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Stressful Conditions Affect Seed Quality in Glyphosate Resistant *Conyza bonariensis* (L.)

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Abstract: *Conyza bonariensis* (L.) Cronquist is one of the main glyphosate-resistant weeds in no-till fields in Brazil. Here, the seed quality of glyphosate-resistant (R) and -susceptible (S) *C. bonariensis* biotypes, collected from different sites, was evaluated under stressful conditions by different seed tests. Glyphosate resistance was confirmed by dose-response and shikimate accumulation assays. The resistance factors were 6.9 (R1/S1), 4.5 (R2/S2), and 5.8 (R3/S3). Biotypes S1, S2, and S3 accumulated 2.7, 2.4, and 2.8 times more shikimic acid than biotypes R1, R2, and R3, respectively. Stress-free seed viability and germination potential ranged from 39% to 57% and from 37% to 57%, respectively, with no difference between R and S biotypes within each collection site. Seed incubation at 8 °C over 7 days (cold test) promoted greater germination in S biotypes (54% to 79%) compared to R ones (28% to 39%). In the accelerated aging tests (incubation at 42 °C over 48 hours), the germination decreased in both S (11% to 27%) and R (6% to 16%) biotypes. In the high-temperature stress tests, there were no differences in germination within biotypes at 35 and 45 °C; however, at 60 °C, the germination of the S1, R1, S2, R2, S3, and R3 biotypes was reduced by approximately 51%, 54%, 63%, 59%, 40%, and 30%, respectively. Under non-stressful conditions, germination potential and seed viability were similar in R and S biotypes; however, under cold or heat stress conditions, R biotypes reduced their germination rates, revealing that glyphosate resistance causes a fitness penalty in *C. bonariensis* at the seed level. However, because seed viability was not determined after experiments, it cannot be stated that such reduction in germination was due to the death or only a dormant phase of the seeds.

Keywords: fitness penalty; germination rate; high-temperature stress; seed viability

1. Introduction

Weeds of the *Conyza* genus are very prolific species that produce small seeds that are easily dragged by the wind because their pappus reduces the speed of settlement [1], contributing to the dispersion of seeds to adjacent and long-distance areas before they fall to the ground [2]. *Conyza* species are native to South and North America, but today are widespread in a wide variety of agronomic crop systems worldwide [3,4]. These weeds self-pollinate and can produce between 100 and 200 thousand winged seeds, a feature that, combined with their invasive behavior and competitiveness [1,5], makes these species some of the most difficult to manage worldwide [3].

Glyphosate (N-(phosphonomethyl)-glycine) is a broad-spectrum herbicide widely used to control weeds in both agricultural and non-agricultural areas [6]. This herbicide is absorbed through foliar tissues and translocated via phloem to meristematic tissues [7], where it inhibits the enzyme 5-enolpyruvyishikimate 3-phosphate synthase (EPSPS). The inhibition of this enzyme in the shikimate pathway prevents the biosynthesis of three essential amino acids (phenylalanine, tyrosine, and tryptophan) [8]. Because the EPSPS inhibition by glyphosate causes shikimic acid accumulation, monitoring of shikimate levels is a widely used parameter to determine glyphosate resistance [9].

Conyza species occur in the Southern, Southeastern, and Midwestern regions of Brazil, making them weeds of agricultural concern [10]. Among the *Conyza* species, *C. bonariensis* (L.) Cronquist (hairy fleabane) is the major weed due to its high adaptability to the main production system (no-till) employed in the country, as well as its ability to select for glyphosate resistance [11]. In 2017, it was estimated that glyphosate-resistant *Conyza* species infested at least 7.7 million ha of soybean alone [12], which corresponded to ~23% of the area planted with this crop in Brazil and ~10% of the total planted area in the country [13].

Knowledge of the biology and ecology of weeds plays an important role in planning weed management strategies [10,14]. Great efforts have been made to characterize the factors involved in the glyphosate resistance, dispersal, and management of *C. bonariensis*, including growth analysis and bioecological aspects [10]. In considering its seed biology, there is abundant information regarding *Conyza*'s seed germination growing degree days requirements for different sowing dates (autumn, winter, and spring) and agricultural environments [10], as well as its seed viability [11,15], emergence times over a growing season [16], seed longevity [17], and some aspects related to seed quality, such as factors related to seed production, dead seeds, empty seeds, and dormant seeds, among others parameters [18,19]. However, studies on how stress conditions (cold or heat) affect the seed quality of glyphosate-resistant *C. bonariensis*—which may contribute to its management by reducing seed persistence, reducing the soil seed bank, and consequently preventing the emergence of weeds at critical times [20]—are scarce.

