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Article

Effects of Environmental Factors on Seed Germination and Emergence of Glyphosate Resistant and Susceptible Sourgrass

Efeitos do Ambiente na Germinação e Emergência de Capim-Amargoso Resistente e Suscetível ao Glyphosate

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ABSTRACT - Sourgrass is one of the most problematic weeds of Brazil. It is an aggressive species in the allocation of resources from the environment and reported to be glyphosate resistant in several states of the country. This work has aimed to verify environmental effects on seed germination and seedling emergence of sourgrass. Laboratory and field trials were conducted to determine the effects of temperature, light, photoperiod, water availability and depth of burial in germination and emergence of seeds. The maximum germination was greater than 80% for seed originated from resistant plants between 15 and 30 °C. For the susceptible ones, the maximum germination occurred only at 30 °C. Germination occurred for both biotypes independent of the presence of light. The best photoperiod for germination of both biotypes was between 8 and 14 hours. Seeds originated from resistant plants showed higher germination levels at low temperatures in the presence of light and in less water availability. There was no germination from -0,8 MPa for both biotypes. The emergence for both biotypes was low, indicating that seed burial could be used in the management of the species. Seeds from resistant plants showed higher and larger germination in temperatures, water content and depth of burial ranges than from originated from susceptible plants.

Keywords: *Digitaria insularis*, water availability, photoperiod, seeds, temperature.

RESUMO - O capim-amargoso é uma das espécies mais problemáticas da agricultura brasileira. É uma espécie agressiva na alocação de recursos do meio, além de apresentar resistência ao glyphosate relatada em diversos Estados do País. Este trabalho teve o objetivo de verificar efeitos ambientais sobre a germinação ou emergência de sementes e plântulas do capim-amargoso. Foram conduzidos experimentos em laboratório e campo para determinar os efeitos de temperatura, fotoperíodo, disponibilidade hídrica e profundidade de semeadura. Todos os experimentos foram instalados em delineamento inteiramente casualizado com quatro repetições. A germinação máxima foi superior a 80% para sementes originadas de plantas resistentes ao herbicida glyphosate entre 15 e 30 °C. Para o suscetível ao herbicida, a melhor germinação ocorreu a 30 °C. A germinação para ambos os biótipos ocorreu independentemente da presença da luz. O melhor fotoperíodo para germinação de ambos os biótipos se localizou entre 8 e 14 horas. Sementes originadas de plantas resistentes mostraram maiores níveis de germinação para baixas temperaturas na presença de luz e para menor disponibilidade hídrica. Não houve germinação a partir de -0,8 MPa em ambos os biótipos. As sementes originadas de plantas resistentes germinam mais e em maiores faixas de temperatura, déficit hídrico e profundidade de semeadura do que as originadas de plantas suscetíveis.

Palavras-chave: *Digitaria insularis*, disponibilidade hídrica, fotoperíodo, sementes, temperatura.

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INTRODUCTION

Sourgrass (*Digitaria insularis*) is a perennial cycle Poaceae with a type C4 photosynthetic metabolism that presents populations resistant to glyphosate (Carvalho et al., 2012). This species is found contaminating areas of pasture, grains and orchards and in ruderal areas, being considered one of the most problematic weeds in Brazil (Carvalho et al., 2011).

Integrated weed management is a challenge for today's agriculture, mainly due to the increase in the number of cases of herbicide resistant plants in recent years. There are currently 35 species resistant to glyphosate in the world, of which seven are found in Brazil (Heap, 2016). The adoption of an integrated management has as one of the major limitations the lack of knowledge on the species biology and ecology (Carvalho, 2005).

Germination is one of the most critical stages in plant establishment. Factors such as temperature, light, soil moisture and depth of seed seeding are known to affect the species germination and emergence (Koger et al., 2004; Tang et al., 2015). Knowledge about the environmental requirements for germination of weed seeds, not yet studied for several species, is critical for the interpretation of their ecophysiological behavior in the field, in addition to enabling the development of seed bank reduction strategies in the cultivated areas (Gomes and Christoffoleti, 2008). Moreover, the differential expression in the percentage of germination among seeds originated from resistant and susceptible plants has already been studied for other species, such as goosegrass (*Eleusine indica*) (Ismail et al., 2002).

