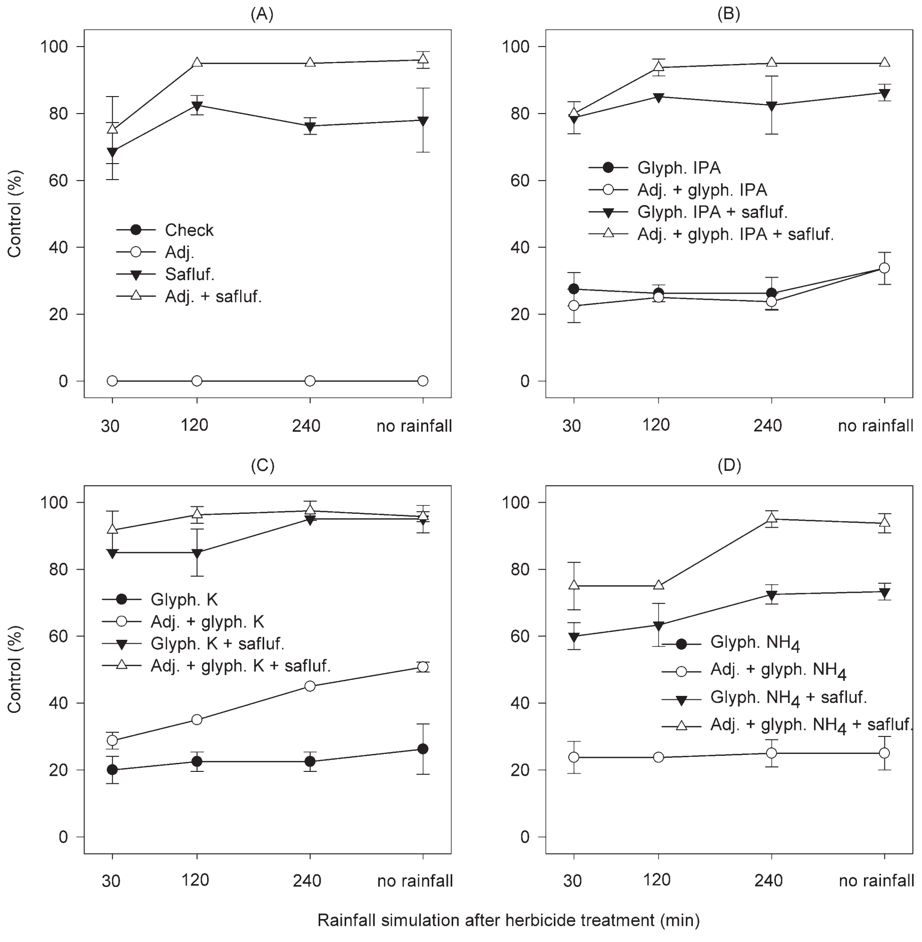

Figure 1. Hairy fleabane control at 7 days after treatment, in response to saflufenacil in association with glyphosate formulations, adjuvants and simulated rainfall. A: without glyphosate; B: glyphosate isopropylamine salt (IPA); C: glyphosate potassium salt (K); and D: glyphosate ammonium salt. Error bars smaller than the size of the symbol are not shown.

At 14 DAT, the application of saflufenacil with adjuvant resulted in 100% control, regardless the simulation of rainfall after application (Fig. 2, A). However, without the addition of adjuvant, the control was 5 to 10% lower, according to the time of simulated rainfall. The addition of glyphosate IPA (Fig. 2, B) and K (Fig. 2, C) salts to saflufenacil, even without adjuvant, also resulted in satisfactory control, approaching 100%, despite the occurrence of rainfalls after herbicide application. In the case of glyphosate NH_4 salt in mixture with saflufenacil, the control reached 100% only with the addition of adjuvant, except in the occurrence of rain at 30 min after spraying, in which the control was close to 90% (Fig. 2, D). The addition of adjuvant to glyphosate, as at 7 DAT evaluation, only increased the control in the plants treated with glyphosate K salt (Fig. 2, C). The occurrence of simulated rainfall significantly reduced glyphosate efficiency, regardless its formulation. The control reached approximately 65% with absence of simulated rainfall, and it was reduced 10 to 25% with the occurrence of rain 30 minutes after application.

