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Influence of environmental factors on seed germination and emergence of *Iresine diffusa*

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Summary

Iresine diffusa has become more abundant under no-till soyabean in Argentina. The influence of temperature, light, cold-wet storage, osmotic potential, dry storage and depth of seed burial on germination and emergence of *I. diffusa* was examined in a growth chamber experiment. *Iresine diffusa* seeds germinated at the highest proportion (>0.80) in all fluctuating day/night temperatures tested. Conversely, under a constant temperature regime, maximum germination rates occurred at 15 (0.78) and 20°C (0.82), and minimum germination rates occurred at 10 (0.19) and 30°C (0.36). Seed germination was not influenced by light exposure. However, germination decreased after 12 (0.76) and 16 (0.65) weeks in cold-wet storage. To reduce germination significantly, −0.4 MPa of osmotic potential (induced by PEG-6000) or 120 mmol L^{-1} of salt (NaCl) concentration was required. Seeds of *I. diffusa* showed high viability (0.85) after 720 days of dry storage. Low emergence was recorded for seeds buried at 2 cm, and seedling emergence was completely inhibited when seeds were buried at 5 and 10 cm. *Iresine diffusa* seeds had high viability and were capable of emerging in a broad range of environmental conditions. The thermal germination conditions, shallow soil depths and high moisture conditions in germination phase for *I. diffusa* are congruent with the conditions in Argentina no-tillage soyabean. Thus, no-tillage could provide better conditions for germination than conventional tillage systems. However, due to the fact that *I. diffusa* can reproduce by rhizomes, further research should be conducted to understand the relative importance of the vegetative reproductive strategy in relation to the presence and persistence of this weed in fields.

Keywords: Juba's bush, temperature, cold-wet, light, osmotic potential, salinity, weed seed.

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Introduction

Iresine diffusa Humb. & Bonpl. ex Willd. (Amaranthaceae) is native from the American continent and is widely distributed in tropical and subtropical environments (Robertson, 1981; Pedersen, 1987; Sanchez del Pino *et al*., 1999) and in Argentina it is found in 17 provinces (Pedersen, 1987). It has been cited as a major invasive species in secondary successions in different regions of America (Kellman, 1980).

Iresine diffusa, commonly called Juba's bush, is a dioecious perennial herb (Robertson, 1981). It has

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numerous erect branches or supporters (Robertson, 1981; Pedersen, 1987) and has a C4 photosynthetic pathway (Acosta, 2012). Seed production per plant ranges from 5000 to 30 000 and long basal trichomes in the utricle mean that seeds are easily dispersed by wind (Acosta, 2012).

In Argentina, the soyabean (*Glycine max* (L.) Merr.) crop area under no-tillage cultivation systems has been expanding in the past two decades (SIIA, 2013). In addition, glyphosate-resistant (GR) soyabean cultivars have been rapidly adopted by farmers in Argentina and consequently, a high proportion of agricultural fields are under a no-tillage cropping system with GR varieties (Scursoni & Satorre, 2010). The associated agricultural practices to GR soyabean cultivars (i.e. lack of soil inversion, large quantities of crop residues and glyphosate herbicide) have strongly modified the soil surface disturbances, resource availability patterns for weed species (Mas *et al.*, 2010) and changed the weed communities (Vitta *et al.*, 2004; Puricelli & Tuesca, 2005). Some of the population shifts could be attributed to environmental changes that would influence weed seed dynamics (Puricelli & Tuesca, 2005).

