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Can seed quality of hairy fleabane be reduced due to glyphosate resistance?

A qualidade de sementes de buva pode ser reduzida devido à resistência a glyphosate?

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ABSTRACT

The resistance to glyphosate may provide a penalty on plant growth and physiology. We investigate if the resistance to glyphosate reduces the seed quality of *Conyza bonariensis* by performing seed viability and vigor tests under a completely randomized design with four replicates of 50 seeds for both glyphosate-susceptible (S) and -resistant (R) biotypes. Low seed viability (<50%) was found in both S and R biotypes. No difference occurred between either S and R biotypes on seed germination and embryo viability. The percentage of seed germination of the S biotype was higher than the R biotype in the cold test (~58%), accelerated aging test (~84%), and high-temperature stress test (~45%). This is the first time one found evidences that resistance to glyphosate may be responsible for a penalty on seed quality of *C. bonariensis*.

KEYWORDS: *Conyza bonariensis*, herbicide resistance, seed vigor.

RESUMO

A resistência a glyphosate pode proporcionar penalidade ao crescimento e à fisiologia das plantas. Foi investigado se a resistência a glyphosate reduz a qualidade da semente de *Conyza bonariensis* por meio de testes de viabilidade e vigor de semente, utilizando-se de delineamento inteiramente casualizado com quatro repetições de 50 sementes tanto para biótipos suscetíveis (S) quanto resistentes (R) a glyphosate. Observou-se baixa viabilidade de sementes (<50%) nos biótipos S e R. Não houve diferença entre os biótipos S e R na germinação de sementes e na viabilidade do embrião. A porcentagem de germinação de sementes do biótipo S foi maior que o biótipo R no teste de frio (~58%), no teste de envelhecimento acelerado (~84%) e no teste de estresse por alta temperatura (~45%). Esta é a primeira vez que se observa que a resistência a glyphosate pode ser responsável por penalidade na qualidade da semente de *C. bonariensis*.

PALAVRAS-CHAVE: *Conyza bonariensis*, resistência a herbicida, vigor de semente.

Resistance to glyphosate is one of the main issues in the current agriculture. Glyphosate-resistant weeds occur worldwide where fleabanes (*Conyza* spp.) assume an outstanding place as one of the main herbicide-resistant weeds. Hairy fleabane [*Conyza bonariensis* (L.) Cronq.] is a cosmopolitan species presents in each continent showing herbicide resistance. According to HEAP (2017), eleven cases of simple herbicide resistance to glyphosate in *C. bonariensis* were reported around the world, except in Asia; in addition, one case of multiple herbicide resistance to glyphosate and paraquat were found in the United States of America. Due to the current increase in cases of glyphosate-resistant weeds, efforts have to be done to study plant biology and plant physiology, allowing researchers to understand the impact of herbicide resistance on population dynamics and then to associate this knowledge with control practices to prevent resistance evolution and to manage resistant weed populations more efficiently.

Authors had found the resistance to glyphosate providing a penalty for *C. bonariensis* resistant biotypes regarding on plant growth (MOREIRA et al. 2010) and gas exchange (GALON et al. 2013). In addition, glyphosate resistance also reduced the seed production of *Lolium multiflorum* L. (VARGAS et al.

2005, FERREIRA et al. 2008) and *Lolium rigidum* Gaud. (PEDERSEN et al. 2007). Differences in plant fitness associated to resistance to ACCase-inhibiting herbicides were found in seed physiological characteristics, reducing seed vigor, germination speed, and seed longevity of *Phalaris minor* Retz. (TORRES-GARCÍA et al. 2015). However, we found no information on the comparison of seed quality between glyphosate-susceptible and -resistant biotypes. Thus, we hypothesized the physiological seed quality might be different between glyphosate-susceptible and -resistant plants. The objective was to investigate if the resistance to glyphosate reduces the seed quality of *C. bonariensis*.