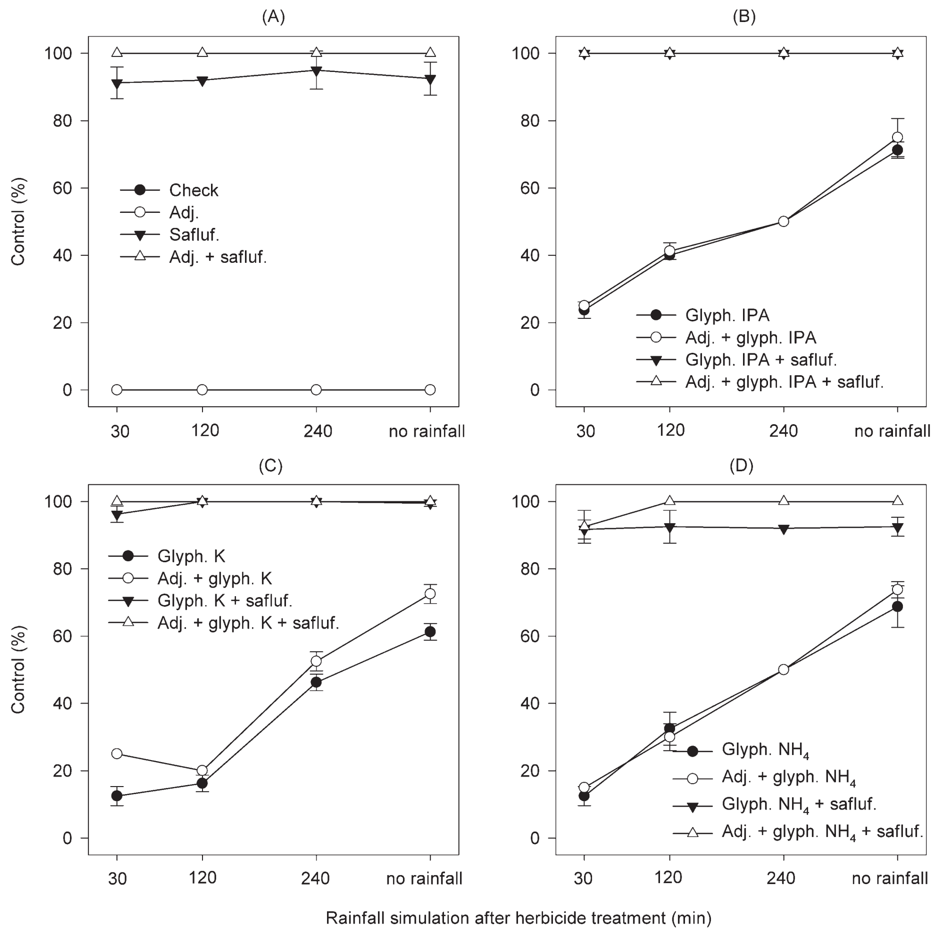

Figure 2. Hairy fleabane control at 14 days after treatment, in response to saflufenacil in association with glyphosate formulations, adjuvants and simulated rainfall. A: without glyphosate; B: glyphosate isopropylamine salt (IPA); C: glyphosate potassium salt (K); and D: glyphosate ammonium salt. Error bars smaller than the size of the symbol are not shown.

At 21 DAT the control was similar to the observed on 14 DAT (Fig. 3). The addition of adjuvant to saflufenacil resulted in total control of the plants, even with rainfall simulation at 30 min after herbicides spraying (Fig. 3, A). In the treatments that combined saflufenacil with glyphosate, the control was total, independent of glyphosate formulation, addition of adjuvant, and simulated rainfall occurrence (Figs 3, B, C, D).

