

ORIGINAL ARTICLE

## Low temperature reverses the resistance to glyphosate in hairy fleabane (*Conyza bonariensis*)

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### Abstract

Environmental factors and the addition of adjuvants to the spray tank mix may interfere with glyphosate efficiency in hairy fleabane control. The objective of this study was to evaluate the effect of air temperature and the addition of ammonium sulfate  $(\text{NH}_4)_2\text{SO}_4$  to glyphosate in the control of glyphosate-resistant (GR) and -susceptible (GS) hairy fleabane. Treatments consisted of air temperatures of 12°C and 25°C, six doses of glyphosate from zero to 2,880 g · ha<sup>-1</sup>, the presence or absence of  $(\text{NH}_4)_2\text{SO}_4$  in the spray solution, and one GS and another GR biotype. At the lowest tested dose (180 g · ha<sup>-1</sup>), control of the GR biotype was 91% and 20% when the plants were kept at 12°C and 25°C, respectively, reducing the resistance factor (RF) by 9.30 times and was associated to the reduction of temperature. The addition of  $(\text{NH}_4)_2\text{SO}_4$  increased the control by 10–20% at high glyphosate doses and at 25°C. The resistance of hairy fleabane to glyphosate was completely reversed when the plants were maintained at 12°C. At this temperature, resistant plants were controlled even at doses well below that recommended for the control of this species. At 25°C, a dose four times higher than that recommended was required for satisfactory control. At the field level, under situations of low temperatures, it was possible to improve the efficacy of glyphosate applications in hairy fleabane control, if there were no other mechanisms of resistance involved.

**Keywords:** ammonium sulfate, EPSPS, non-target-site-resistance, vacuolar sequestration, translocation

## Introduction

*Conyza bonariensis* (L.) Cronq. (hairy fleabane) is one of the most important weeds in several crops, due to the difficulty of control and the evolution of resistance to glyphosate, the main herbicide used globally, as well as its resistance to other important herbicides (Okada *et al.* 2014; Sammons and Gaines 2014; Kleinman and Rubin 2017). The occurrence of hairy fleabane increased with the adoption of the no-tillage system and the introduction of glyphosate-resistant soybean in the late 1990s (Christoffoleti *et al.* 2008). This species is adapted to no-tillage conditions with insufficient amounts of residues on the soil surface (Yamashita and Guimarães 2011). In addition, hairy fleabane is a positive photoblastic species, and the permanence of the seeds on the soil surface favors germination, which

may occur throughout the year (Shrestha *et al.* 2016). Each plant of this species can produce approximately 600,000 seeds, which are easily dispersed by the wind (Kaspary *et al.* 2017). Competition with hairy fleabane can reduce soybean yield by up to 80% at high weed densities. Depending on the soybean cultivar, a single plant per m<sup>2</sup> may result in a 25.9% productivity reduction (Agostinetto *et al.* 2017).

The herbicide glyphosate is currently the main weed management strategy in glyphosate-resistant soybean, and it is an important tool for burndown management in the no-tillage system. Currently, worldwide glyphosate resistance in *Conyza* genus, including *C. bonariensis*, *C. canadensis* and *C. sumatrensis* (Heap 2019), has been reported in 16 countries. *Conyza* species are the

most widespread glyphosate-resistant weeds in the world (Bajwa *et al.* 2016). In Brazil, there are several cases of hairy fleabane resistance to glyphosate, resulting in difficulties in controlling this weed in soybean and other summer crops. Initially restricted to southern Brazil, hairy fleabane resistant to glyphosate is now widely distributed over other regions of the country. Nowadays, the area of *Conyza* species resistant to glyphosate in Brazil is estimated to be 14 million ha, which corresponds to approximately 40% of the total soybean area, resulting in an increase of control costs of US \$15.00 per ha and US \$ 210 million yearly (Adegas *et al.* 2017).

The interaction of glyphosate in plants' metabolism is composed of several steps, culminating with the inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). The glyphosate must cross the structures of the leaf cuticle and the epidermis, apoplast, and mesophyll cells before reaching the phloem to be transported to growing tissues and to reach the site of action (Bromilow and Chamberlain 2000). The restriction of the entrance of glyphosate to the cytoplasm, and consequently in the chloroplasts, can result in a mechanism of resistance to this herbicide (Shaner 2009). Reduced translocation has been reported as the mechanism of *Conyza* resistance to glyphosate (Ge *et al.* 2014; Moretti and Hanson 2017). Glyphosate resistance was observed in horseweed that in plants exposed to low temperature the amount of glyphosate diminished significantly due to a minor sequestration of the herbicide (Ge *et al.* 2011).

