Response of *Digitaria insularis* seed germination to environmental factors

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Abstract. *Digitaria insularis* (sourgrass) is a weed problem emerging in importance in agricultural fields from the north of Argentina and has recently been reported as resistant to glyphosate. Understanding the germination of local biotypes of *D. insularis* could help to reduce invasion and improve the long-term management strategies for this weed. The objective of this work was to study the effect of environmental factors on germination of *D. insularis* seeds from two different populations of Argentina. Three experiments were performed in germination chambers by using recently dispersed seeds. Seeds with or without pre-chilling treatments had 95% germination, suggesting the absence of dormancy in freshly harvested seed. Germination at constant temperature of 25°C was ~55% lower than germination at fluctuating temperature of 20°–35°C. At constant 25°C, germination was higher for seeds from Santiago del Estero than seeds from Córdoba, and as the number of hydration–dehydration cycles increased. Germination was reduced with exposure to far-red light for 1 h. Any crop management decision that reduces soil thermal fluctuations and/or far-red : red ratio (such as stubble or cover crops) could reduce seedling field emergence for this species.

Additional keywords: environmental factors, grass weed, management decisions, seedling emergence, seed origin.

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Introduction

*Digitaria insularis* (L) Mez ex Ekman (sourgrass) is a native species of tropical and subtropical America, distributed from the southern United States to the centre of Argentina (Pyon \textit{et al.} 1977). It has been reported as a glyphosate-resistant weed in soybean (*Glycine max* L.) and maize (*Zea mays* L.) crop fields from Brazil, Paraguay and Argentina (Heap 2016). In Argentina, *D. insularis* was reported as a problem in agricultural fields since 2013 (Papa and Tuesca 2015). The mechanism involved in glyphosate resistance of the Brazilian biotype is related to a limited herbicide translocation, rapid degradation of glyphosate, and changes by mutation of two amino acids in the enzyme EPSPS (de Carvalho \textit{et al.} 2012). This weed has a very high infestation potential because it is able to reproduce by both seeds and rhizomes. As in many perennial grass species, seeds are the principal means of introduction into new areas, whereas rhizomes may be the primary means of dispersal within the field (Mitskas \textit{et al.} 2003). In particular, the high production of small, easily dispersed seeds with a high germination percentage allows the colonisation of new sites (de Carvalho \textit{et al.} 2011). In tropical climates, the seedbank of *D. insularis* is renewed continuously, owing to plants producing seeds throughout the year and temperatures allowing seedling emergence at any time (Lacerda 2003). There is limited information about this species in temperate climates where low temperatures during winter could affect seed production and seedling emergence. Therefore, a better understanding of the germination ecology of local biotypes of *D. insularis* will help to improve the long-term management of this weed in temperate croplands favouring the reduction of dispersal and invasion.

Germination timing and extent is related to seed dormancy. Dormancy release dictates the time of seedling emergence and can influence the number of plants that establish. Seed dormancy is a common characteristic of most grassy weeds, acquired during evolution by selection for the ability to survive in unfavourable environments. However, seeds of some species never experience a deep dormancy, or the range of environmental conditions permissive of germination is very wide (Benech-Arnold \textit{et al.} 2000). The number of plant species with seed dormancy tends to increase with geographical distance from the equator (Bewley \textit{et al.} 2013). Nevertheless, even in tropical environments, especially those with cyclic wet and dry seasons, seed dormancy is widespread (Garwood 1989).

Environmental factors affecting seed dormancy could be divided into dormancy-level-regulating factors (i.e. temperature and moisture) and dormancy-terminating factors or germination-initiating factors (i.e. fluctuating temperatures, light flux density and quality, nitrate concentration, etc.) (Benech-Arnold \textit{et al.} 2000; Forcella \textit{et al.} 2000). Dormancy-level-regulating factors are related to seed germination during the favourable season for plant growth and reproduction (Bewley \textit{et al.} 2013), whereas dormancy-terminating factors or germination-initiating factors...
are signals of canopy gaps and depth of seed burial, thus avoiding futile germination (Fenner 1980; Thompson and Grime 1983; Batlla et al. 2000; Kruk et al. 2006). The presence of a crop canopy or abundant stubble could reduce terminating factors such as fluctuating temperatures and hydration–dehydration cycles of soil surface, and the red : far-red (R : FR) ratio of the light reaching the soil surface. Soil water potential in the first few centimetres of soil is higher under a crop canopy than without crop canopy (Norsworthy and Oliveira 2007). Therefore, before crop-canopy coverage, seeds on the soil surface would be exposed to more hydration–dehydration cycles and more susceptible to germination in the field. After crop canopy closure, germination is lower for those seeds requiring fluctuating temperatures (Benech-Arnold et al. 1988; Huarte and Benech-Arnold 2003), red-enriched light (Bewley et al. 2013) or fluctuations of the soil-water content (Downs and Cavers 2000) to terminate seed dormancy. This, in turn, can affect the dormancy status of the buried seeds (Batlla and Benech-Arnold 2006), reducing the germination percentage (Martínez-Ghersa et al. 1997) and rate (Lush et al. 1984; Kagaya et al. 2005). Accordingly, management decisions could be decided in order to modify soil factors and reduce or delay seed germination and, thus, seedling emergence on the field.