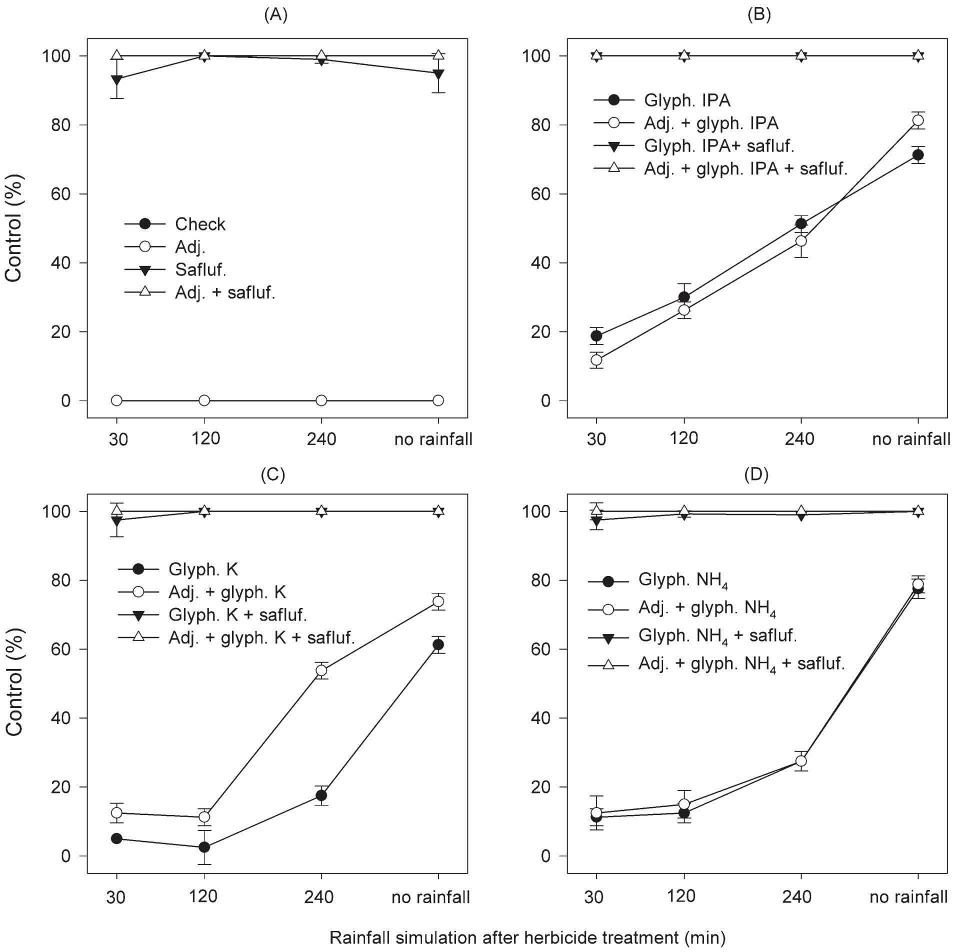

Figure 3. Hairy fleabane control at 21 days after treatment, in response to saflufenacil in association with glyphosate formulations, adjuvants and simulated rainfall occurrence. A: without glyphosate; B: glyphosate isopropylamine salt (IPA); C: glyphosate potassium salt (K); and D: glyphosate ammonium salt. Error bars smaller than the size of the symbol are not shown.

The evaluation at 28 DAT (Fig. 4) was the most important, because provides information about complete plants death or regrowth after herbicide application. The results showed that the addition of adjuvant is essential when saflufenacil is applied alone (Fig. 4, A) or in combination with glyphosate K salt (Fig. 4, C), and occurrence of rainfall 30 min after application. Plants that received these treatments without addition of adjuvant have survived and were able to regrowth. For other glyphosate formulations, the control was 100%, regardless the addition of adjuvant and simulated rainfall occurrences after application (Figs 4, B, D). As in other evaluations, the addition of adjuvant significantly increased the control over plants that were treated with glyphosate K salt (Fig. 4, C). With the occurrence of simulates rainfall at 240 min after the application of this herbicide, the addition of adjuvant resulted in increase of approximately 40% in the hairy fleabane control. For other glyphosate formulations, the addition of adjuvant increased the control at 10 to 15%, varying according with rainfall occurrences after application.