Several studies have been performed to evaluate the contribution of different variables on glyphosate efficacy, such as dose, spray volume, water quality and adjuvants (Jordan *et al.* 1997; Pratt *et al.* 2003; Ramsdale *et al.* 2003; Gauvrit 2003; Nurse *et al.* 2008). The addition of nitrogen sources to the spray solution has been related to cation antagonism in hard water, resulting in better absorption and translocation of the glyphosate (Aliverdi *et al.* 2014).

Soon after application, the herbicide is exposed to several interactions with the environment that can affect its effectiveness. Factors which interact with the herbicide molecule from the moment it is applied to the plants until its arrival at the site of action may be biotic or abiotic. An understanding of the factors that affect a herbicide's effect, as well as the processes triggered by them is important for the adoption of strategies aimed to increase the efficiency of the herbicides and decrease the evolution of herbicide resistance. In addition, understanding environmental effects is also important for the correct diagnosis of herbicide failures mainly in situations where herbicide resistance can also occur.

Although the effect of temperature and the addition of nitrogen sources to glyphosate have already been

evaluated in isolation, the present study deals with the investigation of the interaction of these two factors on hairy buffer control with this herbicide. Therefore, the objective of this study was to evaluate the effect of atmospheric temperature and the addition of ammonium sulfate ( $(\text{NH}_4)_2\text{SO}_4$ ) to the glyphosate spray solution on the glyphosate-susceptible (GS) and glyphosate-resistant (GR) hairy fleabane.

## Materials and Methods

### Plant material

The plant material corresponded to two populations of hairy fleabane. One population was resistant to the herbicide glyphosate, and the other was susceptible. The resistant population was sampled in Dois Irmãos das Missões, RS, and the susceptible population in Santa Maria, RS, both in southern Brazil. Seed dormancy breaking was obtained by soaking the seeds in water for 4 days at 6°C. After that, the seeds suspended in water were deposited on trays containing organic substrate and kept in a greenhouse at 24°C and a photoperiod of 12 : 12 h (night : day) until the two leaf stage. Seedlings were transplanted into pots of 250 ml (one plant per pot) containing a mixture of Haplic Gleisol and organic compound (10 : 1), and 2.5 g · kg<sup>-1</sup> of the 5–20–20 NPK.

### Experimental design and treatments

The experiment was arranged in a factorial ( $2 \times 2 \times 6 \times 2$ ) split-plot design, with four replications. The main plots (factor A) were the temperatures of 12°C and 25°C, allocated in growth chambers (Conviron ATC 40). Factor B included the GS and GR hairy fleabane biotypes. Factor C consisted of the following doses: 0, 180, 360, 720, 1,440 and 2,880 g · ha<sup>-1</sup> of glyphosate (Gliz 480 SL, 408 g · l<sup>-1</sup>). Finally, factor D was composed of the absence or presence of ammonium sulfate ( $(\text{NH}_4)_2\text{SO}_4$ ) applied at a dose of 20 mg · l<sup>-1</sup> of spray solution. Treatments were applied to 15 cm tall plants. Plants were exposed to temperature treatments for 1 week prior to herbicide spraying until the end of the evaluations at 28 days after the treatment (DAT). The plants were grown in a photoperiod of 12 : 12 h (day : night), and light intensity of 240  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ .

Herbicide treatments were applied in a Greenhouse Spray Chamber (Model Generation III, Devries Manufacturing, Hollandale, MN), pressurized with compressed air at a constant pressure of 40 lbs in<sup>-2</sup>, Teejet® 8002 E nozzle, and speed of 1.16 m · s<sup>-1</sup>, resulting in a volume of 200 l · ha<sup>-1</sup>. After application, plants were immediately relocated to their respective temperature conditions (12 or 25°C).

## Data evaluation and analysis

The evaluations were plant control at 7, 14 and 21 DAT, and the shoot dry mass (*SDM*) at 28 DAT. Plant control was evaluated based on a zero to 100% visual grading, where zero corresponded to no damage and 100% to plant death. Plant shoots were collected and deposited in a dryer at 60°C until a constant mass was reached. Data were analyzed with respect to normality and homogeneity of variance. The analysis of variance (ANOVA) was performed by the *F* test ( $p < 0.05$ ). The experiment was repeated twice. The analysis of variance indicated that there were no significant differences between the two runs, and then data were combined to be statistically analyzed.