Therefore, knowing these aspects and associating them with the adoption of adequate control practices is information of great importance (Guo and Al-Khatib, 2003). The objective of this study was to verify the effects of temperature, light, photoperiod, water availability and sowing depth on the germination or emergence of sourgrass seeds from populations resistant and susceptible to glyphosate.

MATERIAL AND METHODS

Origin and proof of resistance

Seeds of sourgrass (*Digitaria insularis*) biotypes were collected in areas reported to having resistance – Brazilian city Matão, SP (21°35'8.35" S and 48°23'12.87" W) – and susceptibility to glyphosate – Brazilian city Santa Helena do Goiás, GO (17°50'19.04" S and 50°34'14.82" W). And the resistant biotype was previously described for having EPSPs (5-enolpyruvylhikimate-3-phosphate) mutations, differential translocation and glyphosate metabolism (Carvalho et al., 2012). Plants of both biotypes were submitted to the application of increasing doses of glyphosate (Roundup Original, 360 g a.e. L^{-1}) in the stage of 4 leaves (0, 75, 150, 300, 600, 900 and 1,200 g a.e. L^{-1}). Subsequently, the required amount of herbicide was calculated for visual control of 50% (I_{50}) of biotypes at 28 days after application using nonlinear log-logistic regressions and the resistance factor (RF) between biotypes was calculated by the relation between the I_{50} of the resistant biotype and the susceptible biotype ($I_{50}R/I_{50}S$).

General germination procedure

Hundred seeds of each biotype were put to germinate in Gerbox®-type plastic boxes (11 x 11 x 3 cm) on two sheets of germinating paper moistened with distilled water 2.5 times the mass of the dry papers (13 mL). The boxes were sealed with film paper to prevent loss of water by evaporation. In order to maintain moisture, more water was added when visually needed. Germination was evaluated daily for 30 days. Seeds with a visible radicle larger than 2 mm were considered germinated. At the end of the germination period, at 30 days after sowing, the percentage of germination (%G) was calculated (Maguire, 1962).

Effects of temperature and light

Eight constant temperatures (5, 10, 15, 20, 25, 30, 35 and 40 °C) and five alternating temperatures (10/20, 15/25, 20/30, 25/35 and 15/35 °C) were tested for their effect on germination. Photoperiod was established in 12 hours under a photosynthetically active light intensity of 105 ?mol m^{-2} s^{-1}. The same treatments were repeated for absence of light covering the in Gerbox® boxes with aluminum foil and the evaluations were carried out under green light.

Effects of photoperiod

Eight light periods were used (0, 6, 8, 10, 12, 14, 16 and 18 hours) in the photosynthetically active intensity of 105 ?mol m^{-2} s^{-1} under the temperature of 30 °C, which in the previous experiment was the one that provided the highest percentage of germination.

Effects of water availability

Six osmotic potentials were used (0.0; -0.2; -0.4; -0.6; -0.8; and -1.0 MPa) adjusted by the use of polyethylene glycol 6000 (Villela et al., 1991). Temperature of 30 °C and a 12 hour photoperiod were used.

Effects of sowing depth

Fifty seeds of each biotype were sown at the depths of 0, 1, 2, 3, 4, 5, 7, 10 and 15 cm below the soil surface in 2 L pots filled with clayey soil with the following characteristics: pH: 5.2; P: 42 mg kg^{-1}; K, Ca, Mg, H+Al, SB, T and V (%): 2.5; 25; 12; 31; 39.5; 70.5 $mmol_c$ dm^{-3}; and 56%, respectively. Clay, silt and sand contents were 428, 91 and 481 g kg^{-1}, respectively. The experiment was conducted under uncontrolled environmental conditions. Pots were irrigated whenever necessary. Seedlings emerged were counted up to 30 days after sowing.

Statistical analysis

In all tests, a completely randomized design with four replications was used. For light and temperatures, the data were submitted to a nonlinear Gauss-like regression analysis in which y = y0+A*exp(-0.5*((x-xc)/w)². For analysis of germination at alternating temperatures, a 2x5 factorial arrangement was used, in which 2 are the biotypes and 5 are the alternating temperatures. In the photoperiod evaluation, a 2x8 factorial arrangement was used, in which 2 are the biotypes and 8 the photoperiods. Data were submitted to analysis of variance (ANOVA) and means compared by the Tukey's test at 5% ($p \leq 0.05$). To evaluate germination in water deficit and different seeding depths, the data were submitted to nonlinear log-logistic regressions, Y = a/(1+exp(-k*(x-xc))), where Y represents the percentage of germination or emergence; *a* corresponds to the highest value observed/ k is the curvature of the line/ and *xc* is the osmotic potential or depth of sowing with a 50% effect on variable *Y*. Emergence data at different depths were transformed into root (x + 0.5) in order to obtain homogeneity of variance. In the analysis of variance, software Agroestat® was used and in the regression analysis, software Origin®.