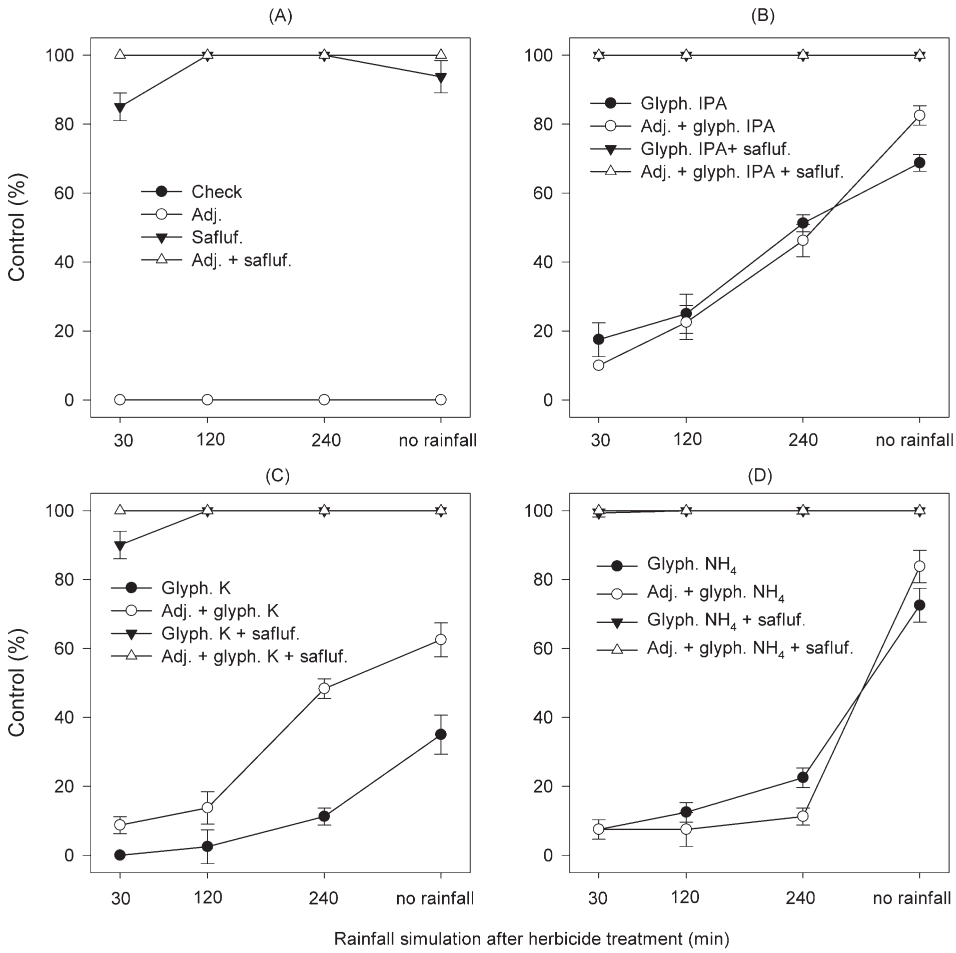

Figure 4. Hairy fleabane control at 28 days after treatment, in response to saflufenacil in association with glyphosate formulations, adjuvants and simulated rainfall occurrence. A: without glyphosate; B: glyphosate isopropylamine salt (IPA); C: glyphosate potassium salt (K); and D: glyphosate ammonium salt. Error bars smaller than the size of the symbol are not shown.

Rainfall simulation significantly reduced glyphosate efficiency in all evaluated formulations (Fig. 4). When rainfall was simulated 30 min after glyphosate spraying, the control was less than 20%, regardless glyphosate formulation and addition of adjuvant. In plants that did not receive rainfall after application, the controls were approximately 80%, 60% and 85% for IPA, K and NH_4 salts, respectively, considering the treatments that contained adjuvant. With the simulation of rainfall after 240 min, controls were reduced to 50%, 50% and 20% for the same treatments. These results indicate that it takes more than 4 hours between its application and the occurrence of rainfall for maintaining glyphosate efficiency in hairy fleabane. Glyphosate NH_4 salt was the most sensitive to the rainfall occurrences after application (Fig. 4, D).

The reduction of shoot dry mass (SDM) (Fig. 5) corroborates the visual control evaluation. The application of saflufenacil, with or without adjuvant, reduced significantly the SDM, except in plants that were exposed to simulated rainfall 30 min after herbicides application, reflecting the occurrence of regrowth in these conditions.

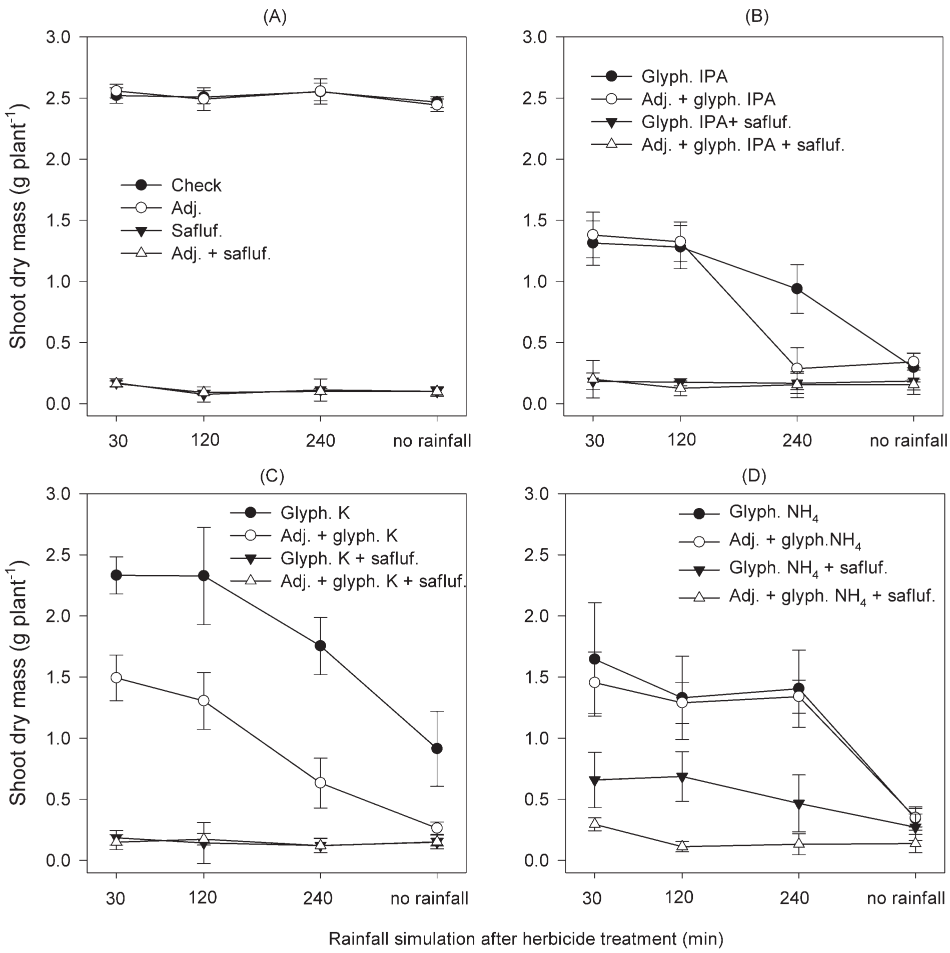

Figure 5. Shoot dry mass (SDM) of hairy fleabane in response to saflufenacil in association with glyphosate formulations, adjuvants and simulated rainfall occurrence. A: without glyphosate; B: glyphosate isopropylamine salt (IPA); C: glyphosate potassium salt (K); and D: glyphosate ammonium salt. Error bars smaller than the size of the symbol are not shown.