The objectives of this study were to evaluate the seed quality of glyphosate-resistant and -susceptible *C. bonariensis* biotypes, collected from different sites of South and Southeast Brazil, subjected to stressful conditions (cold, accelerated aging, and high-temperature) after having characterized the glyphosate resistance by dose–response and shikimate accumulation assays.

2. Materials and Methods

2.1. Seed Collection and Biotypes Isolation

Seeds of *C. bonariensis* biotypes, based on their biology and ecophysiology traits [21], were collected from several sites in South and Southeast Brazil. The seeds of biotypes with suspected glyphosate resistance were collected from surviving plants after a glyphosate treatment in annual and perennial crop fields. On the other hand, the seeds of susceptible (S) biotypes were collected from plants growing on roadsides near their respective fields where suspected resistant (R) plants occurred. The seed collection was performed during the final flowering stage and seeds were stored in paper bags. The seeds were maintained at room temperature for three months in the laboratory.

The seeds of suspected R-plants were sowed in 10-L plastic pots filled with a mixture of a clay-textured soil and a commercial organic substrate (3:1; *v*/*v*). Then, glyphosate (Roundup Original DI, isopropyl amine salt, 360 g L^{-1} of acid equivalent (ae), Monsanto do Brasil Ltda.) was applied to four-leaf plants at 720 g ae ha^{-1} to eliminate susceptible individuals from the field-collected seeds. Surviving plants were grown separately under greenhouse conditions, and the seeds produced were collected, stored in paper bags, and labelled according to their specific collection site. These purified seeds were used for further experiments. Six biotypes (S1, R1, S2, R2, S3, and R3) that originated from three sites (A, B, and C) were used in this study (Table 1).

Table 1. Geographical locations of the Brazilian sites of seed collection of *Conyza bonariensis* biotypes.

Site	Municipality/State	Biotype	Coordinates
A	Passo Fundo, Rio Grande do Sul	S1	−28°20′03″ S and −52°17′00″ W
		R1	−28°19′56″ S and −52°17′10″ W
B	Palmeira, Paraná	S2	−25°24′26″ S and −50°00′13″ W
		R2	−25°24′32″ S and −50°00′11″ W
C	Matão, São Paulo	S3	−21°36′05″ S and −48°25′56″ W
		R3	−21°36′18″ S and −48°26′10″ W

Seeds of R and S *C. bonariensis* biotypes were sowed in 500-mL plastic pots (11 cm diameter) filled with a mixture of a clay-textured soil and a commercial organic substrate (3:1; *v*/*v*). Plants (one plant per pot) were grown in a growth chamber under 25 °C temperature, 14-h photoperiod, and 60% relative humidity. Plants at the rosette stage were used for the herbicide treatments.

2.2. Dose–Response Assay

R and S *C. bonariensis* biotypes from each collection site were contrasted by dose–response assays. Glyphosate was applied on plants at the doses of 0, 22.5, 45, 90, 180, 360, 720, 1440, 2880, and 5760 g ae ha^{-1}. A backpack sprayer pressurized with CO_2 equipped with two fat flan nozzles XR 110.02 VS was used for herbicide application, delivering a 200 L ha^{-1} spray volume. Ten plants per herbicide dose were treated, and 30 days after application, plants were cut close to the soil. The shoots were stored in paper bags and then dried in a forced air oven for five days at 60 °C. After that, dry mass was measured using a semi-analytical balance (±0.01 g). Data were expressed as a percentage in relation to the untreated control (0 g ha^{-1}).

2.3. Shikimic Acid Assay

The quantification of shikimic acid accumulation after herbicide application was used to confirm the glyphosate resistance in the R biotypes, according to the methods employed by Carvalho et al. [22]. Glyphosate was applied to ten *C. bonariensis* plants at 0 and 720 g ae ha^{-1}. A backpack sprayer pressurized with CO_2 and equipped with two fat flan nozzles 110.02 vs. was used for herbicide application, delivering a 200 L ha^{-1} spray volume. Seven days after herbicide application, green leaves were gathered, stored in paper bags, and then dried in a forced air convection oven for seven days at 60 °C. Then, dried material was ground with a Willey mill grinder (20-mesh), stored in plastic bags, and frozen at −20 °C until analyses. The content of shikimic acid was determined with a high-performance liquid chromatography and mass spectrometry system. Data are expressed as the accumulation of shikimic acid after glyphosate application determined by the difference in the concentration of shikimic acid between treated and untreated plants.