In recent years, reports have shown an increase of *I. diffusa* in GR soyabean production (Vitta *et al.*, 2004; Mas *et al.*, 2010). This species can reproduce sexually by seeds or asexually by rhizomes (Acosta, 2012). Seed germination and seedling emergence are critical events for the success of any weed in the agroecosystem (Forcella *et al.*, 2000). To develop an effective, integrated weed management system for specific species, it is critical to have good information about factors affecting seed germination and seedling emergence (Mennan & Ngouajio, 2006). Nevertheless, weed species with asexual reproduction strategies such as *Sorghum halepense* (L.) Pers. may produce large quantities of seeds and rhizomes, but plants that emerged from rhizomes are more competitive and problematic than seedlings (Mitskas *et al.*, 2003). Bud break and establishment from rhizomes in *I. diffusa* may also be an important consideration, but this study focusses on seed germination. To date, there are no published studies about which of the reproductive strategies are more relevant for *I. diffusa*. Studies about seed biology have been cited for related species, e.g., *Amaranthus quitensis* H.B.K. (Faccini & Vitta, 2005), but information on seed germination as well as studies to identify the reasons of success for *I. diffusa* as a weed is scarce.

The objectives of this research were (i) to quantify the influence of temperature, light, osmotic potential, salinity, cold-wet storage and dry storage on seed germination and (ii) to examine the effect of burial depth on *I. diffusa* seedling emergence, to link *I. diffusa* seed germination and seedling emergence requirements to weed management insights and opportunities.

Materials and methods

Seeds of *I. diffusa* were collected in May 2009, from seven soyabean fields near to Esperanza city, Santa Fe Province, Argentina (31.26°S, 60.56°W). Seed populations were collected from fields in close proximity, within an area of 3 km^2, sharing more than 10 years under no-tillage cropping systems. Inflorescences containing seeds were randomly harvested and then these multiple collections were bulked into a single population. Seeds were separated from the inflorescence, cleaned and stored at low relative humidity (≤15%) and room temperature (18–20°C) in a packed, sealed opaque sterile box until used in the research. The weight of one thousand seeds was 51.8 ± 4.5 mg. While the dry storage experiment started immediately after seed harvest, all of the others experiments were carried out 3–5 months after the seeds were harvested.

General protocol for germination tests

Unless otherwise specified, seed germination was determined by distributing 50 seeds in a 9-cm Petri dish containing two pieces of Qualy® filter paper soaked in 5 mL of distilled water (or other solutions appropriate for the experiment). According to a tetrazolium chloride test, *I. diffusa* seed viability was 84% (±7%). Distilled water (or other test solution) was changed daily to keep suitable humidity inside of each Petri dish. Dishes were incubated at alternating 15/25°C temperatures with a 12-h photoperiod, the light phase coinciding with the higher temperature. Photosynthetic photon flux density of 150 μmol m^{-2} s^{-1} was obtained using cool white fluorescent lamps. Germination was monitored daily for 3 weeks and germinated seeds were removed from dishes after each counting. At the end of the experiment, viability of non-germinated seeds was checked with a 0.4% tetrazolium chloride solution. A viable seed showed pink to reddish colour in the embryo after 4 h.

Temperature

Germination was determined in growth chambers under constant (5, 10, 15, 20, 25 and 30°C) or fluctuating temperatures (night/day temperatures: 5–15, 10–20, 15–25, 20–30°C). The photoperiod was established at 12 h. The high-temperature phase coincided with the light period. Fluctuating temperatures regimes correspond to mean daily high and low temperatures for

the months of June, September, March and January, respectively, in Santa Fe Province.

Light exposure

Four different treatments consisting in 0 (complete darkness), 1, 10, and 720 min of light exposure were used to determine the influence of the duration of light exposure on seed germination. The source of light and photon flux density was cited above. The Petri dishes were covered with aluminium foil to maintain complete darkness after light exposure. In addition, germination counts and watering were performed in a dark room under indirect green light. To ensure the proper implementation of the complete darkness conditions, 20 photoblastic seeds of *Gomphrena perennis* L. were included as an internal control in each Petri dish (Acosta *et al*., 2013).

Osmotic stress

Osmotic stress was provided by incubating seed in six aqueous solutions of 0, −0.3, −0.4, −0.6, −0.9 and −1.3 MPa. Polyethylene glycol (PEG) 6000 was dissolved at 0, 154, 191, 230, 297 and 350 g in 1 L of distilled water, respectively, to provide the desired osmotic potentials. The precise amount of PEG 6000 needed was obtained by means of the equation of Michel (1983). To maintain constant MPa values in each Petri dish, PEG solutions were changed daily during the experiment.