ANOVA of the regression was also performed using the log-logistic equation of three parameters (Eq. 1) (Seefeldt *et al.* 1995):

$$y = a / (1 + (X/X_{50})^b), \quad (\text{Eq. 1})$$

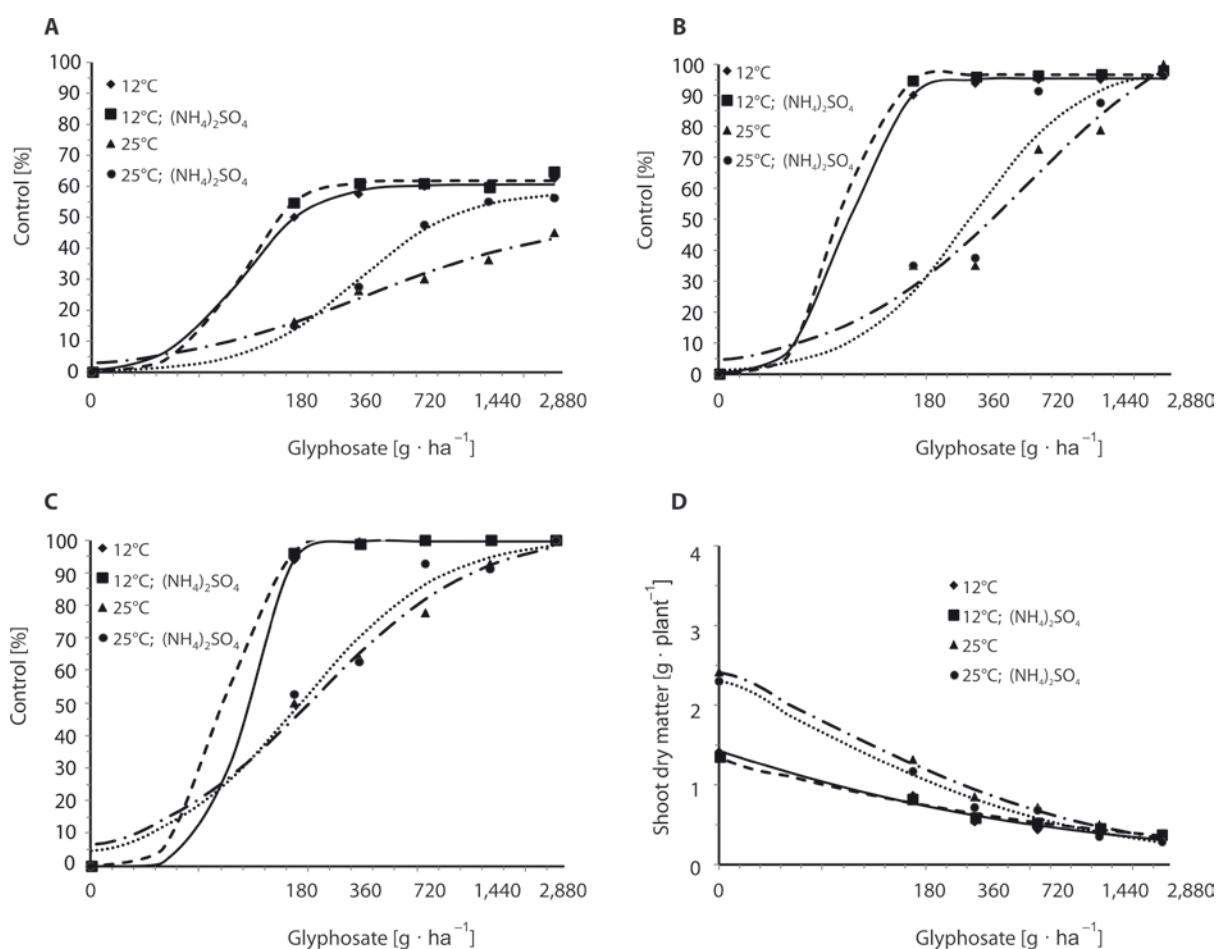
where:  $y$  = dependent variable (control or *SDM*);  $X$  = independent variable (herbicidal dose in  $\text{g} \cdot \text{ha}^{-1}$ );  $a$  = maximum asymptote;  $b$  = slope of the curve;  $X_{50}$  = herbicidal dose ( $\text{g} \cdot \text{ha}^{-1}$ ) responsible for reducing the dependent variable to the level corresponding to 50% of the maximum asymptote value ( $a$ ). For the

adjustment of the regressions, determination of the parameters and construction of the figures, the program SigmaPlot version 12.0 was used.