2.4. Seed Viability by Tetrazolium Test

Tests with *C. bonariensis* seeds were performed according to the methods used by Costa et al. [23]. The seeds used in these experiments were taken at random to avoid biases regarding the seed quality found in the field. In the tetrazolium test, embryos were extracted after seeds were soaked in water for 24 h. After removal, embryos were submerged into a 0.6% tetrazolium solution overnight at 30 °C. Embryos were observed under a stereo microscope (40× magnification) regarding the presence (viable embryos) or absence (unviable embryos) of a red/rose colored area. Data are expressed as a percentage of viable embryos.

2.5. Seed Stress Tests

For the germination potential, cold, and accelerated aging tests, seeds were placed on a sheet of blotting paper in plastic boxes (11 × 11 cm), moistened with distilled water at a volume equivalent to

2.5 times the weight of the dry paper, and then the plastic boxes were sealed with a plastic film. In the high-temperature stress tests, seeds were firstly put inside aluminum capsules and incubated at 35, 45, and 60 °C for 48 h, before placing them in the boxes. Cold test seeds were incubated for 7 days at 8 °C, while the accelerated aging test seeds were incubated at 42 °C for only 48 h. After applying the different procedures for each test, the boxes were incubated at 25 °C and a 12-h light photoperiod under 35 μmol m^{-2} s^{-1} flux rate delivered by cool white fluorescent tubes. Germination of seed was evaluated 4 days after incubation.

All experiments were performed in a completely random design with four replicates (with each replicate consisting of one box with fifty seeds) per biotype, and experiments were repeated twice. Data are expressed as a percentage of viable embryos in the tetrazolium test and as a percentage of normal germinated seeds.

2.6. Statistical Analysis

Dose–response data were fitted to a nonlinear (log-logistic) regression model: Y = min + {(max − min)/[1 + (x/EC_{50})Hillslope]} [24], where Y is the shoot dry mass expressed as a percentage of the untreated control, min and max are the coefficients corresponding to the lower and upper asymptotes, Hillslope is the slope of the line, EC_{50} is the herbicide dose at the point of inflection halfway between the upper and lower asymptotes (i.e., the GR_{50}), and x is the herbicide dose. The dose required to reduce shoot dry mass by 50% (GR_{50}) was expressed by the EC_{50} adjusted to a decimal place, and the dose required to reduce shoot dry mass by 80% (GR_{80}) was estimated by using the adjusted equation for both R and S biotypes. The resistance factor (RF) was calculated for GR_{50} and GR_{80}, relating glyphosate-S and -R biotypes within the same collection site, as follows: RF_{50} = GR_{50}(R)/GR_{50}(S) and RF_{80} = GR_{80}(R)/GR_{80}(S).

Because local environmental conditions affect seed quality [25], the R and S *C. bonariensis* biotypes were analyzed only within collection sites. For the shikimic acid assay, tetrazolium test, germination potential test, cold test, and accelerated aging test, data were analyzed by *t* test, while for the high-temperature stress test, data were firstly analyzed by ANOVA in a factorial design (two biotypes × three temperatures), comparing S and R biotypes originating from the same site. Values of $p > 0.05$ denoted significant differences and the means were compared by the Tukey test at 95% probability.

3. Results

3.1. Dose–Response Assay

For each seed collection site, glyphosate reduced the shoot dry mass of the S *C. bonariensis* biotypes at lower doses than the R biotypes at 30 days after herbicide application (Figure 1). The GR_{50} values were 91 and 628 (S1 and R1), 79 and 350 (S2 and R2), and 88 and 511 g ae ha^{-1} (S3 and R3), respectively. The GR_{80} values were approximately 145 and 1030 (S1 and R1), 162 and 698 (S2 and R2), and 165 and 745 g ae ha^{-1} (S3 and R3), respectively. The resistance factors were found to be 6.9 and 7.1 (R1/S1), 4.5 and 4.3 (R2/S2), and 5.8 and 4.6 (R3/S3), considering the RF_{50} and RF_{80} values, respectively (Table 2).

