ENIRONMENTAL FACTORS AFFECTING SEED GERMINATION OF COMMON TEASEL (Dipsacus fullonum)

ABSTRACT - Common teasel is a widespread, invasive species that has been introduced in Buenos Aires province, Argentina, where it alters the species composition of protected areas and native grasslands. A better understanding of seed germination behavior is essential for predicting its invasion potential in new areas and would be useful for developing effective management strategies. This research, conducted under laboratory conditions, evaluated the influence of several environmental factors such as pH, constant temperature, osmotic stress, salt stress and dry storage, on germination and rate of germination of common teasel seed. Between-year variation in germination responses was also examined. Seed germination was not affected by different pH levels. Although common teasel seeds germinated over a range of temperatures from 6 to 36 °C, the optimum temperature was 22 °C. Common teasel seed germination showed moderate tolerance to osmotic stress and a relatively high tolerance to salt stress. No seed germination was observed at -1 MPa and 640 mM of osmotic potential and salt concentration, respectively. Seed germination remained high (> 90%) after all the dry storage treatments. Besides, the response of seeds to germination parameters showed between-year variation. It was shown that common teasel is able to germinate over a broad range of environmental conditions. This capacity would explain, at least partially, why this species is so widely distributed and its great potential to invade new areas.

Keywords: invasive weed, dry storage, osmotic stress, pH, salt concentration, temperature.

RESUMO - O cardo comum é uma espécie invasora disseminada que foi introduzida na província de Buenos Aires, Argentina, onde altera a composição de espécies de áreas protegidas e pastagens nativas. Uma melhor compreensão do comportamento germinativo das sementes é essencial para prever seu potencial de invasão em novas áreas e seria útil para o desenvolvimento de estratégias de manejo eficazes. Esta pesquisa, conduzida em condições laboratoriais, avaliou a influência de diversos fatores ambientais, como pH, temperatura constante, estresse osmótico, estresse salino e armazenamento seco, sobre a germinação e a taxa de germinação de sementes de cardo comum. A variação interanual nas respostas de germinação também foi examinada. A germinação das sementes não foi afetada por diferentes níveis de pH. Embora as sementes de cardo comum tenham germinado em uma faixa de temperaturas de 6 a 36 °C, a temperatura ideal foi de 22 °C. A germinação de sementes de cardo comum mostrou tolerância moderada ao estresse osmótico e tolerância relativamente alta ao estresse salino. Não houve germinação de sementes a -1 MPa e 640 mM de potencial osmótico e concentração de sal, respectivamente. A germinação das sementes permaneceu alta (> 90%) após todos os tratamentos.

Keywords: invasiva, armazenamento seco, estresse osmótico, pH, concentração de sal, temperatura.

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INTRODUCTION

*Dipsacus fullonum* (common teasel, Dipsacaceae) is a species native to central and southern Europe, western Asia, and boreal Africa. In several countries of America, such as the USA, Canada and Argentina, common teasel has been classified as an invasive plant in pastures and natural communities, including several sites like prairies, savannas, and sedge meadows (Solecki, 1993; Werner, 1975b). Thereby, it is present in 43 states in the USA, being categorized as an invasive weed in twelve of them and as a noxious one in five (Harizanova et al., 2012). In Canada, common teasel populations are found in five provinces and they are considered noxious in one of them, Manitoba (Harizanova et al., 2012).

Common teasel is a species with a competitive and aggressive growth habit, together with a high reproductive performance (Solecki, 1993). In Argentina, *D. fullonum* has infested many localities, also becoming an invasive weed. Giolitti et al. (2009) have indicated that it is highly abundant in the Pampas region.

