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Article

Glyphosate Resistance Affect the Physiological Quality of *Conyza bonariensis* seeds

A Resistência ao Glyphosate Afeta a Qualidade Fisiológica das Sementes de ***Conyza bonariensis***

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ABSTRACT - High seed production and dispersal capacity and glyphosate resistance are among the main factors that have made hairy fleabane (*Conyza bonariensis*) one of the most important and dispersed weeds in the world. Herbicide-resistant weeds populations may have fitness cost due to molecular, physiological, and biochemical changes. This study aimed to evaluate the physiological quality of seeds of *Conyza* spp. and determine whether glyphosate resistance causes fitness costs for resistant biotypes. Seeds from six hairy fleabane biotypes, three glyphosate-resistant and three -sensitive, with a resistance factor average of 11.7 were studied. Among the studied biotypes, five were identified as *C. bonariensis* and one as *C. blakei*. Seed analyses were performed in a completely randomized design with ten replications of 100 seeds each (1,000 seeds per biotype). The analyzed seeds were originated from second-generation self-pollinating plants with known segregation rate. The mean results indicate that, in relation to sglyphosate-sensitive biotypes of *C. bonariensis*, seeds from -resistant biotypes showed a 16% reduction in vigor and 13% in germination, a 44% increase in the number of empty seeds and approximately five times more viable dormant seeds. The lower physiological quality of glyphosate-resistant hairy fleabane seeds regarding to -sensitive indicates that resistance affect the seed's physiology and causes a fitness costs. It implies a reduction of invasive potential and persistence in the environment of resistant biotypes. Therefore, in the absence of glyphosate as a selective factor, the frequency of resistant biotypes tends to decrease and increase the predominancy of glyphosate-sensitivre biotypes. However, the highest number of viable dormant seeds of resistant biotypes supplies the soil seed bank. All biotypes of *C. bonariensis* showed higher physiological quality of seeds when compared to *C. blakei*, indicating that the first species is more adaptably evolved for survival in agricultural and disturbed areas.

Keywords: herbicide resistance, integrated weed management, weed biology, soil seed bank, *Conyza blakei*.

*RESUMO - A alta capacidade de produção e dispersão de sementes e a resistência ao glyphosate estão entre os principais fatores que tornaram a buva (**Conyza bonariensis**) uma das mais importantes e dispersas plantas daninhas no mundo. Populações de plantas resistentes a herbicidas podem apresentar penalidades em função das alterações moleculares, fisiológicas e bioquímicas. O objetivo deste trabalho foi avaliar a qualidade fisiológica das sementes de biótipos de **Conyza** spp. e determinar se a resistência ao glyphosate causa penalidades aos biótipos resistentes. Foram avaliadas sementes de seis biótipos de buva, sendo três resistentes e três sensíveis ao glyphosate, com fator de resistência de 11,7. Entre os biótipos estudados, cinco foram identificados como **C. bonariensis** e um como **C. blakei**. As análises das sementes foram realizadas no delineamento completamente*

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casualizado com dez repetições de 100 sementes cada (1.000 sementes por biótipo). As sementes utilizadas nas análises foram originadas de plantas de segunda geração de autopolinização com taxa de segregação conhecida. Os resultados médios indicam que, em relação aos biótipos sensíveis de ***C. bonariensis****, as sementes dos biótipos resistentes apresentaram redução de 16% no vigor e 13% na germinação, aumento de 44% no número de sementes vazias e em torno de cinco vezes mais sementes dormentes viáveis. A menor qualidade fisiológica de sementes da buva resistente ao glyphosate indica que a resistência causa penalidade. Essa penalidade implica redução do potencial invasivo e persistência no ambiente dos biótipos resistentes. Assim, na ausência do fator seletivo glyphosate, a frequência de biótipos resistentes tende a diminuir e retornar a suscetibilidade. Entretanto, o maior número de sementes dormentes viáveis dos biótipos resistentes abastece o banco de sementes do solo. Todos os biótipos de* ***C. bonariensis*** *apresentaram maior qualidade fisiológica das sementes em comparação ao biótipo de* ***C. blakei****, indicando que a primeira espécie é mais evoluída do ponto de vista adaptativo para sobrevivência em áreas agrícolas.*

Palavras-chave: resistência a herbicidas, manejo integrado de plantas daninhas, biologia de plantas daninhas, banco de sementes do solo, *Conyza blakei*.

INTRODUCTION

Weed management is one of the major challenges for agricultural sustainability (Bajwa et al., 2016). Weed interference on crops has caused agricultural losses of about 34% annually (Oerke, 2006). Weed resistance to herbicides is a major threat to agriculture, as it increases management costs and yield losses, in addition to making control difficult. If weeds are not controlled, agricultural yield losses are estimated at 50%, which would represent about US$ 43 billion per year for corn and soybean only in the United States alone (Jeschke, 2016; van Wychen, 2016).