Salinity

Seeds were located in Petri dishes with eight increasing salinity levels. Each dish was watered with 5 mL of solution of 0, 30, 50, 60, 80, 100, 120 and 180 mmol L^{-1} sodium chloride (NaCl) and incubated under general protocol guidelines. All ungerminated seeds at highest NaCl concentrations were rinsed with distilled water and placed in a new set of Petri dishes with distilled water and incubated under general protocol guidelines, and germination was monitored daily for 21 days to determine whether saline conditions had impacted seed viability.

Cold-wet storage

Seeds imbibed in 5 mL of distilled water inside dishes sealed with Parafilm were exposed to either 0 (non-chilled control), 4, 8, 12 or 16 weeks of cold-wet storage (4°C) in darkness. After each cold-wet storage time, dishes were incubated according to the methodology described above in the general germination protocol, and seed germination was recorded for 3 weeks.

Dry storage

To determine the effect of dry storage on seed germination, 50 seeds were placed inside each of 60 paper bags. Then, bags were stored at room temperature (20 ± 2°C) for 0, 90, 180, 360 and 720 days after harvest. After the storage period, seeds were incubated according to the methodology described above in the general germination protocol, and seed germination was recorded for 3 weeks.

Seed burial depth

Plastic pots of 1 L with a diameter of 15 cm were filled with silt loam soil (2.1% organic matter and a pH of 6.3) that was collected from GR soyabean fields. The soil was autoclaved and sieved through a 2-mm mesh screen. A compaction pressure of 2.0 MPa was achieved using a hydraulic press for providing a uniform density of 1 g cm^3 in all the pots. Subsequently, 50 seeds were set on the soil surface or buried at depths of 0.5, 1, 2, 5, and 10 cm. Pots were exposed to fluctuating temperatures (15/25°C) with a 12-h photoperiod in a growth chamber. Plant pots were randomly arranged inside the growth chamber. The pots were watered three times per week until they reached field capacity and excess water leached from the base. To prevent soil desiccation from evaporation, the top of the pots were covered with plastic film. Seedling emergence was recorded daily. Seedlings were considered emerged when cotyledons were visible, and they were then carefully removed from the pot.

Experimental design and statistical analysis

Experiments were conducted with three replicates for each experimental treatment. The location of Petri dishes was re-randomised within growth chambers on a daily basis. All experiments were conducted twice. Data from the repeated experiments were combined for analyses (two experiments, each of three replications) provided that 'treatment by run' interactions were not significant. In the temperature experiment, owing to each temperature level (constant or fluctuating) requiring a different incubator, the experimental units are the incubators, so Petri dishes inside each incubator were pooled (three Petri dishes into each incubator = 150 seeds for each experimental units). We have repeated this experiment twice, so it has two true replicates for each treatment.

A generalised linear model (GLM) with a binomial error family and logit link was used to test for significant effects on final germination proportion in the experiments. A scale parameter was adopted to correct for overdispersion (quasi-binomial GLM). If all values for some treatment resulted in no germination, the treatment was dropped from the analysis because this distorts the final results. For the GLM, an analysis of deviance was performed to determine significance using the software InfoStat (Di Rienzo *et al.*, 2011) and the chi-squared test or F test for quasi-binomial GLM. Contrasts of model parameters were used to test the difference between treatment means, by applying the Bonferroni's correction to adjust for multiplicity. Significance was confirmed when $P < 0.05$. For each treatment, the fitted mean values of the proportion of *I. diffusa* seeds germinated and their respective 95% confidence interval (CI) were obtained by back-transforming the mean of linear predictor and back-transforming the limits of confidence by the inverse of the link function.