When glyphosate IPA (Fig. 5, B) or K salts (Fig. 5, C) were added to saflufenacil, the reduction of SDM occurred independently of the simulated rainfall after application. However, the addition of glyphosate NH_4 salt to saflufenacil resulted in smaller reduction of SDM, compared to the application of saflufenacil alone or in combination with the other glyphosate formulations (Fig. 5, D). It was observed for all simulated rainfall periods after the application of herbicides without adjuvant, and when the simulated rainfall occurred at 30 min after herbicide treatment when the adjuvant was added to the spray solution. These results reflect the lower control observed in the first evaluation, at 7 DAT (Fig. 1, D), in relation to other treatments. The lower initial control allowed the growth of the plants for a longer period and, consequently, the higher accumulation of SDM. The highest accumulations of SDM, considering treatments that received herbicides (excluding check and adjuvant), were observed in plants treated with glyphosate K salt (Fig. 5, B), especially in the absence of adjuvant and rainfall simulation at 30 min after spraying.

The results demonstrated that saflufenacil herbicide has efficiently controlled 15-cm-height hairy fleabane, even with the occurrence of simulated rainfall 30 min after its application. However, to obtain total control, when simulating rainfall occurred at 30 min after application, it was necessary to add adjuvant or use glyphosate IPA or NH_4 salts (Fig. 4). Adjuvants, especially surfactants, are compounds that accelerate and increase the absorption of post-emergent herbicides through the plant cuticle (Liu, 2004). In addition, these compounds reduce the surface tension of the droplets and improve their contact and adhesion with the leaf surface, improving the herbicide absorption and efficiency (Castro et al., 2018).

Besides the presence of adjuvants in adequate proportions, two other factors are important in the herbicide absorption by the leaves: the physical-chemical characteristics of the herbicides and the foliar surface characteristics of the weeds (Liu, 2004). The absorption of saflufenacil was less impaired by rainfall occurrence compared to that observed for glyphosate herbicide, regardless its formulation. Saflufenacil has a log Kow (octanol/water partition coefficient) value higher than glyphosate (saflufenacil: 2.60; glyphosate: -2.56) (Shaner, 2014). The log Kow indicates the affinity of the active ingredients with the cuticle of the leaves, which normally presents an apolar character. The higher the log Kow value, the faster the herbicide will be absorbed and the lower the losses by rainfall occurrences after application. This may explain the reduction of glyphosate herbicide efficiency, even with rainfall occurring after 240 min (4h).

The addition of adjuvant and glyphosate to saflufenacil had little effect on hairy fleabane final control in absence of rainfall (Fig. 4). It does not mean that adding these compounds into the spray solution is not important. When applied at high doses or in small plants, saflufenacil alone was able to efficiently control *Conyza* plants without the need for adjuvant and glyphosate (Mellendorf et al., 2015; Castro et al., 2018). However, in plants at an advanced stage of growth, or at lower doses of saflufenacil, the addition of adjuvant and glyphosate is important, because they can both increase the efficiency of control and reduce the occurrence of regrowth (Eubank et al., 2013; Dalazen et al., 2015).

The glyphosate application reached a control of 60 to 85%, depending on the formulation (Fig. 4). These levels of control can even be considered high for a resistant population. However, the resistance factor (RF) of the population used in the study can be considered low (FR: 3.98) (Dalazen et al., 2019) and, probably, the effect of glyphosate would be lower in populations with higher RF. Therefore, the addition of saflufenacil to glyphosate has been reported as a good strategy for manage weed species that are difficult to control, including species of *Conyza* (Eubank et al., 2013; Dalazen et al., 2015; Budd et al., 2016). However, the mixture of glyphosate with contact-action PPO inhibitors, such as fomesafen and sulfentrazone, results in antagonism, reducing the efficiency of both herbicides (Shaw & Arnold, 2002). Saflufenacil, however, has some peculiar physicochemical characteristics (pKa of 4.41 and log Kow of 2.6) compared to other PPO inhibitors, which confer to it a systemic character, with translocation via xylem and phloem (Bromilow et al., 1990; Grossmann et al., 2011). In addition, the translocation capacity of saflufenacil may also be related with its lower affinity with PPO enzyme, in comparison with other PPO inhibitors. Therefore, other PPO herbicides rapidly affect vascular tissue, making difficult the translocation. The translocation capacity of saflufenacil makes possible their association with systemic herbicides, such as glyphosate (Dalazen et al., 2015).