Weeds of the genus *Conyza* spp. are among the most problematic in the world. Plants of this genus are invasive, widely distributed, harmful, and highly competitive with agricultural crops (Bajwa et al., 2016). These species are annual cycle, herbaceous plant that reproduces by seeds generated from incomplete self-fertilization (Stewart et al., 2009). They have high seed production, i.e., a single plant can produce more than 200,000 (Bhowmik and Bekech, 1993). Studies conducted in Brazil by Piasecki et al. (2019a) with glyphosate-resistant *C. bonariensis* estimated production of about 120,000 seeds per plant. In addition to high seed production, they have morphological features that favor dispersal, which can exceed the distance of 100 m (99% of seeds up to 100 m and 1% up to 500 m) (Dauer et al., 2007).

High production and dispersion of *Conyza* spp. seeds have favored the selection and dissemination of glyphosate-resistant populations, being currently considered a cosmopolitan plant, i.e., they are present in most environments (Bajwa et al., 2016). These species were the first dicot weed reported to evolve to glyphosate resistance, which was reported in the United States in 2000 (Van Gessel, 2001). There are currently about 52 records of glyphosate resistance of *Conyza* spp. in the world (Heap, 2018). In Brazil, the first record was reported in 2005 (Vargas et al., 2007), and probably in 2018, more than 90% of the plants of these species in agricultural areas are resistant to this herbicide.

Conyza spp. interfere with the development of many crops. However, soybean is one of the most affected. It is due to the coincident growing season of soybean with the highest emergence of *Conyza* spp. plants (spring/summer). If not controlled, a single plant per m^2 can cause soybean yield losses of up to 36% (Trezzi et al., 2015). In addition, the control cost of glyphosate-resistant *Conyza* spp. is estimated to be up to five times higher than that of sensitive plants (Vargas et al., 2016).

The mechanism of glyphosate resistance in *Conyza* spp. is not related to the herbicide target enzyme (5-enolpyruvylshikimate 3-phosphate synthase, EPSPS), but to other molecular, physiological, biochemical, and morphological changes (Peng et al., 2010; Cardinali et al., 2015; Moretti et al., 2017; Hereward et al., 2018; Piasecki et al., 2019b). However, changes that occur in plants due to the resistance process may result in fitness costs for resistant plants in relation to sensitive plants (Yuan et al., 2007; Délye, 2013). Galon et al. (2013) found that a biotype of *Conyza* spp. resistant to glyphosate had physiological disadvantages and lower adaptability potential when compared to a sensitive biotype, while Moreira et al. (2010) demonstrated fitness costs related to plant growth. Pazdiora et al. (2018) observed another type of fitness cost and concluded that a glyphosate-resistant biotype of *Conyza* spp. is susceptible to the phytopathogenic fungus *Podosphaera erigerontis-canadensis*, the causal agent of powdery mildew, while the glyphosate-sensitive biotype is resistant to this fungus.

Studies have been conducted on herbicide-resistant weed species aiming at evaluating hypothesized fitness costs as a function of resistance. However, evaluations of most of these studies have not taken into account the effects on seed production and quality, especially in prolific species, which produce very small seeds, such as *Conyza* spp. Changes in the physiological quality of seeds from these species may affect the number of originated plants and soil seed bank. Thus, the hypothesis of this study was that glyphosate resistance in *Conyza* spp. results in fitness costs on the physiological quality of seeds. Therefore, this study aimed to evaluate the physiological quality of *Conyza* spp. seeds and determine whether the resistance process causes fitness costs in glyphosate-resistant biotypes.

MATERIAL AND METHODS

Selection and characterization of biotypes

Experiments for the production and collection of hairy fleabane seeds were conducted in a greenhouse at the Universidade Federal de Pelotas (UFPel), Faculdade de Agronomia Elizeu Maciel, from March 2016 to April 2018. From 54 hairy fleabane biotypes collected in the state of Rio Grande do Sul in 2016, six were selected: three glyphosate-resistant (GR) and three sensitive (GS). Seeds of GR biotypes were collected in agricultural areas with farmers complaints about difficulties in controlling hairy fleabane and where glyphosate was used for weed management for at least five consecutive years. On another hand, seeds of GS biotypes were collected in areas without herbicide application during the same period.

Glyphosate resistance or sensitivity of these 54 biotypes were verified from greenhouse experiments carried out in a completely randomized design with six replications consisted of three hairy fleabane plants, totaling 18 plants per biotype. Plants were cultivated in 2 L pots containing a mixture of soil and commercial substrate (Mac plant – Mec Prec, Brazil) in a 3:1 ratio. These plants were irrigated daily and maintained in a greenhouse (30 °C/20 °C day/night (± 4 °C), with 12 hours photoperiod) until 60 days after emergence (DAE – rosette stage – 6 to 8 cm in diameter). Plants were treated with glyphosate at a dose of 1,480 g a.e. ha^{-1} (Roundup Original DI 370 g a.e. L^{-1}; Monsanto) at 60 DAE. Spraying was carried out using a backpack sprayer pressurized with CO_2 and a volume of 150 L ha^{-1} with flat fan spray nozzles 110015. The treatment was performed on the same day for all biotypes, which were maintained in the same environment (greenhouse) before and after application. A visual control evaluation was carried out at 28 days after glyphosate treatment (DAT), and the surviving biotypes were considered resistant, while non-surviving biotypes were considered sensitive. Biotypes with variations in survival were considered segregating and were not selected. Six genotypes in the F_0 generation were chosen according to the region of origin, to obtain at least one GR and GS from the same geographical region, and uniformity of survival for GR biotypes and death for GS biotypes, i.e., non-segregating (Tables 1 and 2).