Information about seed-germination response to environmental factors is scarce for D. insularis. Most studies come from Brazil and show that seeds germinate better at fluctuating temperatures of 20°–30°C, 20°–35°C and 15°–35°C (cycle 16 h–8 h) than at constant temperatures (Mondo et al. 2010; de Mendonça et al. 2014). Some authors reported that light is not required for germination of D. insularis (Mondo et al. 2010), whereas others reported the opposite (de Mendonça et al. 2014). Beside the differences among authors, the effect of hydration–dehydration cycles and light quality on seed germination has not been studied. Moreover, local information on this species is lacking in Argentina, and it is known that different biotypes of a species can differ with regard to germination rate (Kaya Altop and Mennan 2011). These differences could imply differences in seedling emergence in the field and, therefore, different management decisions to be considered.

The objective of this work was to determine the effect of temperature, light–darkness, light quality and hydration–dehydration cycles on the germination of D. insularis seeds from different origins. To achieve this objective, experiments on germination chambers were made.

Materials and methods

Seed collection and germination test

The dispersal unit of D. insularis is usually called a ‘seed’ but it is actually the spikelet composed of the caropsis enclosed within the lemma and palea and all of these enclosed by the glumes. Hereafter, ‘spikelet’ will be referred to as seed unless otherwise specified. Mature seeds were collected during April 2014, the season of natural dispersal, from two sites located in northern central Argentina, Villa María del Río Seco, Córdoba province (29°55′S, 63°45′W), and Zanjón, Santiago del Estero province (27°55′S, 64°15′W). After collection, seeds were bulked and stored in paper bags at room temperature (20°–25°C) until they were used in the experiments during May 2014.

Three experiments were carried out in germination chambers at the Faculty of Agronomy of Buenos Aires University, Argentina. For all of them, the experimental units consisted of 36 seeds per replicate placed in a 9-cm-diameter Petri dish with two filter papers (No. 1; Double Rings, Buenos Aires, Argentina), moistened with 4 mL of deionised water, sealed with Parafilm to maintain moisture and then placed in a germination chamber. Germination (>2 mm radicle protrusion through the seed coat) was recorded at regular intervals until no further seeds germinated. At the end of each experimental incubation period, viability of non-germinated seeds was tested with a 1% tetrazolium (2,3,5-triphenyl-2H-tetrazolium chloride) solution (International Seed Testing Association 1999).

Experiment 1: pre-chilling

In order to evaluate the dormancy level of D. insularis, a completely randomised experiment with five replicates was performed with recently dispersed seeds from both populations. Treatments were: (i) seeds pre-chilled at 5°C in darkness for 15 days and then transferred to fluctuating temperature of 20°–35°C (cycle 8 h–16 h) with light; and (ii) seeds without pre-chilling placed at fluctuating temperature of 20°–35°C (cycle of 8 h–16 h) with light.

Experiment 2: temperature, light or darkness, and hydration–dehydration cycles

To test the effects of temperature and light or darkness and hydration–dehydration cycles on germination, a completely randomised factorial experiment with five replicates was performed. Seeds from the two sites (Santiago del Estero and Córdoba) were placed under two temperature regimes (25°C and 35°C) or two light conditions (continuous light and continuous darkness) and three hydration regimes (continuous hydration, one cycle of 3 h hydration–8 h dehydration, or two cycles). For treatments in darkness, Petri dishes were prepared initially in light, but light was excluded by wrapping the dishes in black polypropylene bags before placing them in the germination chambers. Hydration time was estimated by hydrating a group of 100 seeds at 25°C until reaching constant weight. Dehydration time was estimated by removing the lid of the Petri dishes and allowing the seeds to dry to their original weight, in natural light at 25°C (Vincent and Cavers 1978). Once seeds were exposed to different hydration–dehydration cycles, Petri dishes were transferred to the germination chambers.