Results

Temperature

Under constant temperature regimes, significant differences in germination of *I. diffusa* seeds were detected ($F = 46.98$; d.f. = 4; $P < 0.001$; Fig. 1). A high proportion of seeds germinated at 15, 20 and 25°C. However, diminished germination proportions were significant at 30 and 10°C. Germination was completely inhibited at 5°C. When the total viable seeds (=germinated seeds + ungerminated viable seeds) of each constant temperature treatment were analysed, significant differences could not be found ($F = 1.57$; d.f. = 4; $P = 0.31$; Fig. 1).

In alternating temperature regimes, seeds germinated at high proportion in all fluctuating day/night temperature tested [5–15°C: 0.827 (CI: 0.778–0.867); 10–20°C: 0.817 (CI: 0.767–0.858); 15–25°C: 0.877 (CI: 0.832–0.910); 20–30°C: 0.897 (CI: 0.855–0.927)] and significant differences could not be detected among treatments ($F = 1.23$; d.f. = 3; $P = 0.41$).

Light exposure

Seed germination was not influenced by treatments ($\chi^2 = 4.93$; d.f. = 3; $P = 0.31$). The proportion of germination were 0.87 (CI: 0.816–0.905), 0.816 (CI: 0.761–0.862), 0.817 (CI: 0.761–0.862) and 0.86 (CI: 0.808–0.899) in total darkness and light exposure treatments of 1, 10, 720 min respectively. Germination of *G. perennis* seeds, under complete darkness conditions had reached <0.20, while in all light treatments, germination was higher than 0.85 (data not shown).

Cold-wet storage

Germination was influenced by the cold/moist treatment ($\chi^2 = 42.84$; d.f. = 4; $P < 0.001$; Fig. 2). The proportion of germinated seeds after 0, 4, 8 weeks in cold-wet treatments was high, while germination was

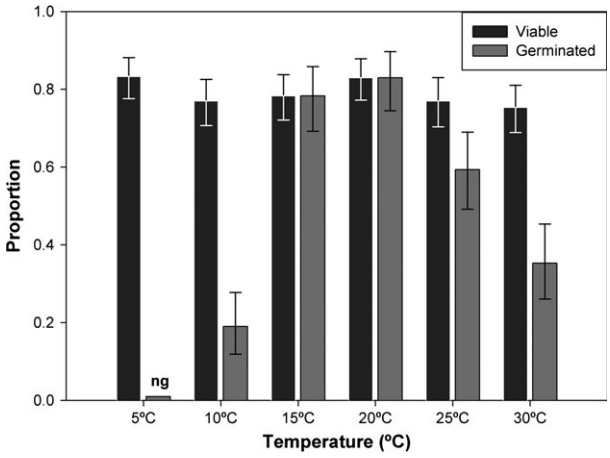

Fig. 1 Effect of constant temperatures on germination and viability of *I. diffusa* seeds after 21 day of incubation. The height of the shaded bar represents the average of the proportion of germinated seeds (black) or viable seeds (grey) as estimated by the fitted GLM, and the error bar represents the 95% confidence interval for this estimate. Treatment labelled 'ng' had no seed germination and was excluded from the analysis.

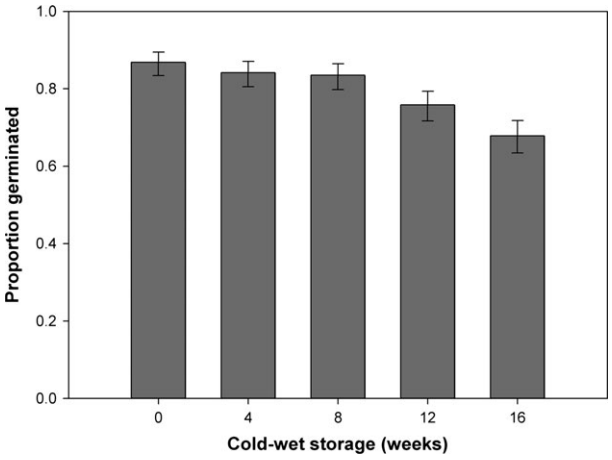

Fig. 2 Effect of cold-wet storage duration on germination of seeds incubated at 25–15°C in a 12-h photoperiod for 21 day. The height of the shaded bar represents the average of the proportion of germinated seeds as estimated by the fitted GLM, and the error bar represents the 95% confidence interval for this estimate.

diminished after 12 and 16 weeks of cold-wet storage. After incubation, the seeds that did not germinate were dissected and treated with tetrazolium chloride to assess viability. Only unstained embryos were recovered and were considered as physiologically decayed seeds. Thus, cold-wet storage of 12 and 16 weeks led to loss of viability, rather than changes in the dormancy/germination status of the *I. diffusa* seeds.