Table 1 **-** Description of the origin site of six biotypes of *Conyza* sp. glyphosate-resistant and glyphosate-sensitive biotypes used in the study

Biotype	Species	Country	State*	County	Latitude	Longitude
1	*C. bonariensis*	Brazil	RS	Pelotas	32°04’05.99” S	52°52’59.43” W
11	*C. bonariensis*	Brazil	RS	Pelotas	32°04’05.91” S	52°52’59.14” W
29	*C. bonariensis*	Brazil	RS	Floriano Peixoto	27°50’48.92” S	52°04’11.15” W
17	*C. bonariensis*	Brazil	RS	Pelotas	31°49’15.15” S	52°27’39.55” W
20	*C. bonariensis*	Brazil	RS	Pelotas	31°49’15.49” S	52°27’39.31” W
48	*C. blakei*	Brazil	RS	Mato Castelhano	27°19’02.59” S	52°10’13.32” W

* RS: Rio Grande do Sul state.

Molecular genotyping analyses were performed using SSR (Simple Sequence Repeats) markers (Abercrombie et al., 2009; Marochio et al., 2017) and morphological analyses for the biotype B48S to determine the *Conyza* species from the six selected biotypes (Table 1). The voucher specimens of biotypes used in the study are deposited in the Herbário Pel of the Federal University of Pelotas (UFPel) under the numbering B01R (No. 26,950), B11R (No. 26,951), B29R (No. 26,952), B17S (No. 26,953), B20S (No. 26,954), and B48S (No. 26,968). Plants non treated with glyphosate of the six biotypes were reproduced for two generations (F_0 and F_1) in a greenhouse by self-pollination. The percentage of segregation with glyphosate application was evaluated in each generation, as described above, with a higher number of plants used in the second generation (F_1) due to the higher seed availability (Table 2).

Table 2 **-** Results of two generations (F_0 and F_1) of segregation experiments in six glyphosate-resistant (B01R, B11R, and B29R) and glyphosate-sensitive (B17S, B20S, and B48S) biotypes of *Conyza* sp. evaluated at 28 days after treatment (DAT) of 1,480 g a.e. ha^{-1} of glyphosate

Biotype	Number of evaluated plants		Survivors	Survivors
	F_0 (1)	F_1 (1)	F_0 (%)	F_1 (%)
B01R	19	112	100	100
B11R	94	220	100	100
B29R	19	78	100	65.4
B17S	94	220	0	0
B20S	18	119	0	0
B48S	16	42	0	0

(1) F_0 and F_1 correspond, respectively, to generations 1 and 2 after seed collection in the field.

F_1 generation plants of these six biotypes were subjected to glyphosate dose-response curve experiments to confirm the resistance and evaluate the resistance factor (RF). Plants were treated with the following glyphosate doses at 60 DAE: 0, 92.2, 185, 370, 740, 1,480, 2,960, 5,920, 11,840, and 23,680 g a.e. ha^{-1}. Cultivation and spraying followed the same procedures described above. Dose-response studies were carried out in a completely randomized design with four replications (each replication consisted of three plants). The shoot dry matter (SDM) was collected from plants at 28 DAT and dried in an air circulation oven at 60 ^{o}C until constant weight; the reduction in dry weight in relation to the untreated control was determined.

The dose responsible for a 50% reduction (GR_{50}) in SDM when compared to the control treatment was obtained through the log-logistic nonlinear regression equation:

$$y = C + (D \text{ “ } C)/[1 + (x/GR_{50})^{b}] \qquad \text{(eq. 1)}$$

where *C* is the minimum limit, *D* is the maximum limit, *b* is the slope angle of the curve in GR_{50}, and GR_{50} is the glyphosate dose required for 50% reduction in SDM. The average resistance factor (ARF) for biotypes under study was calculated by dividing the arithmetic mean of GR_{50} of GR biotypes by the mean of GR_{50} of GS biotypes (Table 3).