3.2. Shikimic Acid Assay

The S *C. bonariensis* biotypes accumulated more shikimic acid than the R biotypes within each seed collection site. The accumulation of shikimic acid was approximately 18,132 and 6808 μg g^{-1} dry mass (S1 and R1), 21,984 and 9198 μg^{-1} (S2 and R2), and 20,064 and 7180 μg^{-1} (S3 and R3), respectively. Thus, biotypes S1, S2, and S3 accumulated 2.7, 2.4, and 2.8 times more shikimic acid than biotypes R1, R2, and R3, respectively (Figure 2).

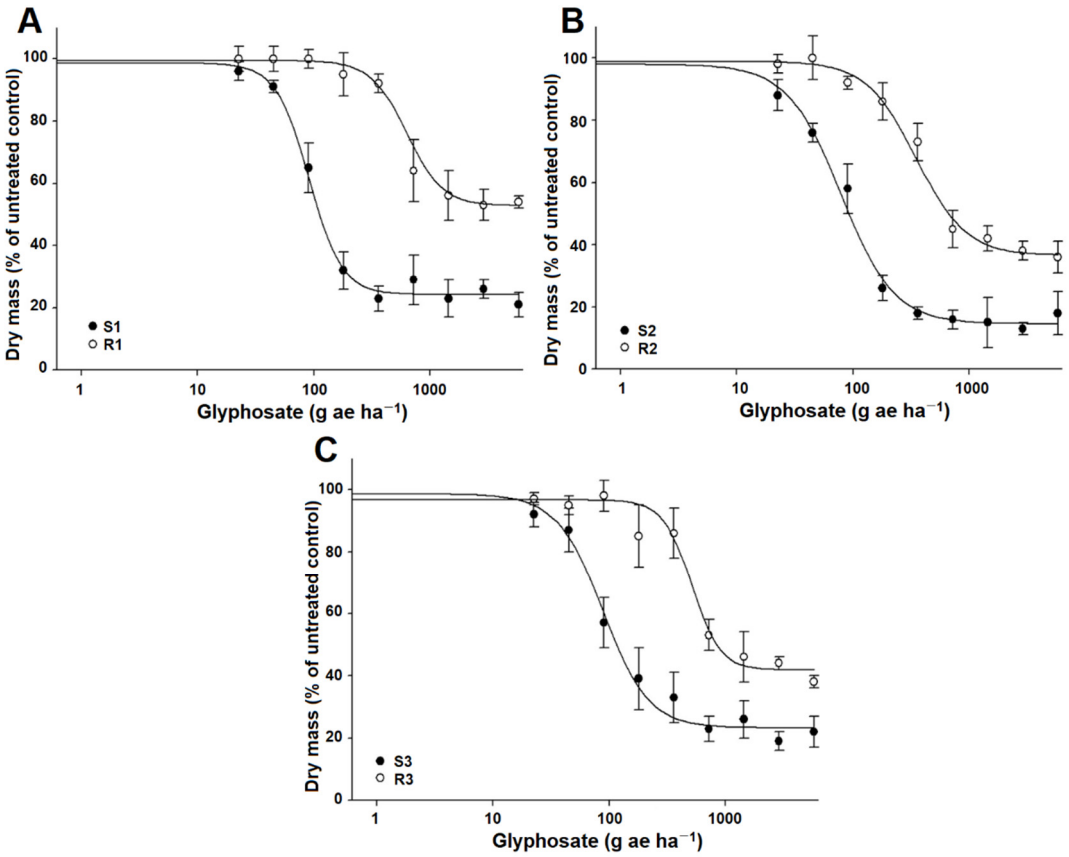

Figure 1. Relation between shoot dry mass and glyphosate dose in dose–response assays for glyphosate-susceptible (S) and -resistant (R) *Conyza bonariensis* biotypes at 30 days after herbicide application. Seeds were collected in Passo Fundo-RS (**A**), Palmeira-PR (**B**), and Matão-SP (**C**), Brazil. Vertical lines represent the standard error of means (n = 10).

Table 2. Equation parameters, coefficient of determination (R^2), significance of regression (p-value), and glyphosate doses required to reduce shoot dry mass by 50% (GR_{50}) and 80% (GR_{80}), as well the respective resistance factor (RF_{50} and RF_{80}), of glyphosate-susceptible (S) and -resistant (R) *Conyza bonariensis* biotypes collected from three seed collection sites in Brazil.