Osmotic stress

Seed germination of *I. diffusa* decreased significantly with reduction of osmotic potential ($F = 103.18$; d.f. = 3; $P < 0.001$; Fig. 3). The highest seed germination proportion was reached at 0 MPa (distilled water), and a diminishing osmotic potential from 0 to −0.4 MPa caused 90% decline in germination. Seed germinating was ≈0.01 at −0.6 MPa, and there was no germination beyond −0.6 MPa.

Salinity

Salt concentration affected the germination of *I. diffusa* seeds ($F = 34.79$; d.f. = 6; $P < 0.001$; Fig. 4). Seeds had the greatest cumulative germination with NaCl treatments from 0 to 100 mmol L^{-1}. However, germination decreased significantly at 120 mmol L^{-1} salt concentration while germination was totally inhibited at 180 mmol L^{-1}.

When non-germinated seeds were removed from 180 mmol L^{-1} NaCl treatment and placed in distilled water, germination was 0.75 (CI: 0.68–0.83). Significant differences could not be detected in total germination between NaCl treatment of 0 and 180 mmol L^{-1} following transfer back into distilled water ($F = 0.41$; d.f. = 1; $P = 0.53$). Therefore, high salt, while inhibiting germination, did not impact seed viability.

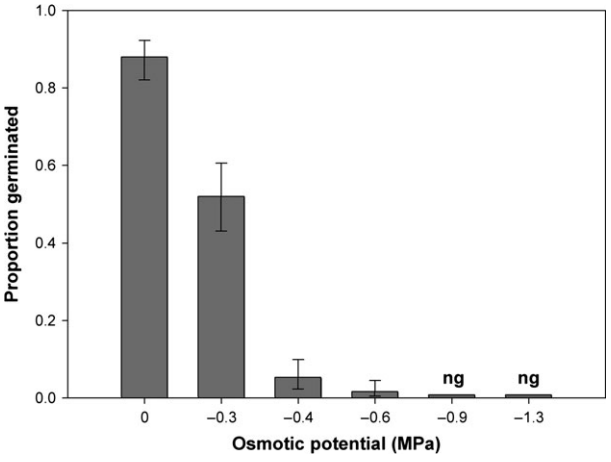

Fig. 3 Effect of osmotic potential on germination of seeds incubated at 25–15°C in a 12-h photoperiod for 21 day. The height of the shaded bar represents the average of the proportion of germinated seeds as estimated by the fitted GLM, and the error bar represents the 95% confidence interval for this estimate. Treatment labelled 'ng' had no seed germination and was excluded from the analysis.

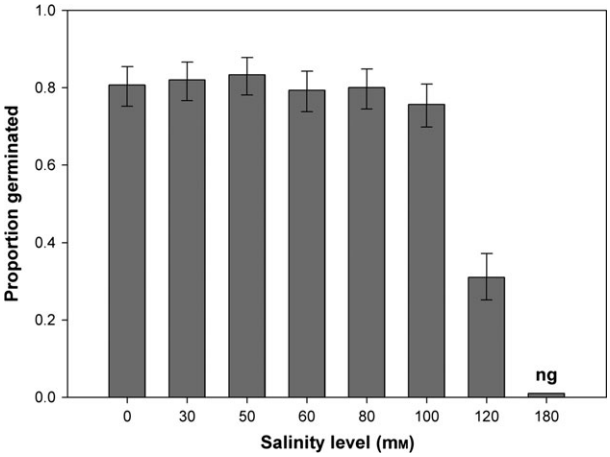

Fig. 4 Effect of NaCl concentration on the germination of seeds incubated at 25–15°C in a 12-h photoperiod for 21 day. The height of the shaded bar represents the average of the proportion of germinated seeds as estimated by the fitted GLM, and the error bar represents the 95% confidence interval for this estimate. Treatment labelled 'ng' had no seed germination and was excluded from the analysis.