Genus *Dipsacus* is known to have caused important negative impacts in infested areas (Bentivegna and Smeda, 2011), such as the decay of available forage, the decline of native species diversity and an increase in crop pathogens by acting as an alternate host. The leaves have rigid spines on the underside of the midrib and small spines on the upper leaf surface (Werner, 1975b); the stems also have several rows of prickles. These characteristics act as a defense against grazing by herbivores (Solecki, 1993) hence negatively affecting grassland destined to livestock production. Additionally, in Argentina, common teasel is found in some of the most important natural conservation areas, forming dense monocultures which displace desirable species, including the “Otamendi Natural Reserve”, (34°14’2’’ S; 58°53’2’’ W) (Cordo, 2004) and “Costanera Sur Ecological Reserve” (34°36’ S; 58°21’ W) (Daddario, pers. obs.). Furthermore, *D. fullonum* is an alternate host to an important sunflower (*Helianthus annuus*) virus known as SuCMoV (Sunflower Chlorotic Mottle Virus). This weed acts as a natural reservoir and, due to its biennial life cycle, it allows the virus to survive through periods when sunflower is absent (Giolitti et al., 2009).

Common teasel is a monocarpic herb commonly categorized as biennial and its reproduction pathway is through achene production (Solecki, 1993). Species of the genus *Dipsacus* usually support 1 to 56 inflorescences and can produce over 33,000 seeds (Bentivegna and Smeda, 2011). Achenes, approximately 1 x 4 mm in size, have no adaptations for dispersal by wind or animals; thus, 99% of the seed produced passively falls to the ground within 1.5 m from the parental plant, generating dense monospecific patches (Werner, 1975a). Possibly, human activities and water are the most likely agents for long-distance teasel seed dispersal. No pre-germination requirements such as freezing, low temperature, scarification or a specific photoperiod have been detected (Werner, 1975a,b). According to Caswell and Werner (1978) mature teasel seeds may germinate immediately, except for a short after-ripening period; thus they have no innate dormancy mechanism.

Germination is one of the most critical stages in weed establishment. Successful germination in the soil seed bank is essential for infestation and establishment at a new site (Tanveer et al., 2013). Several environmental factors, such as temperature, water availability, soil pH, etc., influence seed germination and the optimum conditions necessary for this process to take place vary considerably depending on the species. Temperature and soil water availability are the most important parameters that can delay, reduce, or prevent seed germination (Norsworthy and Oliveira, 2006). Moreover, the ability to germinate under conditions of moisture stress or...
high salt content in soils can enable a weed to take advantage of conditions that limit the growth of other species. In addition, soil pH influences development and competitiveness by affecting the availability of essential minerals and nutrients, the solubility of toxic elements and soil microflora (Chauhan and Johnson, 2008). However, there is little information regarding the parameters affecting seed germination of *D. fullonum*.

Seed germination patterns may also respond to the environmental conditions to which the parental plants were subjected to during growth and seed maturation (Luzuriaga et al., 2006). For any given population of a weed, the occurrence of differences in such patterns between years could indicate the existence of a wide phenotypic plasticity, which would allow the weed to invade a broad variety of areas (Beckstead et al., 1996). Despite this, studies that analyze germination responses do not usually consider between-year variations.

A better understanding of the germination patterns of teasel is going to provide essential knowledge for the development of models which in turn are going to allow to predict periods of maximum germination and potential infestation areas. It is expected that this information is going to help to improve teasel management. Therefore, the objectives of this study were i) to determine the effect of pH, temperature, osmotic stress, salt stress and dry storage on seed germination and germination rate of *D. fullonum*, and ii) to examine whether between-year differences in germination patterns exist.

**MATERIALS AND METHODS**

*Dipsacus fullonum* seeds were collected from a large roadside population of 5 ha located in Bahía Blanca (38°42′ S; 62°16′ W), Buenos Aires province, Argentina. *D. fullonum* was the dominant species in the area. Mean soil pH and salinity was 7.47 and 997 µS.cm⁻¹, respectively. Seeds were collected by shaking the seedheads of 200 randomly selected plants over a plastic box. Recovered seeds were cleaned and stored at low temperature (4 °C) in sealed paper bags for later use. Only healthy, fully-developed seeds were used for experiments.

All experiments were carried out using seeds collected in March 2010 and later repeated once with seeds harvested in 2011 unless otherwise stated. Experiments were conducted 2 months after harvest. The weight of 1000 achenes was 3.26 g and 2.62 g in 2010 and 2011, respectively. According to the tetrazolium test (Copeland, 1976), seed viability was 93.5% and 95.7% in 2010 and 2011, respectively. Weather conditions during the growth cycle were recorded at a meteorological station located at CERZOS-CONICET Bahía Blanca (Figure 1A, B).