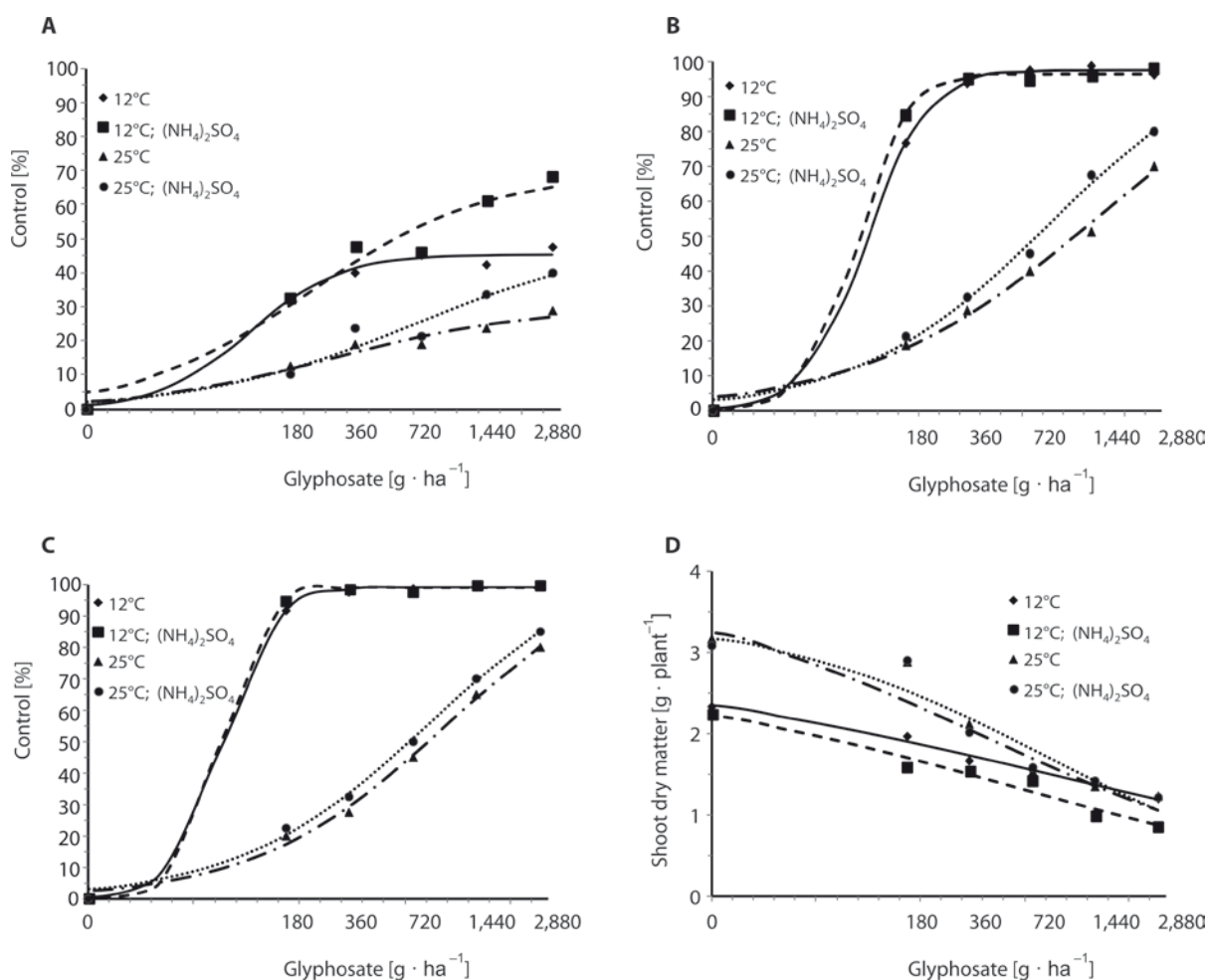
The determination of the  $C_{50}$  (dose value responsible for causing 50% of injury) was based on replacing the  $y$  value by 50 (50% of control) (Ritz *et al.* 2015). For the *SDM* variable, the  $X_{50}$  was referred to as  $GR_{50}$ , which was the dose responsible for causing 50% of growth reduction. The resistance factors (*RF*) for the variables control and *SDM* were calculated by the ratio  $C_{50R}/C_{50S}$  and  $GR_{50R}/GR_{50S}$ , respectively. R and S corresponded to resistant and susceptible populations, respectively.

## Results

The ANOVA indicated a significant interaction between dose, biotype, temperature and  $(\text{NH}_4)_2\text{SO}_4$  for hairy fleabane control and *SDM*. Differences in the control occurred from the first evaluation at 7 DAT, mainly in response to the temperature. In both GS and the GR biotypes (Figs. 1A and 2A, respectively) the control increased when plants were cultivated at 12°C.



**Fig. 1.** Control of glyphosate-susceptible hairy fleabane (*Conyza bonariensis*) at 7 (A), 14 (B) and 21 (C) days after application of treatments and shoot dry matter (D) in response to doses of glyphosate, air temperature (12 and 25°C), and addition of  $(\text{NH}_4)_2\text{SO}_4$  to the spray solution



**Fig. 2.** Control of glyphosate-resistant hairy fleabane (*Conyza bonariensis*) at 7 (A), 14 (B) and 21 (C) days after application of treatments and shoot dry matter (D) in response to doses of glyphosate, air temperature (12 and 25°C), and addition of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> to the spray solution

Susceptible plants presented control greater than 50% at a dose of 180 g · ha<sup>-1</sup> of glyphosate. At a recommended dose of 720 g · ha<sup>-1</sup>, control of susceptible plants was 60% at 12°C and only 30% at 25°C. The addition of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> to the spray solution increased the control of susceptible plants by 10 to 20% only at 25°C and at doses equal to or greater than 720 g · ha<sup>-1</sup>. In the GR biotype growing at 25°C, the control was 19%, whereas at 12°C the control increased to 40% at the dose of 720 g · ha<sup>-1</sup>, without the use of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (Fig. 2A). In this population, regardless of temperature, the addition of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> to the spray solution increased the control by approximately 20% at the doses of 1,440 and 2,880 g · ha<sup>-1</sup> of glyphosate. At lower doses of glyphosate, the addition of the adjuvant did not have a significant effect in this evaluation.