RESULTS AND DISCUSSION

Biotypes

The responses of each biotype to the application of increasing doses of glyphosate were adjusted in a nonlinear log-logistic regression, with regression coefficients of 0.98. There was a difference in visual control of 5.31 times (RF) among the biotypes considering I_{50}. This RF corroborates those of studies with the same and other species in relation to resistance to glyphosate, such as, for example, *Eleusine indica* (Lee and Ngim, 2000; Barroso et al., 2015).

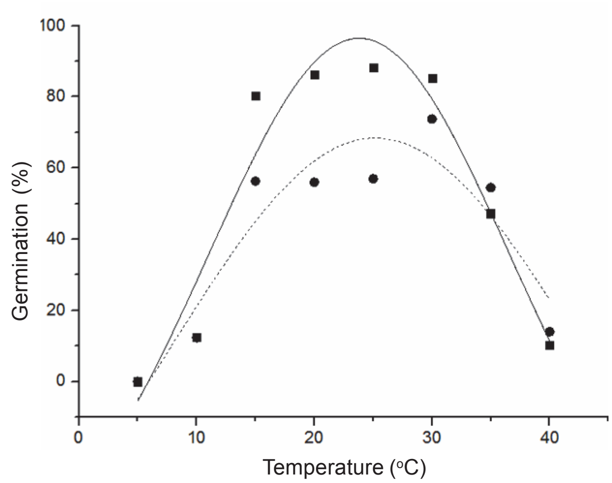
Temperature and Light

The germination responses of each biotype under constant temperature were adjusted in a Gauss-type nonlinear regression with regression coefficients always higher than 0.70. Under constant temperature, there were significant interactions for the germination between biotypes and temperatures. Under a 12 hour photoperiod, sourgrass seeds germinated over a wide temperature range (between 10-40 °C) (Figure 1). Above 40 °C, seed germination rarely occurs as it has negative effects affecting enzymes and other germination processes (Oliveira et al., 2014). In the absence of light, this spectrum was smaller, with germination between 15 and 35 °C (Figure 2).

In the presence of light, the germination percentage (G%) was greater for the resistant (R) biotype between 15 and 30 °C (always higher than 80%) while the susceptible (S) biotype obtained its better germination at 30 °C (lower than 80%). In the absence of light, the highest germination of both biotypes occurred between 25 and 30 °C. In this condition, the germination was lower, reaching the maximum value, 83%, only at 30 °C in the R biotype. Biotype R germinated more than the susceptible one at temperatures between 15 and 30 °C, with or without light, with an exception observed for 25 °C in the absence of light (Figure 2).

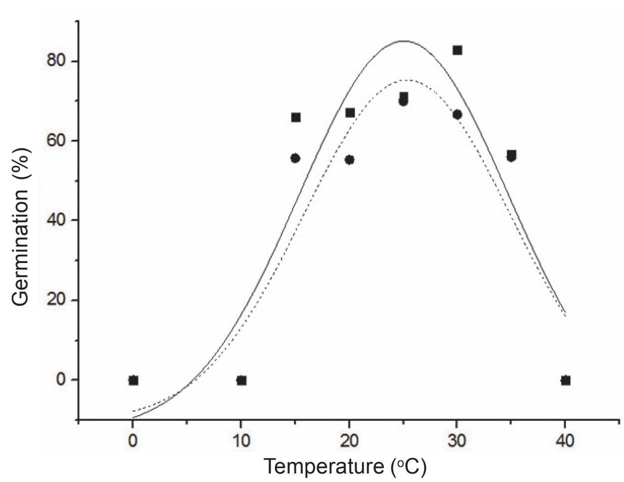
Thus, cardinal temperatures (minimum, optimum and maximum) for resistant seeds were 5 °C, 25 °C and higher than 40 °C for the presence of light and 10 °C, 30 °C and 40 °C in the presence of light. For susceptible seeds, they were 5 °C, 30 °C and higher than 40 °C and 10 °C, 25 °C and 40 °C in presence and absence of light, respectively.