**General protocol for germination tests**

Achenes were surface sterilized by immersion in a 10% solution of sodium hypochlorite for 2 min. After that, the seeds were washed with distilled water and dried with tissue paper.

Five replications of 50 seeds were placed in 9 cm Petri dishes containing a sheet of coarse filter paper and then soaked in 5 mL of distilled water (untreated) or the assigned solution for the experiment (treatments). The Petri dish was considered as the experimental unit. All Petri dishes were placed inside transparent self-sealed plastic bags to minimize water evaporation. Also, both distilled water and the test solution were added on a regular basis to keep a suitable humidity condition. Petri dishes were kept in growth chambers under controlled temperature (24 °C) and total darkness. Seed germination was monitored daily during 30 days. A seed was considered germinated when the radicle length reached 2 mm and it was then removed from the Petri dish. Data are shown as the percentage of germination, i.e., the proportion of germinated seeds over the total seed in each Petri dish, and the rate of germination (Ranal and Santana, 2006):

\[ RG = \frac{\sum_{i=1}^{k} F_i}{\sum_{i=1}^{k} F_i X_i} \]

where *F*<sub>i</sub> is the number of seeds germinated each day *i*, *X*<sub>i</sub> is the number of days from sowing, and *k* is the last day in which germination occurred. Rate of germination values ranged from 0 to 1.
Figure 1 - Daily means of air temperature (dash line) and total monthly rainfall (solid line) recorded for seed collected in 2009-2010 (A), 2010-2011 (B) and 2011-2012 (C) in Bahia Blanca, Argentina.
The higher the value, the greater the number of seeds that germinate faster. Therefore this value provides complementary information on the optimal conditions under which common teasel seeds germinate.

**Effect of pH**

The pH effect on seed germination was studied using ten pH solutions from 3 to 12 at 1 unit intervals. Hydrochloric acid 32% w/v and sodium hydroxide 1N were adjusted with distilled water to obtain the desired solution. The final pH solution was confirmed with a Hach EC10 model 50050 digital pH-meter. On a regular basis, the solution was sucked out of each Petri dish and then replaced by the required liquid in order to keep the pH stable. Petri dishes were incubated at 24 °C, as described earlier for the general germination protocol.

**Effect of constant temperature**

Petri dishes were placed in growth chambers at the following temperatures: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, and 36 °C. Temperatures were selected from the common range which occurs in fall and spring in the study area when most of the weed seeds germinate.

**Effect of osmotic stress**

Seeds were incubated in aqueous solutions with different osmotic potentials. Six treatments were performed including 0 (distilled water), -0.1, -0.2, -0.4, -0.6, -0.8, and -1 MPa. Solutions were obtained by dissolving 56.5, 93.6, 148.8, 192.2, 229.2, and 262 g Polyethylene Glycol (PEG) 6000 in 1 L of distilled water according to the equation of Michel and Kaufman (1973).

**Effect of salt concentration**

Appropriate sodium chloride solutions of 10, 20, 40, 80, 160, 320, and 640 mM were prepared to determine the effect of salt stress on seed germination. These values approximately reflect the salt content of typical soils in Buenos Aires province (Paoloni, 2010). Seeds were exposed to the seven increasing salinity levels cited and distilled water was used as a control in each experimental unit. Then, Petri dishes were incubated under the general protocol guidelines.

**Effect of dry storage**

The dry storage treatment was performed in 2011 with seeds collected that same year and then repeated once with seed harvested in 2012 (Figure 1C). The 1000 seed weight collected in 2012 was 2.22 g and seed viability was 97.2%. Fifty seeds were stored in each of 140 brown paper bags which were placed in darkness in the growth chambers. Treatments included different temperatures, 0, 10, 20, and 30 °C, for a period of 30, 60, 90, 120, 240, and 360 days after the seed was harvested. After each dry storage treatment time, seeds were incubated at 24 °C following the general test protocol.