We used the same susceptible (S) and resistant (R) biotypes previously tested by COSTA et al. (2014) for resistance to glyphosate whose factor of resistance was found by 15.5. After dose-response experiments, plants of both biotypes grew separately under controlled conditions (60% relative humidity, 25 ºC temperature and 14 h photoperiod), and the new seeds produced by these plants were stored in a cold chamber (50% relative humidity and 10 ºC temperature) for further analysis on physiological seed quality.

We performed seed viability tests (germination and tetrazolium tests) and seed vigor tests (cold, accelerated aging, and high-temperature stress tests) in a completely randomized design with four replicates of 50 seeds for both S and R biotypes.

For germination test, we used plastic boxes (11 x 11 cm) and placed the seeds on a sheet of blotting paper previously moistened with distilled water at a volume equivalent to 2.5 times the weight of the dry paper. We placed the boxes in an incubator preset at 25 ºC temperature and 12-h light photoperiod under 35 μmol m^{-2} s^{-1} flux rate delivered by cool-white fluorescent tubes. Evaluation on seed germination performed on the fourth day after placement. We expressed the results as a percentage of normal seedlings.

For tetrazolium test, we extracted the embryos after soaking the seeds in water during 24 h. After embryo removal, we submerged them into a 0.6% tetrazolium solution overnight at 30 ºC. We observed the embryos under a stereo microscope (40x magnification) regarding on the presence (viable embryos) or the absence (unviable embryos) of a red/rose colored area.

For the cold test, we also used plastic boxes (11 x 11 cm) and placed the seeds on a sheet of blotting paper, previously moistened with distilled water at a volume equivalent to 2.5 times the weight of the dry paper. We firstly placed the boxes in a refrigerator at 8 ºC temperature during seven days. After that, seeds germinated and evaluations performed as described in the germination test.

For accelerated aging test, we distributed the seeds on aluminum screens and fixed them inside plastic boxes (11 x 11 cm), adding 40 mL of distilled water. We sealed the boxes and kept them in an aging chamber at 42 ºC temperature during 48 h. After that, seeds germinated and evaluations performed as described in the germination test.

For the high-temperature stress test, we firstly put the seeds inside aluminum capsules and then placed in an air convection oven at 35, 45, and 60 ºC temperatures during 48 h. After that, seeds germinated and evaluations performed as described in the germination test.

Normality of data was not verified by Kolmogorov-Smirnov test, so that we transformed the data into an arcsine of (x/100)0.5 for the following analysis of variance by t test (germination, tetrazolium, cold, and accelerated aging tests) or F test (high-temperature stress test). Due to the significance of F test, we compared the means by using Tukey test. For all tests, we assumed the level of significance by 5%.

The seed viability of *C. bonariensis* was evaluated by germination and tetrazolium tests. The percentage of both seed germination (Figure 1A) and embryo viability (Figure 1B) was generally less than 50% for both biotypes, and no difference occurred between either S and R biotypes.

The seed vigor of *C. bonariensis* was evaluated by cold, accelerated aging, and high-temperature stress tests. The percentage of seed germination of the S biotype was higher than the R biotype in each test (Figures 2 and 3). The seed germination of the R biotype reduced under cold test conditions (Figure 2A). The seed germination reduced more in the R biotype than in the S biotype under accelerated aging test conditions (Figure 2B). The seed germination of both biotypes reduced at 60 ºC temperature in the high-temperature stress test (Figure 3).

We found evidences seed vigor reduced in the R biotype (Figure 1), while seed viability was similar between S and R biotypes (Figures 2 and 3). No differences on seed viability indicate glyphosate resistance did not influence on the potential of seed germination. However, similar results in germination tests are not a guarantee for seed quality, requiring further analysis on potential seed performance. Thus, differences on seed vigor indicate the seed quality was lower in the R biotype, showing a worst potential seed performance than the S biotype. The decrease in seed vigor could affect the establishment of seedlings, which may explain a lower success of resistant biotypes under no herbicide selection pressure (TORRES-GARCÍA et al. 2015).