Plant cultivation and seed collection for analysis

Seeds produced by F_1 generation plants of each biotype were sown in trays containing soil and substrate and maintained in a greenhouse, as described above. F_0 generation plants used in seed production for F_1 generation, as well as those of F_1 generation used in seed production for the analyses of the present experiment (F_2), were not exposed to glyphosate. Seedlings of each biotype were individually transplanted at 30 days after sowing (DAS) to 5 L buckets containing soil and substrate, as described. Twenty plants per biotype were cultivated until the end of the reproductive stage, except for B48S, which had eight plants due to lower germination. Seeds of the six biotypes were sown on the same day

Table 3 **-** Parameter estimates of glyphosate dose resulting in 50% reduction of shoot dry weight (GR_{50}) in glyphosate-resistant (B01R, B11R, and B29R) and sensitive (B17S, B20S, and B48S) biotypes of *Conyza* sp. determined at 28 days after treatment, and average resistance factor (ARF)

Biotype	Glyphosate GR_{50} (1)
B01R	648 a
B11R	954 a
B29R	1,258 a
B17S	65 b
B20S	97 b
B48S	82 b
ARF(2)	11.7

GR_{50} values with different letters are different by the Tukey's test at p≤0.05. Coefficient of variation (CV%) was 14.2. (1) GR_{50}, glyphosate dose causing 50% of shoot dry weight reduction; (2) Average resistance factor = GR_{50} (R)/GR_{50} (S).

and remained under the same environmental conditions throughout the experimental period.

Seeds from plants of each biotype, at full physiological maturity and disconnected from the mother plant, were collected daily and placed in properly identified paper bags. Collections were carried out manually by introducing hairy fleabane branches containing the reproductive structures into the paper bags and then shaking them briefly so that the seeds could come off the clusters. During the collection period, paper bags containing the seeds previously collected remained in the same greenhouse until the end of the experiment. Subsequently, the seeds were refrigerated (~4 ^{o}C) for three months.

Analysis of the physiological quality of hairy fleabane seeds

The experiments were conducted from July to August 2018, at the Laboratório Didático de Análise de Sementes (LDAS) of the Universidade Federal de Pelotas (UFPel). Except for B48S, which had eight plants, the remaining seeds were originated from 20 plants per biotype. Seeds from plants of each biotype were homogenized before the beginning of the analyses. Treatments consisted of six hairy fleabane biotypes, being three resistant to glyphosate (B01R, B11R, and B29R) and three sensitive (B17S, B20S, and B48S). The experimental design was a completely randomized design with ten replications. Each replication consisted of 100 hairy fleabane seeds randomly selected with tweezers under a 40X magnifying glass. In the total, 1000 seeds per biotype were analyzed.

Germination test was conducted on blotter paper, which was weighed and placed inside gerbox boxes and moistened with distilled water in an amount equivalent to three times its weight. The experiment was conducted under 24 hours photoperiod of light at 20 ^{o}C in a BOD germination chamber (Vidal et al., 2007). The percentage of germination was determined at 14 days after sowing (DAS), in which the number of normal seedlings was evaluated. Together with the germination test, the first germination count was carried out at 7 DAS to determine seed vigor (Vivian et al., 2008). After the evaluation of the number of normal seedlings and germination test, the number of empty, dead, abnormal, and dormant seeds were counted, with results were expressed as a percentage.

The viability of non-germinated seeds was evaluated at the end of the germination test by the tetrazolium test with the salt 2, 3, 5-triphenyl-tetrazoliumchloride at 1.0%. A viable seed was that with pink or crimson color in the whole seed (Brasil, 2009), being characterized as dormant. For this, seeds were placed in tetrazolium solution in a transparent glass container closed with aluminum foil to prevent light from entering for 24 hours at ± 30 ^{o}C (Brasil, 2009). Seeds were evaluated in a 40X magnifying glass. The percentages of viable and non-viable seeds were calculated based on the number of dormant seeds in the germination test.

Statistical analyses

Regression parameters for obtaining GR_{50} from dose-response curve data were obtained using the software Sigma Plot® (v. 12.5, SPSS Inc., Chicago, IL, USA). The results of GR_{50} and variables related to seed quality were subjected to the analysis of variance (ANOVA) and Tukey test at $p \leq 0.05$ probability using the CoStat Statistics Software v. 6.451 (http://www. cohort.com/ costat.html).

RESULTS AND DISCUSSION

Experiments were conducted with six selected biotypes originated from two regions with different edaphoclimatic conditions from the state of Rio Grande do Sul (RS), Brazil. The biotypes B29R and B48S were originated from the northern region (plateau), while B01R, B11R, B17S, and B20S were originated from the southern region (lowlands) (Table 1). In addition to the different climate between regions, differences can be found between crops, and consequently, weed management. Different management practices have the potential to alter the levels of segregation and resistance between biotypes. However, among the six biotypes, although a previous segregation test was carried out in F_0 generation, F_1 generation presented the biotype

B29R with about 1/3 of plants sensitive to glyphosate (Table 2). This percentage of segregation of about 3:1 indicates a segregating biotype, according to Mendel's laws. The other biotypes evaluated in the present study showed no variation for glyphosate resistance or sensitivity in the evaluated generations (Table 2). Segregation evaluations are important in studies of resistance to herbicides to assess the genetic stability of biotypes according to the presence or absence of alleles responsible for resistance. Non-segregating resistant biotypes show a stable phenotype.

The correct identification of hairy fleabane species is necessary to ensure that the same species is being investigated, although it is not the aim of this study. The literature indicates that hairy fleabane has about 15% cross-pollination and that glyphosate resistance is nuclear and dominant. Thus, genes that confer glyphosate resistance can be transmitted by pollen via gene flow (Zelaya et al., 2004, 2007). Gene flow can cause a mixture of morphological traits between biotypes and lead to misidentification of the species.