Site /1	Biotype	Parameters /2					GR_{50}	RF_{50}	GR_{80}	RF_{80}
		min	max	Hillslope	R^2	p-Value				
A	S1	24.3	98.7	3.0	0.9773	<0.001	90.6	6.9	145.1	7.1
	R1	52.8	99.4	2.8	0.9765	<0.001	628.0		1030.1	
B	S2	14.6	97.9	1.9	0.9756	<0.001	78.5	4.5	161.8	4.3
	R2	36.6	98.9	2.0	0.9747	<0.001	349.5		697.9	
C	S3	23.3	98.6	2.2	0.9722	<0.001	88.1	5.8	164.9	4.6
	R3	41.9	96.7	3.6	0.9691	<0.001	510.8		754.9	

/1 Seeds were collected in Passo Fundo-RS (A), Palmeira-PR (B), and Matão-SP (C), Brazil. /2 min and max are the coefficients corresponding to the lower and upper asymptotes, GR_{50} is the herbicide dose at the point of inflection halfway between the upper and lower asymptotes, and Hillslope is the slope of the line.

3.3. Embryo Viability and Germination Potential

The embryo viability and seed germination potential of *C. bonariensis* ranged from 37% up to 57%, with no difference between S and R biotypes originating from the same seed collection site. The lowest embryo viability and germination potential, which ranged between 37% and 43%, were found in seeds from site A. At the B and C sites, these parameters ranged from 51% to 57% (Figure 3).

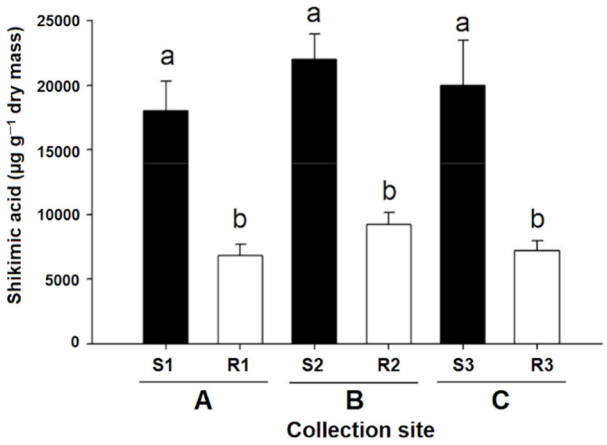

Figure 2. Shikimic acid accumulation in leaf tissues of glyphosate-susceptible (S) and -resistant (R) *Conyza bonariensis* biotypes at seven days after application of 720 g ae ha^{-1} glyphosate. Seeds were collected in Passo Fundo-RS (**A**), Palmeira-PR (**B**), and Matão-SP (**C**), Brazil. Vertical lines represent the standard error of means (n = 10). Different letters denote statistical differences within a collection site according to the 95% Tukey test.