At 14 DAT, control of GS (Fig. 1B) and GR biotypes (Fig. 2B) demonstrated that temperature was critical to glyphosate efficiency. In the susceptible population, when plants were grown at 12°C, even at the lowest doses of glyphosate (180 g · ha<sup>-1</sup>) the hairy fleabane control was above 95%. However, at 25°C, the effect of glyphosate at this dose resulted in only 35% of control.

At the dose of 720 g · ha<sup>-1</sup>, control was 95% and 72.5% for temperatures of 12°C and 25°C, respectively. In the resistant population, at 12°C, the application of 180 g · ha<sup>-1</sup> caused a control of 76%. However, at 25°C the control was only 19%. Consequently, the values of C<sub>50</sub> and RF also varied according to the temperature (Table 1). The C<sub>50</sub> for the GR biotype was approximately 119 g · ha<sup>-1</sup> of glyphosate at 12°C and 1,192 g · ha<sup>-1</sup> of glyphosate at 25°C, corresponding to a 10.05-fold higher RF related with the temperature effect. The addition of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> to the spray solution in both biotypes increased the control at higher doses of the herbicide only at 25°C. When doses higher than 720 g · ha<sup>-1</sup> were applied, the control increased by about 15–20%. When the plants were grown at 12°C, the addition of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> to the spray solution was not significant.

In the evaluation performed at 21 DAT, the results were similar to that observed in previous evaluations. Both GS (Fig. 1C) and GR (Fig. 2C) biotypes were markedly more controlled at 12°C, and a greater effect was observed in the resistant population. At this temperature, when the recommended dose of glyphosate

was applied ( $720 \text{ g} \cdot \text{ha}^{-1}$ ), both susceptible and resistant populations had controls close to 100%. However, when the plants were kept at  $25^\circ\text{C}$ , the control of resistant plants was only 37%, and for the susceptible population the control reached about 70%.

At 21 DAT, the  $C_{50}$  for GR at  $25^\circ\text{C}$  was  $849.8 \text{ g} \cdot \text{ha}^{-1}$  of glyphosate. However, for the same population at  $12^\circ\text{C}$ , the  $C_{50}$  was reduced to  $91.42 \text{ g} \cdot \text{ha}^{-1}$  of glyphosate (Table 1). This means that glyphosate doses of 9.3 times greater were required to obtain control of 50% at  $25^\circ\text{C}$  compared to  $12^\circ\text{C}$ . In other words, it can be said that reducing the temperature from  $25^\circ\text{C}$  to  $12^\circ\text{C}$  reduces hairy fleabane resistance to glyphosate by 9.3 times. The addition of  $(\text{NH}_4)_2\text{SO}_4$  to the spray mixture resulted in an increase in the control, which reached approximately 10%, depending on the dose of glyphosate. This represented a reduction in  $RF$  by approximately 1.6-fold compared to resistant plants that did not receive the nitrogenated adjuvant in the spray solution.

The effect of temperature on the control of GR hairy fleabane plants at 21 DAT is indicated in Figure 3. A pronounced effect was observed in plants kept at  $12^\circ\text{C}$  (Fig. 3A), even at the lower doses used. At this temperature, the dose of  $180 \text{ g} \cdot \text{ha}^{-1}$  of glyphosate, that corresponds to 25% of the recommended dose, resulted in total control. However, at  $25^\circ\text{C}$  (Fig. 3B) adequate control (greater than 80%) was observed only at the highest evaluated dose ( $2,880 \text{ g} \cdot \text{ha}^{-1}$  glyphosate), corresponding to four times the recommended dose.