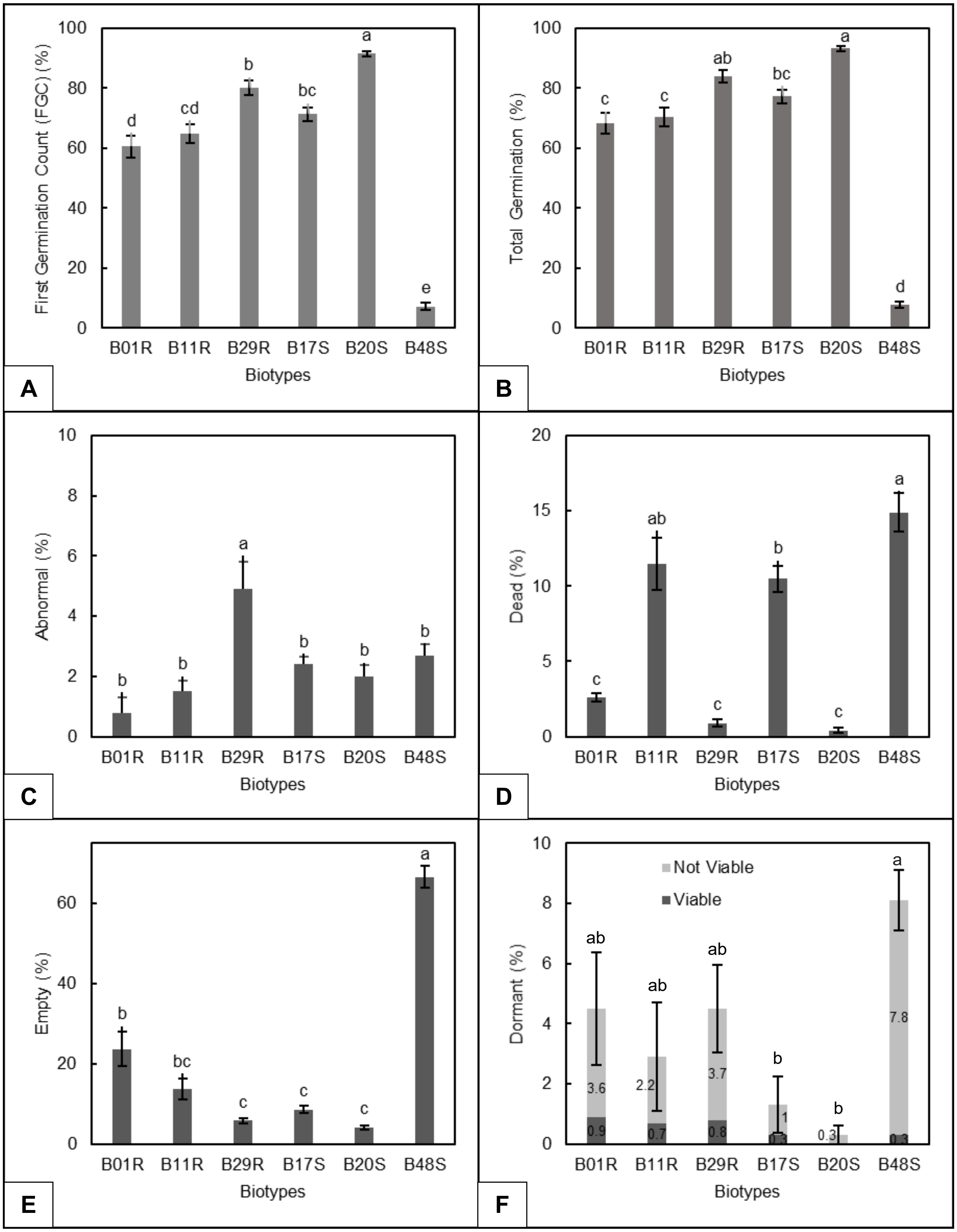
Molecular identification of species of the biotypes used in the present study indicated that B01R, B11R, B29R, B17S, and B20S are from the species *C. bonariensis*. B48S presented different molecular pattern in genotyping analyses, being necessary the complementation with morphological analysis, which was performed by an expert in the field, who concluded that the species in question is *C. blakei* (Cabrera) Cabrera (Table 1) (Piasecki et al., 2019a). According to Lorenzi (2000), the genus *Conyza* includes approximately 100 species, which are distributed throughout the world, although they are originated from the Americas. The main *Conyza* species occurring in agricultural areas are *C. bonariensis*, *C. canadensis*, and *C. sumatrensis*, all of them reported with glyphosate resistance (Heap, 2018). Given the high number of species of this genus, the presence of *C. blakei* among the biotypes under study indicates potential for adaptation of new *Conyza* species for infestation of agricultural areas. Although *C. blakei* has been characterized as herbicide sensitive, it may acquire resistance as it evolves through alleles responsible for resistance, inherited from another *Conyza* species by gene flow.