These results are consistent with those obtained by Mendonça et al. (2014) and Mondo et al. (2010), who have classified sourgrass seeds as preferential positive photoblastics because they do not depend on light for germination, although this one has been higher in its presence (Vázquez-Yanes and Orozco-Segovia, 1993). This characteristic is common in weed seeds because it is a species adapted to different edaphoclimatic conditions, as observed in *Aegilops tauschii* and *Urochloa subquadripara* (Teuton et al., 2004; Fang et al., 2012).



Equations: GR(%) = -57.40 + 153.88*exp(-0.5*((x-23.81)/12.75)²); Gs(%) = -83.18 + 151.64 * exp(-0.5*((x-25.18)/17.54)²).

Figure 1 - Effect of temperatures on germination percentage of sourgrass resistant (R■) and susceptible (S●) to glyphosate, submitted to a 12-hour photoperiod for 30 days.



Equations: GR(%) = -12.73 + +97.86*exp(-0.5*((x-25.03)/9.67)²); Gs(%) = -10.23 + +85.56 * exp(-0.5*((x-25.33)/9.53)²).

Figure 2 - Effect of temperatures on germination percentage of sourgrass resistant (R■) and susceptible (S●) to glyphosate, submitted to absence of light for 30 days.

Under alternating temperature there are also significant interactions between biotypes and temperatures. Germination was higher for R biotype at temperatures 15/25 and 20/30 °C. For the S biotype germination presented a similar behavior and higher results in the temperatures 15/25, 20/30 and 25/35 °C. In the dark, the highest germination for both biotypes occurred at 20/30 °C (Table 1). It should be noted that germination with alternating temperatures was lower than that observed for constant temperature both in presence and absence of light. Seed germination of the R biotype was higher than that of the S biotype at all tested temperatures, except for 25/35 °C under light and 10/20 °C in the dark. Similar results were observed for other species of the genus *Digitaria* (Mondo et al., 2010). Apparently, germination in the dark minimizes the differences observed in germination between the R and S biotypes in the alternating temperatures.

Sourgrass seeds germination occurred, therefore, under different temperatures and light periods, although the latter increased the percentage germinated. The R biotype presented advantages over the S biotype at temperature conditions commonly found in regions where the species occurs (30-35 °C). The higher germination of R seeds in relation to S seed favors the propagation of this biotype in agricultural regions such as Brazilian states São Paulo and Paraná. In this case, the temperature variation did not increase the germination observed at constant temperatures. Thus, systems such as direct planting, where there is lower temperature fluctuation, may favor the germination of the species when compared to conventional cultivation systems.

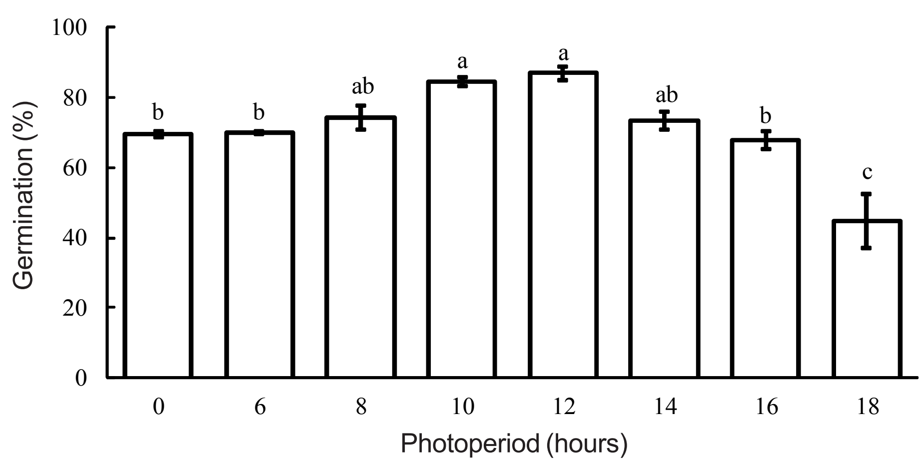
Table 1 - Effect of temperature alternation on seed germination percentage of sourgrass resistant (R) and susceptible (S) to glyphosate, submitted to a photoperiod of 12 hours or absence of light for 30 days.