The glyphosate salts which were used in this study showed different hairy fleabane control in some situations. Considering all evaluated factors, the association of glyphosate IPA salt and saflufenacil presented the best results, in relation to both plant control at 28 DAT and SDM evaluations (Figs 4, 5, respectively). Although the addition of glyphosate NH_4 salt resulted in 100% control, the lower initial control and higher accumulation of SDM would result in higher interference on the culture. The simulation of rainfall after 30 min for the glyphosate K salt resulted in plant regrowth (Fig. 4, C). This would require an additional herbicide application in order to obtain complete weed control.

Several studies evaluate the effect of different glyphosate salts on weed control. Recently, Travlos et al. (2017) have done an extensive review on the efficiency of different glyphosate salts formulations and adjuvants on various weed species. In most cases, the final control provided by the formulations becomes equal, since glyphosate's acid equivalent amounts are the same. However, their absorption and translocation rates vary according to each one's formulation and species, which may be important in adverse conditions, such as rainfall occurrence after herbicides application. Li et al. (2005) reported that, although the final control was not affected, the initial absorption of glyphosate IPA salt was higher in common waterhemp (*Amaranthus rudis*) compared to di-ammonium salt formulation. However, in morningglory (*Ipomoea lacunose*), the initial uptake of glyphosate was higher for the di-ammonium salt. These differences are associated with the cuticle composition of each weed species, which may vary, as well as its polarity and affinity with the herbicides.

The occurrence of rainfall after application, even after 240 min, reduced the efficiency of all glyphosate formulations, although it was more harmful to both K and NH_4 salts formulations, compared to IPA (Fig. 4). In another study, the occurrence of rainfall at 6 h after application reduced the efficiency of glyphosate (Pedrinho Júnior et al., 2002). However, the authors did not report which salt formulation was used in this research. Similarly, using the same salts of glyphosate which were used in the present study, Costa et al. (2017) observed higher control of alexandergrass (*Urochloa decumbens*) in plants that were treated with glyphosate IPA salt in comparison to K and di-ammonium after rainfall simulation. Even with rainfall only after 15 min, the IPA formulation resulted in 100% control at 28 DAT, whereas, for both K and di-ammonium formulations, the controls were approximately 20 and 85%, respectively. In the case of sicklepod (*Senna obtusifolia*), both the formulation and the rainfall occurrence after 15 min did not interfere in the control (Souza et al., 2014), confirming the variation in the responses according to the weed species. Moreover, these responses may also to vary according to the sensitivity of different populations of the same weed species, as well as the occurrence of resistance should be considered.

In addition to the type of glyphosate salt, it is important to emphasize that the type of adjuvants and other compounds of the formulation are also fundamental to the prediction of absorption of this herbicide. However, these information are many times not provided in the technical reports or herbicide labels. Thus, although the active ingredient is the same among trademarks, they may have formulation differences, which may result in variations in uptake, translocation and amount of molecules that will reach the EPSPS enzyme. These considerations may explain the increased efficiency only of for glyphosate K salt when the adjuvant was added (Fig. 5, C).

CONCLUSION

In summary, the absorption of saflufenacil by hairy fleabane occurs rapidly and the simulated rainfall from 30 min after application does not result in loss of efficiency, when the herbicide is applied with adjuvant. The association of glyphosate isopropylamine (IPA) and ammonium salts (NH_4) to saflufenacil provides increased control and prevents the occurrence of regrowth when rainfall occurs soon after the application. The occurrence of simulated rainfall after 240 min considerably reduces glyphosate efficiency, regardless its salt formulation. In addition, the use of adjuvant improves the efficiency of glyphosate potassium (K) salt.

REFERENCES

Ashigh, J.J. & Hall, C. 2010. Bases for interactions between saflufenacil and glyphosate in plants. *Journal of Agriculture Food and Chemistry* **58**, 7335–7343.

Benbrook, C.M. 2016. Trends in glyphosate herbicide use in the United States and globally. *Environmental Sciences Europe* **28**, 1–15.

Bromilow, R.H.K., Chamberlain, K. & Evans, A.A. 1990. Physicochemical aspects of phloem translocation of herbicides. *Weed Science* **38**, 305–314.

Budd, C.M., Soltani, N., Robinson, D.E., Hooker, D.C., Miller, R.T. & Sikkema, P.H. 2016. Glyphosate-resistant horseweed (*Conyza canadensis*) dose response to saflufenacil, saflufenacil plus glyphosate, and metribuzin plus saflufenacil plus glyphosate in soybean. *Weed Science* **64**, 727–734.

Castro, E.B., Carbonari, C.A., Velini, E.D., Gomes, G.L.G.C. & Belapart, D. 2018. Influence of adjuvants on the surface tension, deposition and effectiveness of herbicides on fleabane plants. *Planta Daninha* **36**, e018166251.