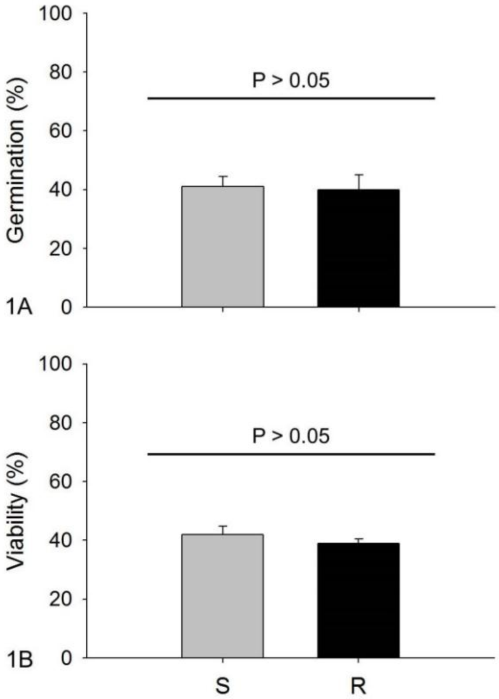

Figure 1. Seed germination (1A) and embryo viability (1B) in glyphosate-susceptible (S) and glyphosate-resistant (R) biotypes of *Conyza bonariensis*. Vertical lines indicate the standard error of the mean.

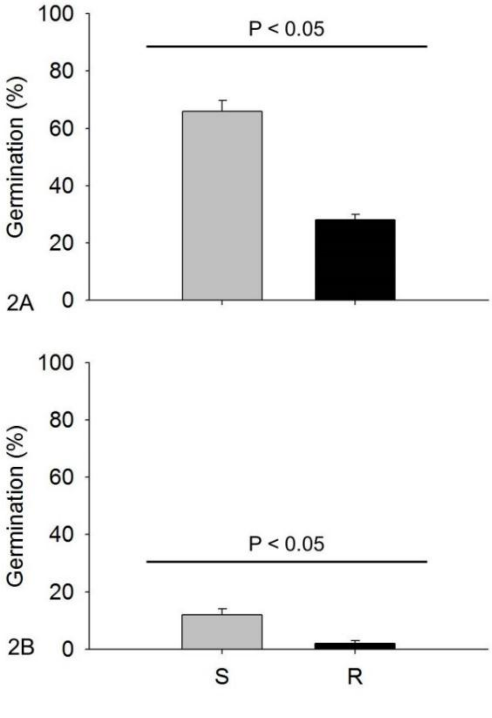

Figure 2. Seed germination under cold test (2A) and accelerated aging test (2B) conditions in glyphosate-susceptible (S) and glyphosate-resistant (R) biotypes of *Conyza bonariensis*. Vertical lines indicate the standard error of the mean.

Herbicide resistance may involve ecological costs related to seed persistence and plant fitness. Differences in seed quality may determine a lower seed persistence of herbicide-resistant biotypes in the soil seed bank (GHERSA & MARTÍNEZ-GHERSA 2000). The size of the embryo and longevity are measures of seed vigor that may determine plant establishment success (WEAVER & THOMAS 1986, THOMPSON et al. 1994). As the longevity may be estimated by the accelerating aging test, we concluded the R biotype shows lower seed persistence than the S biotype in *C. bonariensis*. In addition, as the longevity is related to the size of the embryo (TORRES-GARCÍA et al. 2015), we may affirm the R biotype probably shows a smaller embryo than the S biotype, which will influence on the potential seedling performance and, as a consequence, the plant fitness of *C. bonariensis* resistant to glyphosate.

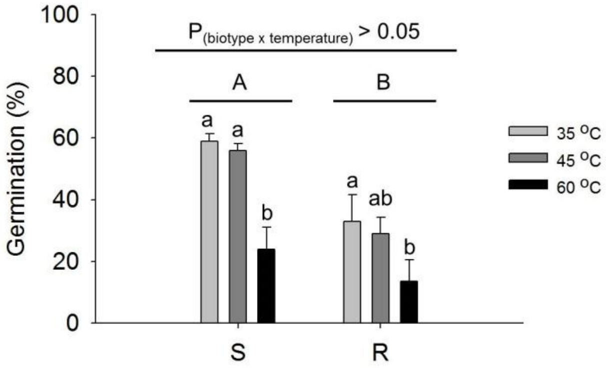

Figure 3. Seed germination under high-temperature stress test conditions in glyphosate-susceptible (S) and glyphosate-resistant (R) biotypes of *Conyza bonariensis*. Vertical lines indicate the standard error of the mean. Interaction between biotypes and temperatures was not significant (P>0.05). Capital letters indicate differences between biotypes and same lowercase letters represent no difference among temperatures for each biotype.