The difference in geographical origin did not affect the glyphosate dose required for 50% reduction in SDM (GR_{50}) between GR and GS biotypes (Table 3). Because of this, the results were evaluated individually per biotype. The mean GR_{50} for glyphosate sensitive plants was 81 g a.e. ha^{-1}, while resistant biotypes presented 953 g a.e. ha^{-1}. The GR_{50} mean results were used to calculate the average resistance factor (ARF) between biotypes, which was 11.7, being considered a high resistance factor (RF) since it is higher than 10 (Herbicide Resistance Action Committee, 2012; http://hracglobal.com/ herbicide-resistance/confirming-resistance).

The correct identification of the species of biotypes under study and the evaluation of segregation and resistance factor between biotypes (GR X GS), together with a high number of replications used in seed analysis of the six biotypes, allow us to draw robust conclusions about the effect of glyphosate resistance on hairy fleabane seeds. First, seed analysis results were analyzed individually for each biotype. It was performed to infer whether the variations as a function of glyphosate sensitivity in each biotype were high and analyze the behavior of the biotype B48S (*C. blakei*) in relation to the others (Figure 1). In the second stage, the means between the GR X GS biotypes were analyzed, not considering the results obtained for B48S because it is from another species (Figure 2).

The results of the physiological quality of hairy fleabane seeds presented differences between GR X GS biotypes, with better results for GS, except for B48S. Overall, B20S had the best results among biotypes, while B48S had the worst (Figure 1). Biotypes of *C. bonariensis* showed results higher than 60% for total germination (Figure 1B) and vigor (Figure 1A). These results are considered high in relation to those found by Piasecki et al. (2019a), who reported about 38% germination for glyphosate-resistant *C. bonariensis*. Germination of hairy fleabane seeds is influenced by the environment (light, temperature, salinity, and depth of sowing), with values ranging from 18 to 32% under ideal conditions (Nandula et al., 2006, Wu et al., 2007; Davis and Johnson, 2008). For B48S, the results for the same variables were lower than 10%, being considered low (Figure 1A and B). Thus, in addition to environmental conditions, the species has essential importance for the physiological quality of seeds.

The low values for the physiological quality of B48S seeds when compared to the others are mainly due to the high number of dead and empty seeds, with values of 14.9 and 66.6%, respectively, totaling 81.5% (Figure 1D and E). These results indicate that B48S has low



Bars indicate the standard deviation of the mean. Different letters among biotypes indicate statistical difference by the Tukey's test at p≤0.05. B01R, B11R, and B29R: glyphosate-resistant biotypes of *Conyza* sp. B17S, B20S, and B48S: glyphosate-sensitive biotypes of *Conyza* sp. F) The sum of two interfaces for each biotype indicate the total percentage of dormant seeds, and individual values indicate viable and not viable seeds. (A) First germination count (FGC - vigor) at 7 days after sowing (DAS); and (B) total germination, (C) abnormal seedlings, (D) dead seeds, (E) empty seeds, and (F) dormant seeds (viable and not viable by tetrazolium test) at 14 DAS.

Figure 1 **-** Percentage results per biotype of evaluated variables of seeds of six glyphosate-resistant (B01R, B11R, and B29R) and glyphosate-sensitive (B17S, B20S, and B48S) *Conyza* sp. biotypes.

reproductive efficiency, probably due to difficulties in pollination and/or fertilization of *C. blakei* when compared to other biotypes. Moreover, the total cycle of B48S was about 50 days longer than the others were, which was ~150 days, and this difference may have influenced the results. On the other hand, the low reproductive efficiency and the longer cycle of B48S indicate that *C. bonariensis* may have evolved from a reproductive point of view, and it may explain the fact that this species has greater agricultural importance than *C. blakei*.

The mean results of seed quality for GR X GS biotypes, not considering B48S, indicate that resistant biotypes presented lower values for vigor and germination, in the order of 16 and 13%, respectively (Figure 2A and B). No significant difference was observed for abnormal and dead seedlings (Figure 2C and D). However, GR hairy fleabane showed 44% more empty seeds (Figure 2E) and about five times more dormant seeds than GS (Figure 2F). Therefore, the results for the physiological quality of seeds showed that GR hairy fleabane has a fitness cost in relation to GS (Figure 2). A reduction in germination of seeds from *Ipomoea purpurea* (Van Etten et al., 2016) and *Ambrosia trifida* (Dinelli et al., 2013) resistant to glyphosate have been reported. However, the mechanisms that cause this reduction are unknown.