Experiment 3: temperature and light quality

To test the effect of temperature and light quality on germination, a completely randomised factorial experiment with five replicates was performed. Seeds from the two sites (Santiago del Estero and Córdoba) were placed under two temperature regimes (constant 25°C and fluctuating 20°–35°C for 8 h–16 h) and four light regimes (60R, 60 min red light; 60FR, 60 min far-red light; 30FR, 30 min far-red light; R-FR, a cycle of 60 min red light–30 min darkness–30 min far-red light). After light treatments, seeds were incubated at 20°–35°C (cycle 8 h–6 h) in darkness for 20 days. Petri dishes were prepared initially in light but light was excluded by wrapping them in black polypropylene bags at 25°C, in the dark, for 24 h until
exposure to the different light regimes, when the Petri dishes were unwrapped, exposed to the light, wrapped again and placed in the germination chambers. Red light (calculated proportion of phytochrome as Pfr (Pfr:Pr) 87%, 35 μmol m⁻² s⁻¹) was provided by red fluorescent tubes (Philips 40/15; Philips, Amsterdam). Far-red light (calculated proportion of phytochrome as Pfr (Pfr:Pr) 2.7%, 42 μmol m⁻² s⁻¹) was provided by a 150-W incandescent reflector lamp (Philips R95) in combination with an 8-cm water filter and an RG9 filter (Schott, Mainz, Germany) (Scopel et al. 1991).

Statistical analyses
Analysis of variance (ANOVA) followed by Tukey’s multiple comparison test was done using a general linear model procedure of STATISTICA version 8 (StatSoft, Tulsa, OK, USA) to determine significant differences at \( P = 0.05 \). When factorial analysis showed significant interactions between main effects, factors were analysed separately. The assumptions of the ANOVA (random, homogenous and normal distribution of residuals) were tested. If the assumptions of variance were not met, cumulative germination percentages were square-root-remains for graphical presentation.

Results

Experiment 1: pre-chilling
No significant differences (\( P > 0.05 \)) were observed between seeds with and without pre-chilling. The final germination percentage reached in all the treatments was >95% and the rest of seeds that did not germinate showed 100% viability with the tetrazolium test (data not shown).

Experiment 2: temperature, light or darkness, and hydration–dehydration cycles
Statistically significant site × temperature × light and temperature × hydration–dehydration cycle interactions were observed (Table 1). Therefore, data were analysed separately. Independent of the origin of the seeds and light treatments, germination at constant temperature 25°C was lower than germination at fluctuating temperatures 20°C–35°C. The average germination at 20°C–35°C was 97% for the seeds from Santiago del Estero and 84% for the seeds from Córdoba, without differences between light treatments. At 25°C, germination of the seeds from Santiago del Estero averaged 53% and was significantly higher than germination of the seeds from Córdoba at 17%. The only combination with differences among light treatments was seeds from Santiago del Estero at 25°C, showing lower germination in light than in darkness (Fig. 1).

Independent of the seed origin, at 20°C–35°C germination was >90% without significant differences among hydration–dehydration cycles. By contrast, at constant 25°C germination was different among hydration–dehydration cycles, being on average 14%, 34% and 58%, for continuous hydration, one hydration–dehydration cycle and two cycles, respectively (Fig. 2).

![Graph](image)

Fig. 1. Effect of temperature (fluctuating 20°C–35°C and constant 25°C) and light (light and darkness) on Digitaria insularis seed germination (%), from two sites (Santiago del Estero and Córdoba). Values are the means and vertical bars are standard error. Pairs of columns with the same letters are not significantly different according to Tukey’s test (at \( P = 0.05 \)).

Experiment 3: temperature and light quality
There was a significant temperature × light quality interaction (\( P < 0.01 \)) (Table 2); therefore, data were analysed separated. Independent of the seed origin, germination was higher (\( P < 0.01 \)) at alternating temperatures 20°C–35°C than at constant 25°C in all light-quality treatments (Fig. 3). At constant temperature, seeds in the 60FR treatment had significantly reduced germination (~20% less) compared with seeds in the 60R and 30FR treatments (Fig. 3).

Discussion
The high germination percentage observed in both populations of Digitaria insularis under fluctuating temperatures compared with...
the same populations under constant temperatures suggests that recently dispersed seeds have a low dormancy level. As is true for many summer arable weeds, high dormancy levels are reduced by exposure to low temperatures (Benech-Arnold et al. 2000). The ability to respond to fluctuating temperatures is important for species where the seeds need to germinate close to the soil surface. This is one factor aiding seeds of such species in avoiding germination at depth (Forcella et al. 2000; Fenner and Thompson 2005). Such species are likely to become more prevalent under no-till where seeds remain at the soil surface; however, the presence of abundant stubble can reduce the size of temperature fluctuations at the soil surface (Teasdale and Mohler 1993; Dahlia et al. 2007).