Understanding ecologically and evolutionarily based changes in plant response to biotic and abiotic stresses provides useful insights into the theory of weed community assembly (WEIHER & KEDDY 1999) and the potential for weed invasions into plant communities (SAX et al. 2007). In general, differences in plant growth and seed production between herbicide-susceptible and -resistant weed biotypes could affect the persistence and frequency of herbicide-resistant biotypes in a population; if growth and reproductive fitness of resistant biotypes reduced, the frequency of resistant biotypes will also reduce in the absence of selection pressure (TORRES-GARCÍA et al. 2015). Considering glyphosate-resistant biotypes of *C. bonariensis* may produce low-quality seeds and probably tends to show lower fitness than glyphosate-susceptible biotypes, their invasive potential and persistence reduced. As a consequence, the frequency of resistant biotypes tends to decrease and return to susceptibility when herbicide application is stopped (MAXWELL et al. 1990).

Despite our inferences, herbicide resistance may (GALON et al. 2013, TORRES-GARCÍA et al. 2015, YANNICCARI et al. 2016) or may not (DUFF et al. 2009, TRAVLOS & CHACHALIS 2013, VILA-AIUB et al. 2014) constitute fitness costs (including seed production and vigor) in the resistant populations. In some cases, by the way, the glyphosate-resistant biotype was more competitive than the glyphosate-susceptible biotype, particularly when grown at high densities and under moisture-deficit stress (SHRESTHA et al. 2010). Therefore, it seems rather risky to make generalizations on the relative fitness of herbicide-susceptible and -resistant biotypes since negative, positive, or no impacts of herbicide resistance on plant populations have been reported (TRAVLOS & CHACHALIS 2013). Consequently, fitness evaluations describing the potential success of a biotype should be case specific and based on survival, competition, and reproduction characteristics (HOLT 1990).

The fitness cost (including seed production and vigor) associated with herbicide resistance is evident, but not universal, among plant species (VILA-AIUB et al. 2009). Understanding the differential seed quality consequences of herbicide resistance is important to predict the evolutionary dynamics of herbicide-resistant weeds. Development of integrated management strategies for herbicide-resistant weeds clearly requires an understanding of population dynamics and potential impacts of the resistant biotypes (TRAVLOS & CHACHALIS 2013). Since seed quality is lower in the R biotype and, as a consequence, there probably is a differential fitness between the S and R biotypes, it is likely to be a lesser persistence of glyphosate-resistant *C. bonariensis* plants in the environment without using glyphosate. However, the continued reliance of farmers on glyphosate, the resistant biotype is likely to become an even larger management problem in the area unless alternative management strategies are adopted (TRAVLOS & CHACHALIS 2013).

Finally, we have to consider the biotypes used in this study did not originate at the same site, with the same environmental conditions. We know one of the factors affecting seed quality is the site of seed production (RAO et al. 2017), in spite of, in some cases, no influence may also be observed (HERRERA et al. 2007). However, almost all studies on weed resistance is carried out using biotypes from different places, because is not easy to obtain seeds of susceptible- and resistant-biotypes from the same site. Moreover, despite the S and R biotypes being originated from different sites, the seeds used in this study were produced by plants at the same place (under controlled conditions where we conducted our study, as described above). Therefore, our results evidence the resistance to glyphosate may be responsible for a penalty on seed quality of *C. bonariensis*, but further studies should be performed using susceptible- and resistant-biotypes originated from the same site.

In conclusion, the percentage of seed germination and viable embryos is not dependent on glyphosate resistance. However, the reduced percentage of seed germination under temperature or/and moisture stress conditions indicates a seed quality penalty due to glyphosate resistance in *C. bonariensis*.

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