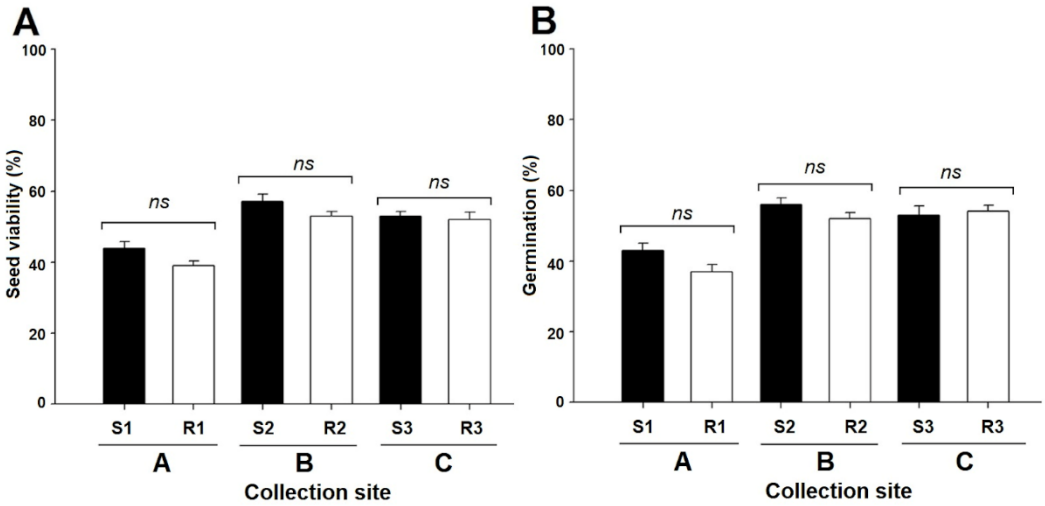

Figure 3. Embryo viability and germination potential in seeds of glyphosate-susceptible (S) and -resistant (R) *Conyza bonariensis* biotypes. Seeds were collected in Passo Fundo-RS (**A**), Palmeira-PR (**B**), and Matão-SP (**C**), Brazil. Vertical lines represent the standard error of means (n = 4) using 50 seeds per replicate. *ns*: no statistical differences within a collection site according to the 95% Tukey test.

3.4. Cold and Accelerated Aging Tests

The incubation period at 8 °C a week (cold test) promoted a greater seed germination of S *C. bonariensis* biotypes (S1 = 67%; S2 = 79% and S3 = 54%) in relation to their R counterparts (R1 = 28%; S2 = 39% and S3 = 38%) (Figure 4A). The germination rates of biotypes S1 and S2 were higher than those observed in the embryo viability and germination potential tests. In the accelerated aging test (48 h of incubation at 42 °C), the germination rates of the R biotypes were lower than the S biotypes, regardless of the collection site. The seed germination was 11% and 6% (S1 and R1), 15% and 7% (S2 and R2), and 27% and 16% (S3 and R3), respectively (Figure 4B).

3.5. High-Temperature Stress Test

The S *C. bonariensis* biotypes showed higher germination rates than the R biotypes within each temperature regime. The germination of the S and R biotypes ranged from 52% to 63% and from 29%

to 43%, respectively, at 35 and 45 °C. However, there were no differences within biotypes for these temperature regimes. The lowest germination rates were observed at 60 °C in all biotypes (except for the R3 biotype, which had a similar germination rate at 35 and 45 °C). In this case, the germination of the S1, R1, S2, R2, and S3 biotypes was reduced by approximately 51%, 54%, 63%, 59%, and 40%, respectively, in comparison to the averages observed at 35 and 45 °C (Figure 5).

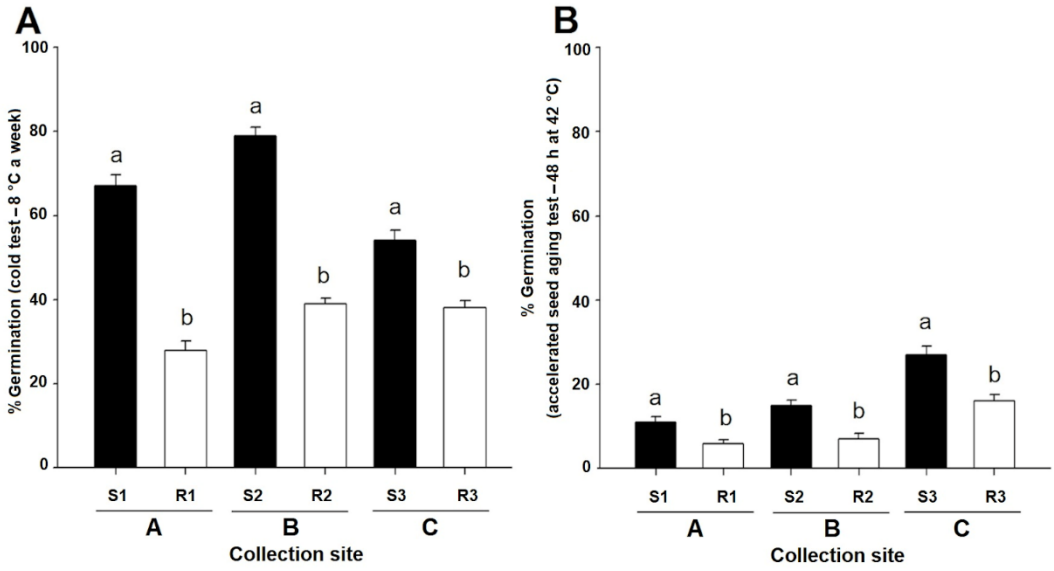

Figure 4. Seed germination under cold (**A**) and accelerated aging (**B**) tests in glyphosate-susceptible (S) and -resistant (R) *Conyza bonariensis* biotypes. Seeds were collected in Passo Fundo-RS (**A**), Palmeira-PR (**B**), and Matão-SP (**C**), Brazil. Vertical lines represent the standard error of means (n = 4) using 50 seeds per replicate. Different letters denote statistical differences within a collection site according to the 95% Tukey test.