Costa, A.C.R., Gonçalves, C.G., Silva Junior, A.C., Cardoso, L. & Martins, D. 2017. Action of rain on formulations of glyphosate mixed with saflufenacil for the control of *Brachiaria decumbens*. *Arquivos do Instituto Biológico* **84**, 1–8.

Dalazen, G., Kruse, N.D., Machado, S.L.O. & Balbinot, A. 2015. Synergism of the glyphosate and saflufenacil combination for controlling hairy fleabane. *Pesquisa Agropecuária Tropical* **45**, 249–256 (in Portuguese).

Dalazen, G., Pisoni, A., Menegaz, C. & Merotto Jr, A. 2019. Low temperature reverses the resistance to glyphosate in hairy fleabane (*Conyza bonariensis*). *Journal of Plant Protection Research* **59**, 433–440.

Dennis, M., Hembree, K.J., Bushoven, J.T. & Shrestha, A. 2016. Growth stage, temperature, and time of year affects the control of glyphosate-resistant and glyphosate-paraquat resistant *Conyza bonariensis* with saflufenacil. *Crop Protection* **81**, 129–137.

Dinelli, G., Marotti, I., Bonetti, A., Catizone, P., Urbano, J.M. & Barnes, J. 2008. Physiological and molecular bases of glyphosate resistance in *Conyza bonariensis* biotypes in Spain. *Weed Research* **48**, 257–265.

Duke, S.O. & Powles, S.B. 2008. Glyphosate: a once-in-a-century herbicide. *Pest Management Science* **64**, 319–325.

Eubank, T.W., Nandula, V.K., Reddy, K.N., Poston, D.H. & Shaw, D.R. 2013. Saflufenacil efficacy on horseweed and its interaction with glyphosate. *Weed Biology and Management* **13**, 135–143.

Frans, R., Talbert, R., Marx, D. & Crowley, H. 1986. Experimental design and techniques for measuring and analyzing plant responses to weed control practices. *In:* Research methods in weed science. Champaign: Southern Weed Science Society Pages 29–46.

Ge, X., d'Avignon, D.A., Ackerman, J.A., Duncan, B., Spaur, M.B. & Sammons, R.D. 2011. Glyphosate-resistant horseweed made sensitive to glyphosate: low-temperature suppression of glyphosate vacuolar sequestration revealed by 31P NMR. *Pest Management Science* **67**, 1215–1221.

Geier, P.W., Sthlman, P.W. & Charvat, L.D. 2009. Dose responses of five broadleaf weeds to saflufenacil. *Weed Technology* **23**, 313–316.

Grossmann, K., Niggeweg, R., Christiansen, N., Looser, R. & Ehrhardt, T. 2010. The herbicide saflufenacil (Kixor™) is a new inhibitor of protoporphyrinogen IX oxidase activity. *Weed Technology* **58**, 1–9.

Grossmann, K., Hutzler, J., Caspar, G., Kwiatkowski, J. & Brommer, C.L. 2011. Saflufenacil (Kixor™): biokinetic properties and mechanism of selectivity of a new protoporphyrinogen IX oxidase inhibiting herbicide. *Weed Science* **59**, 290–298.

Heap, I. 2020. The International Survey of Herbicide Resistant Weeds. http://www.weedscience.org. Accessed: 11.03.2020.

Holländer, H. & Amrhein, N. 1980. The Site of the inhibition of the shikimate pathway by glyphosate: I. Inhibition by glyphosate of phenylpropanoid synthesis in buckwheat (*Gagopyrum esculentum* Moench). *Plant Physiology* **66**, 823–829.

Kaspary, T.E., Lamego, F.P., Cutti, L., Aguiar, A.C.M., Rigon, C.A.G. & Basso, C.J. 2017. Growth, phenology and seed viability between glyphosate-resistant and glyphosate-susceptible hairy fleabane. *Bragantia* **76**, 92–101.

Kleinman, Z. & Rubin, B. 2017. Non-target-site glyphosate resistance in *Conyza bonariensis* is based on modified subcellular distribution of the herbicide. *Pest Management Science* **73**, 246–253.

Li, J., Smeda, R.J., Sellers, B.A. & Johnson, W.G. 2005. Influence of formulation and glyphosate salt on absorption and translocation in three annual weeds. *Weed Science* **53**, 153–159.

Liu, Z. 2004. Effects of surfactants on foliar uptake of herbicides – a complex scenario. *Colloids and Surfaces B: Biointerfaces* **35**, 149–153.

Mahoney, K.J., Shropshire, C. & Sikkema, P.H. 2014. Comparison of glyphosate formulations for weed control and tolerance in maize (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.]. *Agricultural Sciences* **5**, 1329–1337.

Mellendorf, T.G., Young, J.M., Matthews, J.L. & Young, B.G. 2015. Influence of application variables on the foliar efficacy of saflufenacil on horseweed (*Conyza canadensis*). *Weed Science* **63**, 578–586.

Molin, W.T. & Hirase, K. 2004. Comparison of commercial glyphosate formulations for control of prickly sida, purple nutsedge, morningglory and sicklepod. *Weed Biology and Management* **4**, 136–141.