Dry storage

In this study, differences could not be detected among dry storage periods tested ($\chi^2 = 8.13$; d.f. = 4; $P = 0.44$). A high proportion of *I. diffusa* seeds germinated at dispersal time in autumn [0 day: 0.853 (CI: 0.784–0.903)] and in all dry storage treatments [90 day: 0.86 (CI: 0.792–0.908); 180 day: 0.783 (CI: 0.707–0.843); 360 day: 0.817 (CI: 0.744–0.872); 720 day: 0.839 (CI: 0.769–0.892)].

Burial depth

Seedling emergence of *I. diffusa* was strongly influenced by seed burial depth ($F = 61.25$; d.f. = 3; $P < 0.001$; Fig. 5). The highest emergence was for seeds sown on the soil surface. Diminished emergence was significant at 0.5 and 1 cm. Little emergence was recorded for *I. diffusa* seeds buried at 2 cm, while seedling emergence was completely inhibited in seed buried at 5 and 10 cm.

Discussion

The germination of *I. diffusa* seeds at constant temperature was high between 15 and 25°C (Fig. 1). Warmer

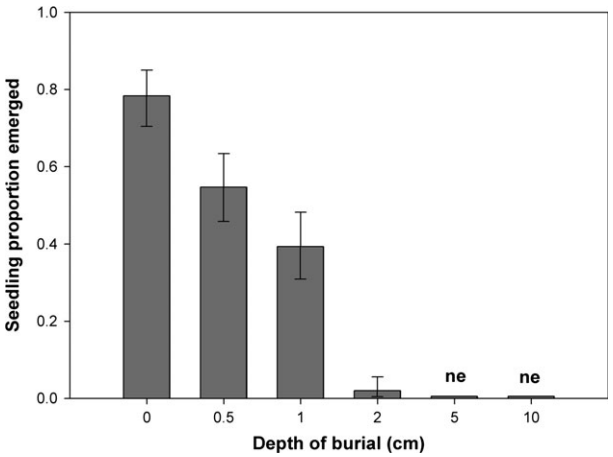

Fig. 5 Effect of seed burial depth on seedling emergence (%) for 21 day. The height of the shaded bar represents the average of the proportion of emerged seedlings as estimated by the fitted GLM, and the error bar represents the 95% confidence interval for this estimate. Treatment labelled 'ne' had no seedling emergence and was excluded from the analysis.

temperature ranges for optimum germination values have been reported in related species, such as 30–37 °C in *A. quitensis* (Faccini & Vitta, 2005). In comparison with other summer weeds frequent in Argentina, the minimum temperature for germination of *I. diffusa* seeds (<10°C; Fig. 1) was lower than *Portulaca oleraceae* L. (no germination at 10°C and 26% germinated at 15°C) (Ferrari & Leguizamón, 2006). Thus, *I. diffusa* seeds would emerge before this species, based on its temperature requirements. In addition, germination of *I. diffusa* seeds was diminished at the highest constant temperatures tested (Fig. 1). Germination of non-dormant seeds may be inhibited in response to unfavourable environmental conditions (Baskin & Baskin, 1998), so germination could be reduced at supra-optimal temperatures (Guillemin *et al.*, 2013). Our study showed that constant temperatures higher than 25°C reduced the germination of *I. diffusa* seeds, but further studies are necessary to understand the effect of high temperatures.