Temperature (°C)	Germination light(1)		Germination dark1	
	R	S	R	S
	(%)			
10/20	65.3 Abc	48.5 Bb	44.0 Ac	18.0 Bc
15/25	70.3 Aab	62.5 Ba	50.0 Abc	52.0 Ab
20/30	77.3 Aa	64.8 Ba	72.3 Aa	71.6 Aa
25/35	58.3 Ac	58.8 Aa	59.0 Ab	56.0 Ab
15/35	59.0 Ac	47.1 Bb	54.0 Abc	54.0 Ab

(1) For each experiment the means followed by the same letter do not show a significant difference according to Tukey's test at level $p \leq 0.05$. Uppercase letters compare temperature effects among biotypes. Lowercase letters compare temperature effects in each biotype.

Photoperiod

There were no significant differences in germination between the two biotypes submitted to the different photoperiods. Only photoperiod effect occurred on seed germination, which was small. Periods of 8 to 14 hours of light provide greater germination of sourgrass seeds (Figure 3). The germination of almost 70% of seeds in the absence of light is highlighted, corroborating the data previously observed.



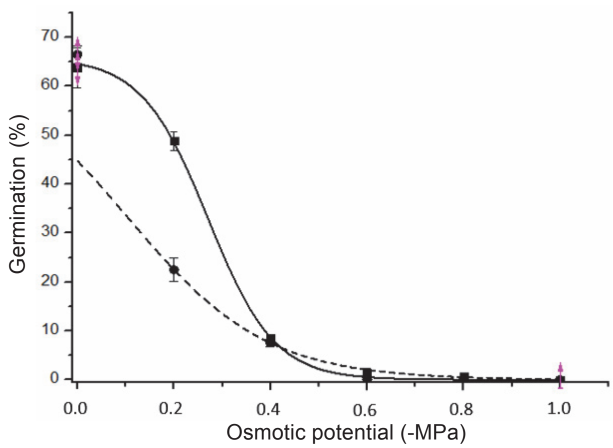
Means followed by the same letter do not show a significant difference according to Tukey's test at level $p \leq 0.05$.

Figure 3 - Effect of photoperiod on the average percentage of sourgrass seed germination at 30 days after sowing. Vertical bars represent the standard error of the means.

Water availability

A nonlinear log-logistic regression model, G (%) = a/(1+exp(-k*(x-xc))), R^2 0.99, was the one that best described the effect of water deficiency on sourgrass seed germination. In general it is observed that the R biotype obtained a higher rate of germination than the S as the concentration of PEG was increased, that is, water restriction decreased seed germination of R and S seeds. Seeds of the R biotype germinated until -0.8 Mpa. And seeds of the S biotype until -0.4 MPa. Under -0.2 MPa, germination of biotype R was almost 50% and of biotype S was 22.5% (Figure 4). This fact indicates seed germination capacity of the R biotype with less availability of water, that is, in dry periods of the year. The same was observed for *Lolium multiflorum* resistant to glyphosate (Nandula et al., 2009).

Similar results with other grasses were obtained by Pereira et al. (2012), which have verified a reduction of 40.2% in the germination of *Urochloa decumbens* and of 23.7% in the germination of *Urochloa ruziziensis* in -0.2 MPa. Bansal et al. (1980) explain that water deficiency can reduce the percentage of germination as the osmotic potential becomes smaller because it slows the metabolic and biochemical processes, which delay or inhibit the seeds germination and interfere in the embryo's cellular imbibition and elongation.



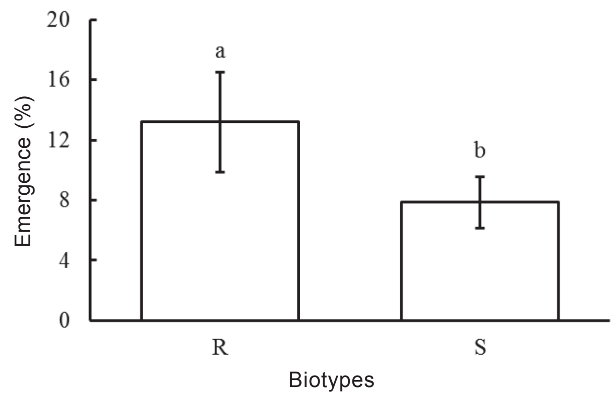
Vertical bars represent the standard error of the means. Equations: GR(%) = 64.90 (1+exp(14.88*(x-0.27))); GS(%) = 196.61 (1+exp(6.81*(x+6.81))).