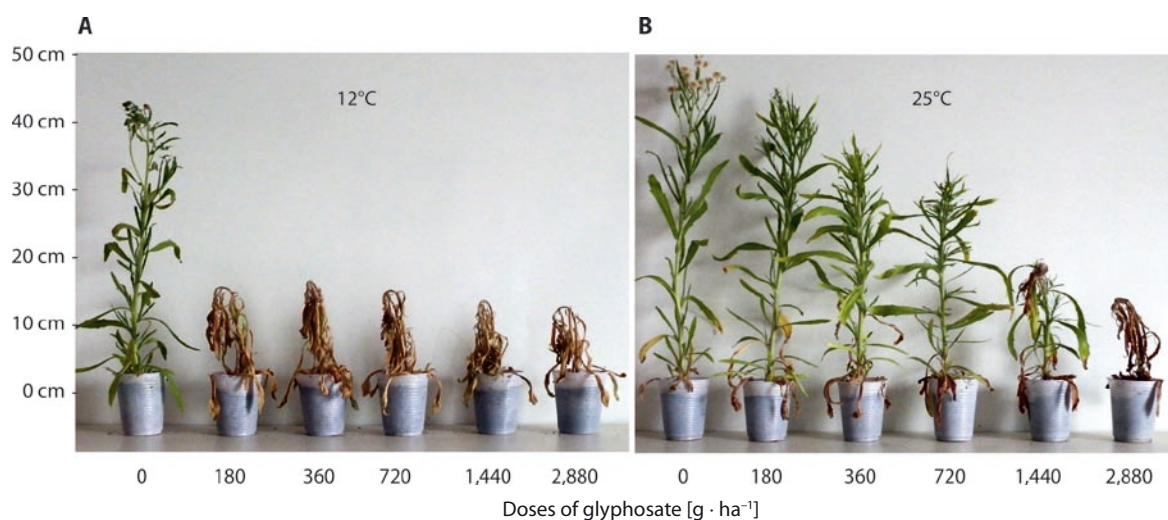
Unusual symptoms to those commonly observed in glyphosate treated plants were observed in plants grown at  $12^\circ\text{C}$ . In the first week after treatment, treated plants presented symptoms that are usually caused by contact herbicides, with rapid necrosis in the leaves and stems, even before the occurrence of chlorosis. These symptoms were observed from the lowest dose of the herbicide ( $180 \text{ g} \cdot \text{ha}^{-1}$ ), especially in the glyphosate-resistant population. However, at  $25^\circ\text{C}$  only the

occurrence of regular symptoms caused by glyphosate, such as chlorosis followed by necrosis, was observed.

Corroborating with the control results,  $SDM$  of plants kept at  $25^\circ\text{C}$  was higher in comparison with  $12^\circ\text{C}$  (Figs. 1D and 2D). The values of  $GR_{50}$  for the GS biotype were  $76.13 \text{ g} \cdot \text{ha}^{-1}$  of glyphosate when the plants were kept at  $12^\circ\text{C}$ , compared to  $135.52 \text{ g} \cdot \text{ha}^{-1}$  of glyphosate at  $25^\circ\text{C}$  (Table 1). In the GR biotype, this effect was also observed, with  $GR_{50}$  values of  $309.73 \text{ g} \cdot \text{ha}^{-1}$  and  $837.39 \text{ g} \cdot \text{ha}^{-1}$  of glyphosate for plants at  $12^\circ\text{C}$  and  $25^\circ\text{C}$ , respectively. This resulted in a 2.70-fold higher  $RF$  in resistant plants kept at the highest temperature in relation to the same population at  $12^\circ\text{C}$ . Similar to the findings for plant control, the addition of  $(\text{NH}_4)_2\text{SO}_4$  to the spray solution caused a reduction in the accumulation of  $SDM$  and, consequently, in the  $GR_{50}$  values (Table 1). The  $RF$  at  $25^\circ\text{C}$  decreased from 6.17 to 4.97 with the addition of  $(\text{NH}_4)_2\text{SO}_4$  in plants at  $25^\circ\text{C}$ . At  $12^\circ\text{C}$ , the effect of  $(\text{NH}_4)_2\text{SO}_4$  on the  $SDM$  was smaller, precisely due to the main effect of the temperature factor (Table 1).

## Discussion

The temperature of  $12^\circ\text{C}$  reversed the hairy fleabane resistance to glyphosate as indicated by the evaluations of plant control and  $SDM$  throughout the entire period of assessment. When the plants were kept at  $25^\circ\text{C}$  the effect of glyphosate was not satisfactory, even at high doses. However, at  $12^\circ\text{C}$  plants were completely controlled at the low dose evaluated ( $180 \text{ g} \cdot \text{ha}^{-1}$ ). When *C. bonariensis* populations were exposed to glyphosate at different temperatures, glyphosate tolerance increased linearly as the temperature was increased (Kleinman *et al.* 2016). Similar results have already been observed in horseweed (*C. canadensis*), whereas plants cultivated at low temperatures ( $10\text{--}12^\circ\text{C}$ )



**Fig. 3.** Symptoms of glyphosate-resistant hairy fleabane (*Conyza bonariensis*) in response to doses of glyphosate, and air temperatures of  $12^\circ\text{C}$  (A) and  $25^\circ\text{C}$  (B)

**Table 1.** Values of  $C_{50}$ ,  $GR_{50}$  and resistance factors ( $RF$ ) from glyphosate-susceptible and -resistant hairy fleabane (*Conyza bonariensis*) in response to the air temperature and addition of  $(NH_4)_2SO_4$  to the spray solution