The ideal conditions for seed germination of *D. insularis* were fluctuating temperatures 20–35°C (cycle 8 h–16 h). Under these temperature conditions, light was not necessary to stimulate seed germination. These results agree with those of Mondo et al. (2010), which showed the highest germination for *D. insularis* seeds from Brazil at 20°–35°C and 15°–35°C, independent of light effect. Under fluctuating temperatures, no differences were observed among the other factors evaluated for germination (light and darkness, hydration–dehydration cycles and light quality). It is known that changes in the degree of dormancy comprise changes not only in temperature requirements for germination, but also in sensitivity to the effects of dormancy-terminating factors (Forcella et al. 2000). Exposure of *D. insularis* to fluctuating temperatures not only removed the constraints for germination, but also reduced the sensitivity to light v. darkness, hydration–dehydration cycles and light quality. These results explain the lack of differences among different light or hydration–dehydration treatments for germination under fluctuating temperatures.

Germination of seeds from Santiago del Estero was higher at constant temperature (25°C) with darkness than with light (Fig. 1). These results contrast with those reported by de Mendoça et al. (2014), who found a higher germination for *D. insularis* seeds with light at 25°C. However, no significant differences among light treatments were observed in seeds from Córdoba (Fig. 1), and these results agree with Mondo et al. (2010). In addition, seeds from Santiago del Estero had greater germination than seeds from Córdoba, independent of the germination treatment. These differences could be due to the growing conditions of the parent plants during seed development, known as maternal effects (Fenner 1991), or could be due to genetic differences between biotypes; de Mendoça et al. (2014) found differences among *D. insularis* biotypes from the same region in germination response to different temperatures.

At constant 25°C, germination increased as the number of hydration–dehydration cycles increased (14% always hydrated, 34% with one hydration–dehydration cycle and 58% with two cycles, Fig. 2). These results agree with Martinez-Ghersa et al. (1997), who found that hydration–dehydration cycles increase germination of some seeds. Hydration–dehydration cycles could have an effect similar to other dormancy-termination factors, and the sensitivity of seeds to this factor is diminished by exposure to fluctuating temperatures. Most grain crops in Argentina are cultivated by using a no-till seeding system,
where weed seeds remain on the soil surface and are subject to repeated hydration–dehydration fluctuations as a result of evaporation cycles in the upper 5–10 cm of the soil when stubble is scarce (Ritchie and Johnson 1990). Considering that immediately after winter the temperature of the soil is lower than the 20°C–35°C cycle, seedling emergence of *D. insularis* by the end of winter could be strongly determined by hydration–dehydration cycles. This could be especially important under a no-till system with limited stubble at the beginning of spring, when soil thermal amplitude is lower than that registered in summer.

On the other hand, at constant temperatures (25°C) the exposure of the seeds to far-red light for 60 min reduced the germination percentage compared with seeds exposed to red light for 60 min or far-red light for 30 min. The inhibitory effect of far-red light on seed germination was reported for several weed species (Ballaré et al. 1992) such as *Lolium multiflorum* (Deregibus et al. 1994), *Silene gallica* and *Brassica campestris* (Batlla et al. 2000), and *Amaranthus palmeri* (Jha and Norsworthy 2009). Results observed in the present study are evidence of the inhibitory effect of low R : FR ratio on the germination of *D. insularis* seeds. Far-red light increases and the ratio R : FR (red, ~645 nm) decreases as plant canopies develop after the summer solstice. As a result, emergence of sensitive species could be inhibited as the crop canopy develops or as the summer progresses (Forcella et al. 2000). Furthermore, the crop canopy reduces not only the R : FR ratio but also the soil thermal amplitude (Huarte and Benech-Arnold 2003; Kruk et al. 2006; Jha and Norsworthy 2009).

Many summer grass species usually show a deep seed dormancy, which is naturally removed by exposure to low temperatures (usually called pre-chilling) during the cool season (Vegis 1964; Benech-Arnold et al. 2000; Adkins et al. 2002). This mechanism allows seed germination under ideal conditions in the following warm season. The lack of a deep dormancy in *D. insularis* could be related to the tropical and subtropical origin of this species, where cold temperatures are rare.

**Implications for management**

From these results, management decisions that affect the environment of the seed could be useful to reduce or delay seed germination and seedling emergence in the field. Such management decisions could include sowing crops or cover crops that generate a dense crop canopy or maintaining abundant stubble on soil surface to reduce soil thermal amplitude, R : FR ratio and hydration–dehydration cycles. These practices, in turn, could help to reduce the colonisation of this weed into new areas (Gugglielmini and Ferraro 2016).

On the other hand, the ambiguous germination response to the light according to seed origin, suggests that seeds burial to reduce seedling emergence could be an ineffective option for *D. insularis*.

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**References**