Figure 4 - Effect of osmotic potential on seed germination of sourgrass resistant (R■) and susceptible (S●) to glyphosate incubated at 30 °C with a photoperiod of 12 hours for 30 days.

Depth of sowing

A nonlinear log-logistic regression model, E(%) = a/(1+exp(-k*(x-xc))), R^2 0.98, was the one that best described the emergence of seedlings in response to different seeding depths. When comparing the average emergence of the two biotypes (R and S), higher emergence (E%) of sourgrass R seeds was observed (Figure 5). Likewise, greater E% was verified when seeds were arranged in depths between 0 and 4 cm and there were no significant differences between biotypes for the depths. The highest emergence is also related to the higher germination previously observed.

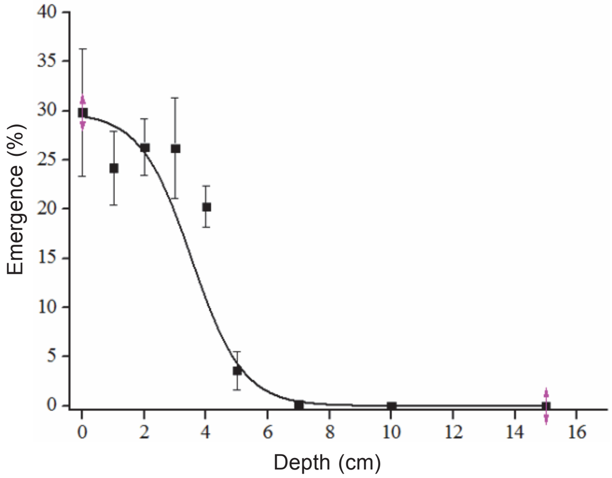
Non-emergence at greater depths may be related to the absence of light. However, as observed, germination of sourgrass seeds occurred in the dark. Emergence reduction may then be related to the small amount of reserves present in the seeds of the species or to the seed lower hydration at greater depths (Dinelli et al., 2013). Decreases in seed germination with increased sowing depth were reported in other species (Rao et al., 2008; Tang et al., 2015). There was no interaction between the biotypes for this environmental characteristic (Figure 6).



Means followed by the same letter do not show a significant difference according to Tukey's test at level p ≤ 0.05.

Figure 5 - Effect of sowing depth on the mean seed emergence of sourgrass evaluated 30 days after sowing.

Likewise, Barbosa et al. (1989) have verified that the highest values of seedling emergence of the genus *Digitaria* were found on the surface and 2 cm deep. For species



Vertical bars represent the standard error of the means. Equation: E (%) = 26.76/(1+exp(2.99*(x-4.37))).

Figure 6 - Effect of sowing depth on the mean seed emergence of sourgrass evaluated 30 days after sowing.

with larger seeds, such as *Cenchrus echinatus*, seedlings emergence occurs in up to 5 cm (Giancotti et al., 2011).

Germination capacity in a greater diversity of temperatures, light and water availability of the seeds of the R biotype compared to the S shows the adaptive advantages of this biotype for different environmental conditions throughout seasons and geographical locations, as already reported for species of *Conyza* spp. (Vidal et al., 2007) – in this case, mainly for low temperatures in the presence of light and for places with lower water availability, as observed in the experiments carried out.

The greater germination of the R biotype compared to the S may have occurred also due to the possible presence of dormancy in the S seeds since the germination of these did not reach 75%. Evolutionarily it would be an advantage for the S biotype to present germination over time in order to guarantee the perpetuation of the species against adverse conditions (Martins et al., 2013). In contrast, seeds of resistant biotypes of *Ambrosia trifida* presented higher dormancy than susceptible seeds. However, in this case, morphological differences of the seeds were also involved (Dinelli et al., 2013).

For the R biotype, the faster germination and in a higher percentage indicates that the possibility of a trade-off in its metabolism, as observed in other species, does not exist or is not involved with the germination of seeds, which is in agreement with the low resistance factor generally observed for the species. Alleles that generate resistance to herbicides can cause pleiotropic effects in the life cycle of a plant, with germination being the sum of the effect of these alleles and environmental effects. This effect has already been reported for some cases of weed resistance to herbicides inhibiting ACCase (Vila-Aiub et al., 2011; Délye et al., 2013). The selection of weed biotypes resistant to glyphosate has increased in recent years (Heap, 2016). This study brings to the seed ecology scope the environmental effects of this selection for sourgrass.

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