Monquero, P.A. & Silva, A.C. 2007. Effect of rainfall on *Euphorbia heterophylla* and *Ipomoea purpurea* control by glyphosate and sulfosate. *Planta Daninha* **25**, 399–404 (in Portuguese).

Moretti, M.L. & Hanson, B.D. 2017. Reduced translocation is involved in resistance to glyphosate and paraquat in *Conyza bonariensis* and *Conyza canadensis* from California. *Weed Research* **57**, 25–34.

Mueller, T.C., Main, C.L., Thompson, M.A. & Steckel, L.E. 2006. Comparison of glyphosate salts (isopropylamine, diammonium, and potassium) and calcium and magnesium concentrations on the control of various weeds. *Weed Technology* **20**, 164–171.

Mylonas, P.N., Giannopolitis, C.N., Efthimiadis, P.G., Menexes, G.C., Madesis, P.B. & Eleftherohorinos, I.G. 2014. Glyphosate resistance of moleculary identified *Conyza albida* and *Conyza bonariensis* populations. *Crop Protection* **65**, 207–215.

Okada, M., Hanson, B.D., Hembree, K.J., Peng, Y., Shrestha, A., Stewart, C.N., Wright, S.G. & Jasieniuk, M. 2014. Evolution and spread of glyphosate resistance in *Conyza bonariensis* in California and a comparison with closely related *Conyza canadensis*. *Weed Research* **55**, 175–185.

Pacanoski, Z. & Mehmeti, A. 2019. Pre-emergence grass weed control in winter wheat (*Triticum aestivum* L.) with soil applied premixed herbicides influenced by precipitations. *Agronomy Research* **17**, 2386–2398.

Pedrinho Junior, A.F.F., Piva, F.M., Martini, G., Felici, G.V. & Durigan, J.C. 2002. Influence of rain on the efficacy of glyphosate in mixture with adjuvants in weed desiccation. *Planta Daninha* **20**, 263–271 (in Portuguese).

Sammons, R.D. & Gaines, T.A. 2014. Glyphosate resistance: state of knowledge. *Pest Management Science* **70**, 1367–1377.

Santos, G., Oliveira Junior, R.S., Constantin, J., Francischini, A.C., Machado, M.F.P.S., Mangolin, C.A. & Nakajima, J.N. 2014. *Conyza sumatrensis*: A new weed species resistant to glyphosate in the Americas. *Weed Biology and Management* **14**, 106–114.

Shaner, D.L. 2014. *Herbicide Handbook.* 10th ed. Lawrence, KS: Weed Science Society of America. 513 pp.

Shaw D.R. & Arnold J.C. 2002. Weed control from herbicide combinations with glyphosate. Weed Technol 16, 1–6

Silva, F.A.S. & Azevedo, C.A.V. 2006. A new version of the Assistat-statistical assistance software. In: *Proceedings of the 4th World Congress on Computers in Agriculture and Natural Resources.* Orlando, Florida: American Society of Agricultural and Biological Engineers, pp 393–396.

Souza, G.S.F., Martins, D., Pereira, M.R.R. & Bagatta, M.V.B. 2014. Action of rain on the efficiency of herbicides applied post-emergence in the control of *Senna obtusifolia*. *Rev. Ciên. Agron*. **45**, 550–557.

Starke, R.J. & Oliver L.R. 1998. Interaction of glyphosate with chlorimuron, fomesafen, imazetaphyr and sulfentrazone. *Weed Science* **46**, 652–660.

Travlos, I., Cheimona, N. & Bilalis, D. 2017. Glyphosate efficacy of different salt formulations and adjuvant additives on various weeds. *Agronomy* **7**, 1–9.

Vanaga, I., Gurkina, J., Treikale, O., Priekule, I. & Pugacova, J. 2006. Influence of Heracleum sosnowskyi control measures on weed diversity in agricultural fields in Latvia. *Agronomy Research* **4**, 433–436.

Vidal, R.A., Queiroz, A.R.S., Trezzi, M. & Kruse, N. 2016. Association of glyphosate with other agrochemicals: the knowledge synthesis. *Revista Brasileira de Herbicidas* **15**, 39–47.

Viirlaid, E., Riiberg, R., Mäeorg, U. & Rinken, T. 2015. Glyphosate attachment on aminoactivated carriers for sample stabilization and concentration. *Agronomy Research* **13**, 1152–1159.

Waggoner, B.S., Mueller, T.C., Bond, J.A. & Steckel, L.E. 2011. Control of glyphosate-resistant horseweed (*Conyza canadensis*) with saflufenacil tank mixtures in no-till cotton. *Weed Technology* **25**, 310–315.

Werlang, R.C. & Silva, A.A. 2002. Glyphosate - carfentrazone-ethyl interaction. *Planta Daninha* **20**, 93–102 (in Portuguese).