Seeds of *I. diffusa* reached high germination rates over the range of fluctuating temperatures tested, suggesting that it could germinate throughout the year in tropical and subtropical regions in Argentina. On the other hand, decreasing early season soil temperature has been mentioned as one of the disadvantages of no-till cultivation (Johnson & Lowery, 1985). Due to the fact that a high proportion of *I. diffusa* seeds germinated at the lower temperature fluctuation regime tested, this species could have a possible competitive advantage over other weed species. For example, *I. diffusa* seeds might germinate earlier in the season than other weed seed, such as *P. oleraceae* (<20% germination at 5–15°C) (Ferrari & Leguizamón, 2006). Thus, a significant population of *I. diffusa* could germinate and emerge early, may affect the crop and may require prompt control.

Our experimental conditions suggest that *I. diffusa* seeds seem not to have a dark dormancy, because the proportion of germinated seeds in darkness was as high as in light. This fact is in contrast to the light requirements for germination found in other related Amaranthaceae weed species, such as *A. quitensis* (Faccini & Vitta, 2005) and *G. perennis* (Acosta *et al.*, 2013), whose seed germination in continuous darkness was lower than in white light treatments. However, it is important to note that *I. diffusa* germination in darkness was only tested under alternating temperature conditions, but temperature fluctuation can substitute the light requirement, as in *Nicotiana tabacum* L. (Toole *et al.*, 1955). The effect of darkness under constant temperature was not tested in our work, so this possibility should not be dismissed in further studies.

Significant decrease in germination of *I. diffusa* seeds in cold-wet storage treatments of 12 and 16 weeks (Fig. 2) was due to diminished seed viability. Germinating seeds of many species, especially those of tropical or subtropical origin, suffer chilling injury when exposed to low but non-freezing temperatures, resulting in poor seedling establishment (Bedi & Basra, 1993). Therefore, although *I. diffusa* could pose a weed threat in tropical and subtropical regions with no rigorous winter, germination could be affected by low temperatures in regions with an extensive winter. Although our study was conducted for a time in which there was only a small reduction ($\approx$ 20%) in germination, the proportion of decayed embryos recorded was significant. So, further studies are necessary to indicate whether chilling injury could be an important factor for seedbank depletion of *I. diffusa*.

Also, results indicated high sensitivity to increasing osmotic stress for *I. diffusa* seeds (Fig. 3). Our data were similar to that in *Amaranthus viridis* (Chauhan & Johnson, 2009). This weed was considered very sensitive to low osmotic potential, because an osmotic potential of −0.2 MPa reduced its germination by 86% compared with the control (Chauhan & Johnson, 2009). In contrast, other weed species were reported as rather tolerant to low osmotic potentials, such as *Conyza bonariensis* (L.) Cronq., with 50% of germination at −0.7 MPa (Zambrano-Navea *et al.*, 2013). Our results showed that low water potential could be a key factor affecting germination time in *I. diffusa*, suggesting that this species could not germinate under moderately water-stressed conditions.

Iresine diffusa has been found in saline soils of México (Sanchez del Pino *et al.*, 1999) and in marshes

and along the coast of the USA (Robertson, 1981). Salt tolerance during the germination phase may be especially critical for establishment of plants in *I. diffusa*, and this characteristic allows it to have an advantage over soyabean cultivars with low tolerance in saline environments. In addition, *I. diffusa* appears to have better adaptation to salt stress than other weeds. For example, *A. viridis* seeds did not germinate at 100 mmol L^{-1} (Chauhan & Johnson, 2009), whereas in the present work a high proportion of *I. diffusa* seeds germinated at a NaCl concentration of 100 mmol L^{-1} (Fig. 4). Moreover, under salt stress, Na^+ and Cl^- may be taken up by the seed and toxic effects of NaCl might appear (Ungar, 1996). However, inhibited *I. diffusa* seeds at the highest salinity treatment were able to germinate at levels similar to those of the control, after rinsing in distilled water and imbibition in control conditions, indicating that germination inhibition was due to an osmotic effect, as opposed to a specific ion toxicity effect. It may be more appropriate to use seed survival under hypersaline conditions rather than germinability as a criterion for success, as recovery germination does occur in seeds of *Atriplex* spp. and other halophytes when the hypersaline conditions are alleviated (Ungar, 1996). Results showed that seeds of *I. diffusa* could withstand high salinity stress and provide a viable seedbank for recruitment of new individuals.