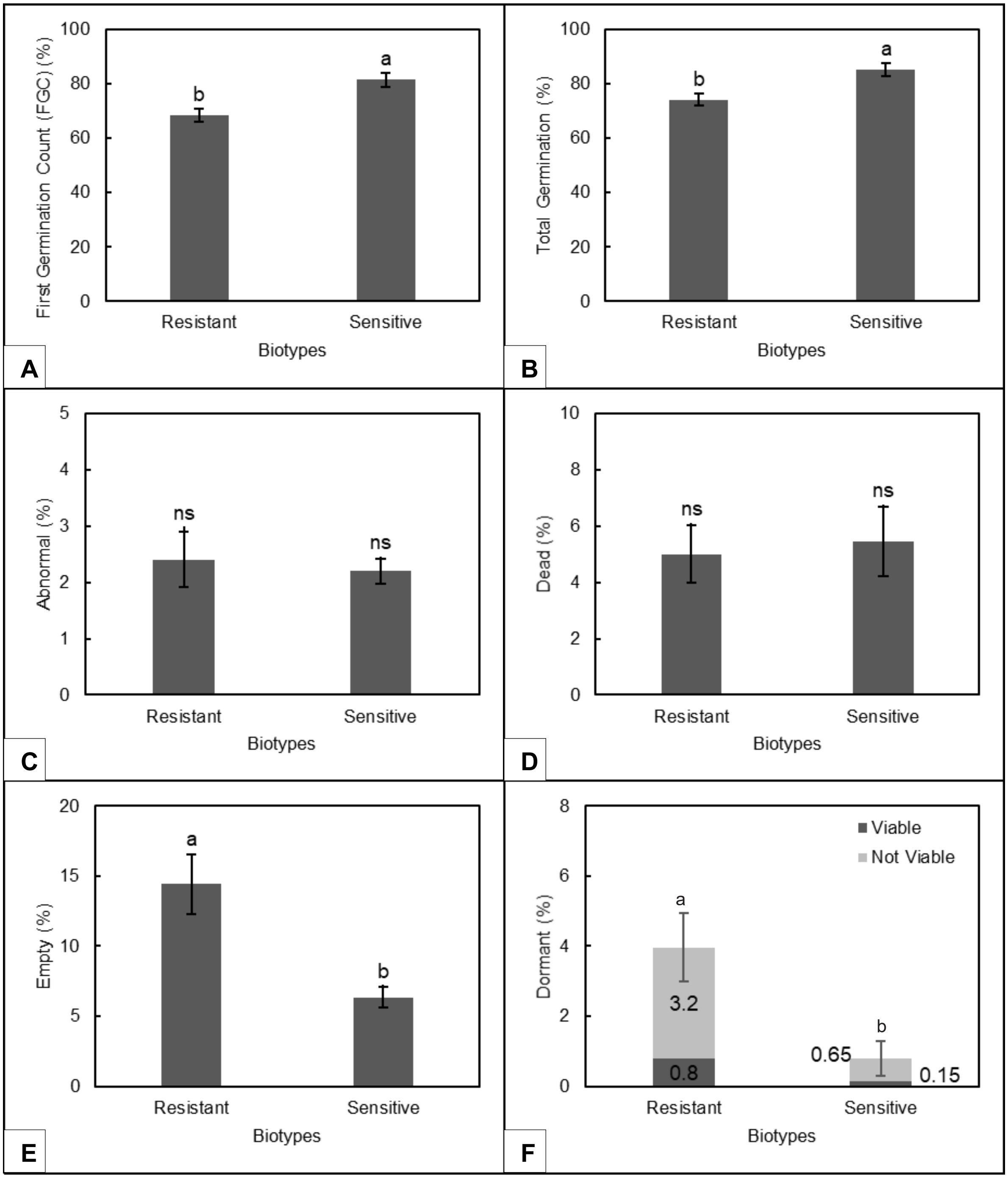
The tetrazolium test (Figure 3) showed that GR had about five times more dormant seeds than GS (Figure 2F). In addition, the viability of dormant seeds from GR was about 5.3 times higher than GS. Probably, seeds of hairy fleabane GR had changes in hormone content involved with seed dormancy and germination, such as gibberellic, indole acetic, and abscisic acids (Taiz et al., 2017). The higher viability of dormant seeds from GR hairy fleabane can be considered an adaptive plant survival strategy. This characteristic allows that GR hairy fleabane shows germinative flushes in time as a function of variations and stimuli received from the environment. Therefore, seeds germinate and emerge only under appropriate conditions. Although the result found for viable dormant seeds is only 0.8% of the total seeds produced for GR hairy fleabane, this number represents thousands of viable seeds, considering the high seed production capacity that hairy fleabane has (more than ~100 thousand per plant). Another interesting point of this result is that the highest number of viable dormant seeds will supply the soil seed bank.

For GS biotypes, about 81.5% of seeds germinate in the first 7 DAS (Figure 2A), which indicate high vigor, and only 3.7% from 7 to 14 days, totaling 85.2% (Figure 2B), whereas only 0.15% remained dormant and viable (Figure 2F). On the other hand, seed germination of GR biotypes was 68.5% in the first 7 DAS and 5.7% from 7 to 14 DAS, totaling 74.2%.

High germination of GS biotypes at the early days of sowing is unfavorable for their survival in the presence of selective factor (glyphosate in this case). Thus, if any control factor is applied in the initial germination period (e.g., herbicides), it will cause a high reduction in GS plant density. However, biotypes of GR hairy fleabane with lower vigor and germination in the initial period germinate at different flows and periods, which favors the escape of initial control factors, produce plants and, consequently, seeds to supply the soil seed bank and ensure the perpetuation of the species.