Treatment	$C_{50}$ susceptible <sup>1</sup>	$C_{50}$ resistant <sup>2</sup>	$RF$ (R/S) <sup>3</sup>	$RF$ (S/S <sub>12°C</sub> ) <sup>4</sup>	$RF$ (R/R <sub>12°C</sub> ) <sup>5</sup>
7 DAT					
12°C	182.73 (±14.49)	>2,880.00 (±21.22)	>15.76	1.00	1.00
12°C + $(NH_4)_2SO_4$	150.56 (±51.36)	568.02 (±86.85)	3.77	0.82	<0.20
25°C	>2,880.00 (±176.20)	>2,880.00 (±147.51)	>1.00	>15.76	>1.00
25°C + $(NH_4)_2SO_4$	933.68 (±27.98)	>2,880.00 (±199.68)	>3.08	5.11	>1.00
14 DAT					
12°C	89.66 (±26.06)	118.51 (±9.04)	1.32	1.00	1.00
12°C + $(NH_4)_2SO_4$	81.83 (±40.75)	105.94 (±24.14)	1.29	0.91	0.89
25°C	435.38 (±115.39)	1,191.93 (±271.86)	2.74	4.85	10.05
25°C + $(NH_4)_2SO_4$	339.09 (±117.17)	777.95 (±180.81)	2.29	3.78	6.56
21 DAT					
12°C	106.10 (±22.60)	91.42 (±13.04)	0.86	1.00	1.00
12°C + $(NH_4)_2SO_4$	86.87 (±14.31)	89.61 (±36.50)	1.03	0.82	0.98
25°C	213.49 (±46.28)	849.80 (±171.01)	3.98	2.01	9.30
25°C + $(NH_4)_2SO_4$	193.76 (±41.13)	697.10 (±159.04)	3.60	1.82	7.62
Treatment	$GR_{50}$ susceptible <sup>1</sup>	$GR_{50}$ resistant <sup>2</sup>	$RF$ (R/S)	$RF$ (S/S <sub>12°C</sub> )	$RF$ (R/R <sub>12°C</sub> )
<i>SDM</i>					
12°C	76.13 (±21.27)	309.73 (±93.25)	4.06	1.00	1.00
12°C + $(NH_4)_2SO_4$	83.81 (±0.70)	298.05 (±93.91)	3.55	1.10	0.96
25°C	135.52 (±46.41)	837.39 (±46.97)	6.17	1.78	2.70
25°C + $(NH_4)_2SO_4$	152.08 (±56.49)	756.34 (±71.98)	4.97	1.99	2.44

<sup>1</sup>dose of glyphosate responsible for providing 50% control ( $C_{50}$ ) or reduction of growth ( $GR_{50}$ ) in susceptible plants; <sup>2</sup>dose of glyphosate responsible for providing 50% control ( $C_{50}$ ) or reduction of growth ( $GR_{50}$ ) in resistant plants; <sup>3</sup>resistance factor obtained by the  $C_{50}$  resistant/ $C_{50}$  ratio susceptible; <sup>4</sup>resistance factor obtained by susceptible  $C_{50}$  at 25°C/susceptible  $C_{50}$  at 12°C ratio; <sup>5</sup>resistance factor obtained by the resistant  $C_{50}$  at 25°C/resistant  $C_{50}$  at 12°C ratio

showed greater control and smaller accumulations of *SDM* than plants cultivated at 25°C (Ge *et al.* 2010). This result was attributed to the distribution pattern of the glyphosate herbicide in the cell. In plants grown at low temperatures, most of the absorbed glyphosate was detected in the chloroplasts, where amino acid synthesis occurs and where EPSPS enzyme is found (Ge *et al.* 2010). However, in plants grown at higher temperatures, most of the glyphosate was present in the vacuole, which is considered a deposit organelle of the cell. Thus, with the sequestration of the glyphosate herbicide in the vacuole, no inhibition of the EPSPS enzymes occurs and the plant survives the herbicide. Similar to horseweed, the resistance of hairy fleabane to glyphosate was also attributed to variation in the subcellular distribution of the herbicide, with a lower presence of the herbicide in the chloroplasts. However, herbicide concentration in vascular tissues was similar in susceptible and resistant plants (Kleinman and Rubin 2017).