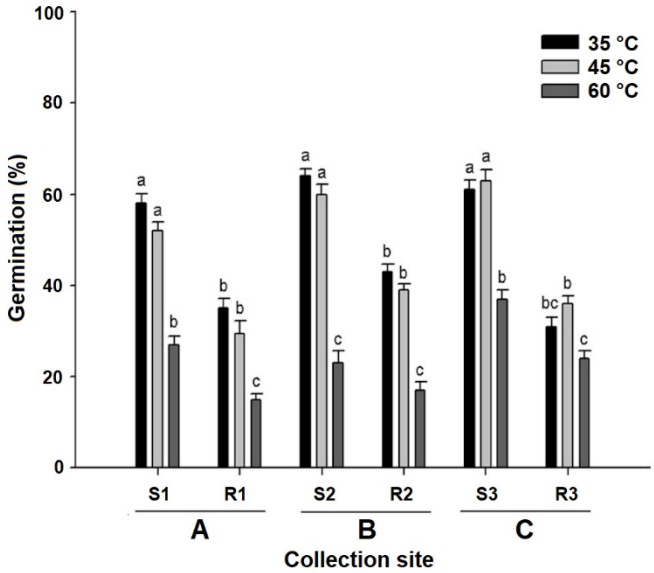

Figure 5. Seed germination under high-temperature stress test for glyphosate-susceptible (S) and -resistant (R) *Conyza bonariensis* biotypes. Seeds were collected in Passo Fundo-RS (**A**), Palmeira-PR (**B**), and Matão-SP (**C**), Brazil. Vertical lines represent the standard error of means (n = 4) using 50 seeds per replicate. Different letters denote statistical differences within a collection site according to the 95% Tukey test.

4. Discussion

The S *C. bonariensis* biotypes showed lower GR_{50} values than the R biotypes. Previous experiments performed with susceptible populations of *C. bonariensis* and *C. canadensis* have shown GR_{50} values below 100 g ae ha^{-1} [16,26], demonstrating that species of the *Conyza* genus are highly susceptible to glyphosate. In addition, the GR_{50} values of the S populations, regardless of the different geographic origins of each biotype, were similar. Piasecki et al. [18] observed a similar phenomenon, with S populations of *C. bonariensis* collected in different regions of southern Brazil showing no differences in their GR_{50} (also less than 100 g ae ha^{-1}). The R biotypes, in turn, presented different GR_{50} values, revealing different profiles of glyphosate resistance; however, the GR_{80} values were below the field dose recommended by the manufacturer for this species (960 g ae ha^{-1}), except for biotype R1 (GR_{80} = 1.030 g ha^{-1}). Different glyphosate-resistant *Conyza* species have shown resistance ratios ranging from 6 to 30 with regard to their S counterparts [4,27,28], depending on the resistance mechanism involved.

Because the EPSPS transforms the shikimate-3-phosphate and phosphoenolpyruvate into EPSP, the inhibition of this enzyme by glyphosate results in the accumulation of shikimic acid in susceptible plants [8]. Both R and S *C. bonariensis* biotypes accumulated shikimic acid (i.e., glyphosate was equally loaded in the phloem and reached the EPSPS in both biotypes) [29]. Similar results have been found in different studies where both R and S biotypes/populations of *Conyza* species accumulated shikimic acid [4,30,31]; however, the accumulation rates of shikimic acid in R plants was always lower than the accumulation observed in S plants. The low accumulation of shikimic acid between our R *C. bonariensis* biotypes suggests different resistance mechanism(s), which reduced the amount of glyphosate that reached or interacted with the EPSPS [27].