On the other hand, in the absence of the selective factor glyphosate, seeds of GS hairy fleabane tend to settle quickly and occupy ecological niches before GR. Thus, late germination of GR biotype seeds establishes seedlings under intense competition with established GS plants (Owen et al., 2014). Differences in seed production and quality between GR and GS biotypes affect the frequency of occurrence and persistence of GR or GS biotypes in the environment (Torres-García, 2015). Thus, lower physiological quality of seeds tends to reduce the frequency of GR biotypes in the environment and return to susceptibility in the absence of glyphosate application (Maxwell et al., 1990).

In agricultural systems, weed seed production plays an essential role in the perpetuation of the species. Weed seeds may remain viable in the soil for more than ten years, producing emergence flows and making management difficult (Radosevich et al., 2007). The longevity of hairy fleabane seeds in the soil is around three years (Wu et al., 2007). However, the high number of seeds produced per plant and dormancy favor the replenishment of soil seed bank. Thus, although there is a fitness cost in the quality of hairy fleabane seeds due to glyphosate resistance, this fitness cost is probably not negatively affecting the reduction of the existing population in agricultural areas using glyphosate. Furthermore, dormancy of viable seeds and probable intermittent germination tend to compensate for lower germination and initial vigor,



Bars indicate the standard deviation of the mean. Different letters among biotypes (R and S) indicate statistical difference by the Tukey's test at p≤0.05. B01R, B11R, and B29R: glyphosate-resistant biotypes of *Conyza* sp. B17S and B20S: glyphosate-sensitive biotypes of *Conyza* sp. F) The sum of two interfaces for each biotype indicate the total percentage of dormant seeds, and individual values indicate viable and not viable seeds. (A) First germination count (FGC - vigor) at 7 days after sowing (DAS); and (B) total germination, (C) abnormal seedlings, (D) dead seeds, (E) empty seeds, and (F) dormant seeds (viable and not viable by tetrazolium test) at 14 DAS. B48S was not considered.

Figure 2 - Average percentage results of evaluated variables of seeds of three glyphosate-resistant (B01R, B11R, and B29R) and two glyphosate-sensitive (B17S and B20S) *Conyza* sp. biotypes.

ensuring that plants develop and produce seeds to replenish soil seed bank, as well as perpetuate and distribute glyphosate-resistant hairy fleabane plants in future generations. In this way, in order to reduce the infestation by GR plants, strategies should be developed to reduce the seed bank and the number of plants surviving the management practices. Alternating weed management methods and times can be an effective strategy.

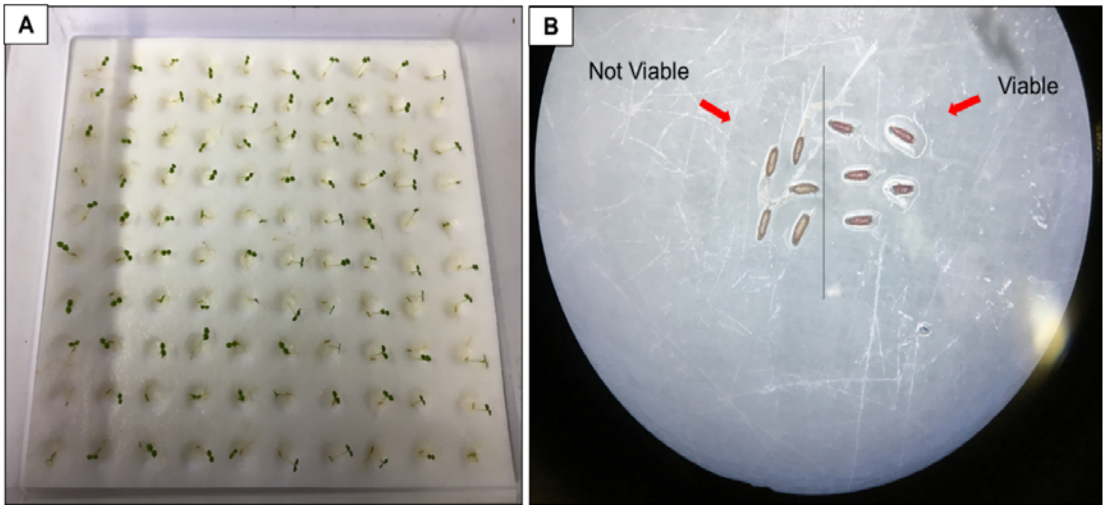


Figure 3 - Details of seeds analysis. A) *Conyza* sp. seed germination and B) tetrazolium test.

Glyphosate-resistant *C. bonariensis* biotypes presented lower physiological quality of seeds when compared to -sensitive biotypes of the same species, characterizing a fitness cost due to the resistance process. Glyphosate-resistant *C. bonariensis* biotypes have higher number of viable dormant seeds than sensitive biotypes. To our knowledge, this is the first report of fitness cost on seed quality of *C. bonariensis* as a function of glyphosate resistance. The results of the present study indicate that *C. bonariensis* presents superior physiological quality of seed when compared to the species *C. blakei.*

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