The hypothesis that the limitation of translocation and subsequent sequestration of glyphosate in the vacuole could be the main mechanism of resistance was enhanced by experiments which showed that the vacuolar sequestration of glyphosate correlated with the

resistance of this herbicide in ryegrass (*Lolium* spp.) (Ge *et al.* 2012). In horseweed it was found that the lowest translocation, together with other mechanisms of resistance, is an important resistance mechanism to glyphosate (Powles and Yu 2010; Gonz ales-Torralva *et al.* 2012). However, studies on *Amaranthus tuberculatus* and *Sorghum halepense* found that resistance is strongly correlated with the lack of absorption of glyphosate in the plant cell (Ge *et al.* 2014). Sequestration in the vacuole is an important resistance mechanism for other herbicides in addition to glyphosate. In *L. rigidum*, resistance to the herbicide paraquat was attributed to the compartmentalization of the herbicide in the vacuole (Yu *et al.* 2010). These examples indicate the importance of restricting translocation as a mechanism of herbicide resistance. Recently the differential effect of temperature has been identified as an important interaction that must be considered for the diagnosis of this problem in experimental and field situations.

The addition of  $(NH_4)_2SO_4$  to the spray solution showed positive results in the control of hairy fleabane plants in some situations. In the treatments that combined the highest doses of the herbicide with the

temperature of 25°C the control increased 10–20% with the addition of this adjuvant. The addition of nitrogenated sources as adjuvant to the herbicide spray solution has been related to the decrease of the cation effect in hard water and with the better absorption and translocation of glyphosate (Aliverdi *et al.* 2014). In *Abutilon theophrasti* the addition of 20 g · l<sup>-1</sup> of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> to the spray solution provided greater absorption and translocation of glyphosate and glufosinate, resulting in a greater effect of these herbicides (Maschhoff *et al.* 2000; Young *et al.* 2003). At the same concentration of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, a higher efficiency of glyphosate in *Sida spinosa* and *Ipomoea hederacea* was observed (Pratt *et al.* 2003; Mueller *et al.* 2006).

There are several reports about differential control of weed species obtained by glyphosate combined with nitrogen fertilizers, especially in studies conducted in North America. In a weed community, the joint application of glyphosate and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> resulted in a 93% reduction in plant biomass, while treatments without this adjuvant reduced biomass by 77% (Mahoney *et al.* 2014). The control of *Urochloa decumbens* with glyphosate was not increased by the use of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, but this combination resulted in greater control in *S. halepense* (Carvalho *et al.* 2012). These results demonstrate that plant responses to the (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> are species-dependent, and can also vary according to the biotype.

According to the responses observed in the present study, mainly in response to the effect of temperature, it is possible to indicate that the resistance mechanism to glyphosate may be similar to the process described by Ge *et al.* (2012), where there was a reduction of glyphosate translocation due to sequestration in the vacuole of cells, in opposition to the presence of the herbicide in the cytoplasm and chloroplasts. Although these studies have been carried out to investigate the mechanism of resistance, the practical application of these results may contribute to greater control efficiency of hairy fleabane plants, susceptible or resistant to glyphosate, especially in regions with prolonged periods of low temperatures, like the south of Brazil. In this case, the application of the herbicide under conditions of adequate temperature can help decrease the evolution of resistance to the glyphosate herbicide. However, it should be noted that resistance to this herbicide may also be associated with other mechanisms (Sammons and Gaines 2014). In those cases, the effect of temperature is not associated with the occurrence of resistance.

In addition, it is necessary to evaluate the effect of temperature on the efficiency of herbicide mixtures normally carried out together with glyphosate. Therefore, the variation of glyphosate efficiency over the years in the same area or application on different days or at different times may be associated with the temperature effect on the resistance mechanism in opposition to other common causes related to the lack

of herbicide efficiency, such as wind, water availability, low doses, or herbicide mixtures.

The association of ammonium sulfate (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> with glyphosate in the spray solution was partially effective as a strategy for hairy fleabane control. The use of ammonium sulfate increased the control by 10–20% in the GS and GR plants, mainly at the highest doses of the herbicide and at the growth temperature of 25°C. In spite of an improvement in control of the GR biotype, the control level remained below 80% (good).

The reduction of temperature resulted in an expressive increase of hairy fleabane control. When plants were maintained at 12°C, the resistance of the plants was markedly reversed, resulting in an increase of hairy fleabane control of more than 80% in comparison with plants grown at 25°C. At this temperature, resistance to glyphosate was evidenced, presenting a *RF* of 9.3 at 21 DAT, compared to plants grown at 12°C. These results indicate that, for this hairy fleabane population from southern Brazil, the resistance to glyphosate is affected by temperature, probably due to the sequestering of glyphosate in the cell vacuole. The addition of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> resulted in a little control increase, being restricted to applications at 25°C and higher doses of glyphosate.

The resistance to glyphosate in hairy fleabane and the interaction with environmental factors, as demonstrated in this study, in addition to other mechanisms of resistance indicate a complex scenario of control in these *Conyza* species. These species are currently one of the main plant protection problems in most cultivated areas worldwide. The high frequency of resistance to glyphosate in hairy fleabane populations, multiple resistance mechanisms, and interaction with environmental conditions in herbicide efficacy and efficiency indicate that special attention should be given to herbicide programs for the control of this species, including the adoption of integrated methods in conjunction with chemical control.

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