**Statistical analysis**

All experiments were conducted as a completely randomized design, except for the dry storage experiment where a split-plot design was used, with temperature as a principal factor and period as a subfactor. Daily, each Petri dish was randomly placed in the growth chamber.

Data were checked before analysis to confirm normality (Shapiro-Wilks p<0.05) and homogeneity of variance (Levene p<0.05). When it was necessary, data were transformed using the arcsine square-root transformation in order to improve variance homogeneity (Snedecor and Cochran, 1956). Transformed data were subjected to ANOVA. Means were separated using Fisher’s Protected Least Significance Difference test at p≤0.05.
In the salt and osmotic stress experiments, regression analysis was used to determine the response of the data (Chauhan et al., 2006). The germination values obtained at different osmotic potentials were fitted to a functional three-parameter logistic model of the form:

\[ G = G_{\text{max}} \left( 1 + \frac{x}{x_{50}} \right)^{-G_{\text{rate}}} \]

Besides, a three-parameter exponential decay curve was fitted to the germination (%) obtained at different salt concentrations of the form:

\[ G = G_{\text{max}} / (1 + \exp (-\frac{x-x_{50}}{G_{\text{rate}}})) / G_{\text{rate}} \]

where, \( G \) represents germination (%) at osmotic potential or salt concentration \( x \), \( x_{50} \) is the osmotic potential or salt concentration to reach 50% of maximum germination, \( G_{\text{max}} \) is the maximum germination and \( G_{\text{rate}} \) determines the steepness of the decay. Rate of germination values obtained at different osmotic potentials and salt concentrations were fitted to the same models, respectively.

All analyses were performed using the statistical software INFOSTAT (Di Rienzo et al., 2008) and osmotic and salt stresses were fitted to the respective models adjusted using the software CurveExpert 1.3 (Hyams, 2005).

RESULTS AND DISCUSSION

Effect of pH

*Dipsacus fullonum* seeds germinated across the whole range of pH studied (Figure 2). There was no interaction between the year of harvest and the pH treatment, as there was no effect of the year of harvest. According to these observations, pH does not affect seed germination \((F=1.03; p=0.42)\), nor their germination rate \((F=0.84; p=0.58)\). Our results indicate that pH is not a limiting factor for a successful germination of *D. fullonum* seeds. Similarly, this kind of pH adaptation has been observed in other invasive species, such as *Urena lobata* (Wang et al., 2009) and *Sonchus oleraceus* (Chauhan et al., 2006). In contrast, other species are known to be sensitive to pH. For instance, *Ipomoea purpurea* (Singh et al., 2012) did not germinate under extreme pH conditions.

Effect of constant temperature

Seed germination of *D. fullonum* was markedly affected by temperature \((F_{2010}=77.14; F_{2011}=51.78; p<0.01; \text{Figure 3A})\). Here there was an interaction between the year of harvest and the temperature on the percentage of germination \((F=3.66; p<0.01)\), and on the rate of germination \((F=1.81; p=0.05)\). Germination was significantly higher in 2011 than in 2010 in the range from 24 to 30 °C. In addition, common teasel seed germination behavior is described by the following types of responses: inhibited, low, elevated and intermediate germination. Germination was inhibited under 6 °C and above 36 °C. Low germination (<40%) occurred between 8 °C and 10 °C while intermediate germination (between 40-70%) occurred at the 12 °C to 16 °C and 32 °C to 34 °C intervals. Finally, elevated germination (>70%) of the seeds occurred in the 18-30 °C range (Figure 3A). Maximum rate of germination was registered in the 20-26 °C range (Figure 3B).