The viability and germination potential of *C. bonariensis* seeds were similar between biotypes within each collection site. The viability of seeds was low (±50% or less) in relation to other studies that have reported viability rates between 65% and 80% in R and S *Conyza* populations [11,19]. However, there are also cases with lower germination rates [2,23]. This discrepancy in the viability and germination rates may be due to seed longevity; in the particular case of the *Conyza* species, longevity is short due to the small size of seeds resulting in few stored reserves [32]; therefore, it is normal to observe the deterioration or death of seeds [15]. This suggests that R and S *C. bonariensis* seeds of site A were older than those collected at sites B and C. In addition, the geographical origin of the seeds could also have contributed to the differences in germination rates, since evolutionary adaptations to local climate influence the germination of *Conyza* species [33,34].

In general, *Conyza* seeds have shallow physiological dormancy [35]; however, in the cold tests (incubation at 8 °C for 7 h), the germination percentage of the S1 and S2 *C. bonariensis* biotypes was higher than in stress-free germination tests, suggesting that seeds of these biotypes had some type of latency, since this secondary dormancy may be responsible for low germination rates [15]. In relation to the aging accelerated tests (incubation at 42 °C for 48 h), all *C. bonariensis* biotypes presented low germination percentages (>30%), but germination was higher in R biotypes. Aging accelerated tests aim to determine the rate of deterioration processes and potential longevity of seeds [36]. These results suggest that seeds of R biotypes deteriorate and die faster than seeds of S biotypes at high temperatures. These results are consistent with those observed by Costa et al. [23] in *C. bonariensis*. At the vegetative level, there is no evidence for fitness cost in glyphosate-resistant *Conyza* species [5,26,28,37]; however, our results suggest that there may be a seed level fitness penalty in *C. bonariensis*. Piasecki et al. [18] also observed a reduction in the vigor and germination rate in seeds of glyphosate-resistant *Conyza* species and, in turn, a significant increase in both empty and dormant seeds. In addition, glyphosate application at the vegetative and reproductive stages impairs the production and viability of glyphosate-resistant *C. bonariensis* seeds [29].

In the high-temperature stress tests, both R and S *C. bonariensis* biotypes reduced their germination potential as the temperature increased. This reduction could be due to the accumulation of abscisic acid in response to exposure to high temperatures, which inhibited the germination and seedling

establishment [38]. High temperatures, in association with other factors, alter the structures enclosing the embryo, preventing radicle emergence, which causes the death of seeds [39], thus explaining the reduction in the germination rates of R and S *C. bonariensis* biotypes collected from different sites. Weed seed thermal death is dependent on the weed species, size of seeds (length × width × weight) [40], as well as the temperature regime and the exposure time [41,42]. *Amaranthus albus, Echinochloa colona, Portulaca oleracea, Sisymbrium irio, Sonchus oleraceus*, and *Solanum nigrum* presented 100% of seed mortality when exposed to 50 °C; however, the exposure time required ranged from 4 h up to 113 h, depending on the weed species [41,42]. In another study, the exposure of *A. retroflexus, E. crus-galli, Galinsoga quadriradiata, P. oleracea, Setaria viridis*, and *S. nigrum* to ±60 °C for 65 s reduced germination by only 10%; but the exposure of the seeds of these same weed species to 70–80 °C for ~90 s caused 100% seed mortality [40]. Our results suggest that thermal treatments could be an alternative for the control of glyphosate-resistant biotypes of *C. bonariensis*; however, there are no data related to the temperature regimes and exposure times needed to cause seed mortality in *Conyza* species.

The results revealed that seeds of R *C. bonariensis* biotypes, subjected to different stressful conditions (cold, heat, and/or accelerated aging), reduced their germination by a greater percentage than that of the S biotypes. However, the seed viability of R biotypes was not determined after these experiments; therefore, it cannot be positively stated that such reduction in germination was due to the death of seeds or if the seeds entered a latent phase, although it is widely known that the dormancy of *Conyza* species is short or non-existent [15,35].

5. Conclusions

Glyphosate resistance was corroborated in the *C. bonariensis* biotypes referred to as R1, R2, and R3 because they showed high GR_{50} values and low shikimate accumulation in relation to their S counterparts. Under stress-free conditions, the germination potential as well as the seed viability were similar between R and S *C. bonariensis* biotypes; however, under stressful conditions of cold, heat, or accelerated aging, the germination rate of R biotypes was reduced, regardless of the collection site, showing that glyphosate resistance affects seed quality in *C. bonariensis* (i.e., there is a fitness penalty at seed level). According to the high-temperature stress tests, thermal treatments could be an alternative for the control of glyphosate-resistant biotypes of *C. bonariensis*.

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