*Dipsacus fullonum* germinated over a broad range of constant temperatures, thus temperature is not a factor that prevents the germination of common teasel seeds in Buenos Aires province. *D. fullonum* seeds showed the highest and fastest germination at intermediate temperatures which are common in fall and spring in the study area. The ability to germinate at low temperatures allows the establishment of common teasel seedlings earlier in the season than other species with higher temperature germination regimes. In a similar study, germination of other weed species, such as *Crassocephalum crepidioides*, occurred over a wide range of temperatures (5 °C and 35 °C), reaching optimum germination at 20 °C (Nakamura and Hossain, 2009).
Effect of osmotic stress

Seed germination of *D. fullonum* was affected by osmotic stress (*F*2010*=231.65; *p*<0.01; *F*2011*=60.03; *p*<0.01; Figure 4). There was an interaction between the harvest year and the osmotic stress treatment. Means of percentage and rate of seed germination for each treatment in 2011 were significantly higher than in 2010, except for the distilled water control (Figure 4). A functional three-parameter logistic model best described the relationship between seed germination of teasel and increasing osmotic stress (Figure 4A). The same model best described the relationship between the rate of germination and increasing osmotic stress (Figure 4B). The osmotic potentials required for 50% inhibition of maximum germination determined from the fitted model were -0.49 and -0.62 MPa in 2010 and 2011, respectively. Seed germination was >75% up to an osmotic potential of -0.4 MPa. Germination decreased 77.5% (2010) and 41.2% (2011) from -0.4 MPa to -0.6 MPa (Figure 4A). The percentage of seed germination was very low (<15%) at an osmotic potential of -0.8 MPa. Common teasel seed germination ceased at -0.8 MPa (2010) and -1 MPa (2011). No differences in the rate of seed germination was found up to an osmotic potential of -0.4 MPa, but it showed a significant reduction of 63.3% (2010) and 58.2% (2011) from -0.4 MPa to -0.6 MPa (Figure 4B).

According to the adjusted equation of germination percentage, a reduction of 26.3% from 0 to -0.5 MPa was calculated. However, in Canada, Beaton and Dudley (2013) have obtained a reduction of 7.2% from 0 to -0.5 MPa. These results could support the idea that germination requirements vary according to the weed ecotype and their ecological habitat (Chauhan et al., 2006; Wang et al., 2009). Since a low proportion of seeds germinated at an osmotic potential of -0.8 MPa, it is suggested that common teasel is moderately tolerant to water stress. Similarly, *Cynara cardunculus* populations exhibited 32-46% seed germination at -0.6 MPa (Raccia et al., 2004). Conversely, other common invasive species in Argentina were sensitive to water deficit. For example, seeds...
of *Diplotaxis tenuifolia* presented a low germination percentage (less than 20%) at -0.6 MPa (Kleemann et al., 2007). At the same osmotic potential, *Coniza canadensis* seed germination was only 2% (Nandula et al., 2006). Besides, other species are much more sensitive to the lack of water in the soil, *e.g.*, *Campsis radicans* seed germination is deeply inhibited at an osmotic potential of -0.2 MPa (Chachalis and Reddy, 2000) and *S. marianum* seed germination is very low at -0.6 MPa (Montemurro et al., 2007). In summary, germination would be high during the rainy seasons, which commonly correspond to fall and spring in Buenos Aires province (Paoloni, 2010); but common teasel seeds can still germinate under low soil water content. This could be a competitive advantage with other sensitive species in dry soils, *e.g.*, the semi-arid regions of Buenos Aires province.

**Effect of salt concentration**

*D. fullonum* seed germination is affected by salt concentration ($F_{2010} = 97.28; p < 0.01$; $F_{2011} = 138.61; p < 0.01$). There was an interaction between the year of harvest and salt concentration on both percentage and rate of seed germination. With increasing levels of salt concentration, seeds harvested in 2011 presented a significantly higher percentage and rate of germination when compared with seeds harvested in 2010 (Figure 5). A three-parameter exponential decay model best described the relationship between both the percentage of germination (Figure 5A) and the rate of germination of teasel seeds (Figure 5B) with increasing salt concentration. The percentage of germination reached a mean value of 96.5% with 0 mM of salt concentration (distilled water), significantly decreasing to 49% in 2010 and 80% in 2011 with a 320 mM solution. Seed germination was totally inhibited in a 640 mM concentration of NaCl solution (Figure 5A). Maximum rate of germination was 0.18 (2010) and 0.19 (2011) at 10 mM and decreased significantly to 0.08 and 0.12 at 320 mM, respectively (Figure 5B).

Increasing salt concentration led to a reduction in the percentage and rate of seed germination in *D. fullonum*. However, seeds can still germinate at a NaCl concentration of 320 mM, which shows that it can withstand a...
high salt content in the soil. Common surface horizons present values of up to 8 dS.m⁻¹ in Buenos Aires province (Paolini, 2010), but according to our results seeds can still germinate at a salinity of approximately 30 dS.m⁻¹. Although common teasel is generally considered a glycophyte species, Beaton and Dudley (2004) have found that teasel roadside ecotypes in Canada showed a high tolerance to salt stress; in accordance with the results presented here, this indicates that teasel can sometimes behave as a halophyte species. Zia and Khan (2002) have also reported that Limonium stocksii, a known halophytic species, had shown germination at a concentration higher than 300 mM NaCl. On the contrary, other weed species cannot tolerate high salt concentrations, such as Sonchus oleraceus, which showed great inhibition at 320 mM (Chauhan et al., 2006) and Coniza canadensis which showed only 4% of germination at 160 mM (Nandula et al., 2006). In conclusion, in Buenos Aires province, D. fullonum seeds showed a halophytic behavior and thus would be able to germinate in habitats where native halophyte vegetation develops naturally.

**Effect of dry storage**

There was no interaction between the year of harvest and the dry storage treatment on seed germination and rate of germination. No effect was detected on seed germination due to the exposure to several different temperatures ($F=1.63; p=0.2$) nor to the different lengths of storage periods ($F=1.5; p=0.19$). Differences in percentage of germination ($F=6.9; p=0.01$) were registered in seeds collected in 2011 compared with seeds obtained in 2012 (Figure 6A). In addition, the rate of germination was not influenced by temperature exposure. On the other hand, there was an interaction between the year of harvest and the different periods of storage on rate of germination ($F=14.17; p<0.01$) (Figure 6B).

The response to dry storage of other species is diverse. In the case of seeds of Onopordon acanthium, a biennial plant with growth characteristics similar to those of D. fullonum, seed dormancy was reduced, therefore leading to an increase in the percentage and rate of germination after storage for long dry periods (Qaderi et al., 2005). Conversely, germination could be strongly reduced after a long storage period, as in the case of Crassocephalum crepidioides (Nakamura and Hossain, 2009). D. fullonum seeds are able to withstand long periods at low and high temperatures without major changes in their germination. This indicates that seeds of common teasel are not recalcitrant and that they are capable of colonizing sites at different latitudes and of persisting in the soil seed bank.

**Between-years variation examination**

Even though both the percentage and the rate of germination estimates exhibited differences between years of harvest under some of the environmental factors tested, the germinating seed curves of D. fullonum showed a similar trendline. Similarly, Beckstead et al. (1996) have demonstrated between-year variation in Bromus tectorum, a species introduced in western North-America.

Fernández-Pascual and Giménez-Alfaro (2014) have indicated that the phenotypic plasticity found in Centaurium somedanum during successive years is related to the temperatures to which plants are subjected during seed maturation. In this study, it was speculated that weather variations during seed setting may have been responsible for the differences in germination behavior found between seeds harvested in two different years.
Unfortunately, it was not possible to find a clear correlation between the weather conditions measured and the germination outcome (Figure 1). According to Beckstead et al. (1996), the variation of seed germination patterns between years is not easily attributed to weather and a proper correlation would require the selection of appropriate variables. Further research is required to fully elucidate the origin of the differences in germination behavior found in seeds of *D. fullonum* from different years and populations.

The germination of seeds of *Dipsacus fullonum* was affected by many factors and occurred under a broad range of environmental conditions. This explains in part why it is so widely distributed in Argentina and suggests common teasel populations would be capable of further expanding its geographical distribution in this country. Teasel withstands a broad range of germinating conditions which adds to its invasive potential. Should an invasive assessment protocol be implemented in Argentina for this species, all the seed germination features recorded in this study should be rated with a high score.

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