The high viability kept until the end of the experiment indicates that *I. diffusa* seeds were not recalcitrant. Besides, germination was high shortly after the seeds were collected. Many weed species rely on innate mechanisms that avoid germination immediately after shedding (Schütz *et al.*, 2002). At dispersal time in autumn, about half of the seeds of *A. quitensis* are dormant and they fail to germinate, even at optimum temperature and light conditions, and the main emergence flush occurs during the following spring (Faccini & Vitta, 2005). The results of our research suggest that high germination rates are probable in *I. diffusa* seeds at dispersal time, so an important emergence flush in autumn could occur, but this possibility would eventually be confirmed by additional studies. In addition, in some species, dry storage can cause seeds to enter dormancy (Baskin *et al.*, 2006), but *I. diffusa* seeds have showed high germination rates after 2 years of dry storage. Furthermore, germination patterns of seeds could change after harvest during dry storage conditions (Cristaudo *et al.*, 2007). It should be noted that all other experiments were performed 3–5 months after harvest and were repeated over time (two different runs for each factor), so seed physiological changes could have occurred during this time. However, in concordance with the results obtained in the dry storage experiment, there were no significant 'treatment by run' interactions in all others experiments. Thus, inferences drawn from the results suggest that germination behaviour of *I. diffusa* seeds did not change during storage for each factor analysed in our experimental conditions.

Seedling emergence of *I. diffusa* was sharply diminished by increasing burial depth. Data from other weed species have shown similar patterns of emergence in small seeded broad-leaved weeds (Mennan & Ngouajio, 2006). In general, small-seeded species might have scarce reserves that constrain them to near-surface emergence (Baskin & Baskin, 1998). Our results suggest that decreased seedling emergence because of increased seed burial depth (Fig. 5) could be related to the small seed size of *I. diffusa*. In no-till practices, there are high concentrations of weed seeds in the upper layers of the soil (Ghersa & Martínez-Ghersa, 2000). The incidence of *I. diffusa* would be high in fields in which no-tillage or minimum-tillage practices are performed, as the depth requirement for germination would be met. So, tillage operations that bury seeds could be an option to limit the germination of *I. diffusa*.

Iresine diffusa combines sexual and vegetative reproduction. In some weed species, such as *Sorghum halepense*, plants that emerged from rhizomes are more competitive and problematic for crops than seedlings, because of their earlier emergence and faster growth rate (Mitskas *et al.*, 2003). Some germination characteristics of *I. diffusa* could be indicative of success in no-till (i.e. burial depth), while others (i.e. soil moisture stress and the ability to germinate in the dark) could be characteristics that favour germination of buried seeds, rather than seed remaining on the surface in no-till systems. The fact that this species can reproduce by rhizomes raises the scenario under which controlling individuals coming from rhizomes (and consequently studying factor affecting rhizomes sprouting) could be more important than seed germination. This clearly deserves further study.

Conclusion

Iresine diffusa seeds have high viability at dispersal (>80%) and are capable of emerging in a broad range of environmental conditions. Alternating temperatures, similar to temperature regimes observed in different seasons in several cultivated regions of Argentina, appear to provide suitable thermal germination conditions for *I. diffusa* seeds. Moreover, germination may be enhanced due to shallow soil depths and high moisture conditions. Although no-tillage could provide better conditions for *I. diffusa* germination than

conventional tillage systems, further research should be conducted to understand the relative importance of the sexual versus asexual reproductive strategy of *I. diffusa*, in relation to the presence and persistence of this weed in the context of no-till systems.

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References

Acosta JM (2012) Arquitectura y modelos de crecimiento de malezas tolerantes a glifosato. Caracterización de las posibles estrategias de escape a la acción herbicida. Tesis de Doctorado, Universidad Nacional del Litoral, Santa Fe, Argentina.

Acosta JM, Bentivegna DJ, Panigo ES, Dellaferrera I & Perreta MG (2013) Factors affecting seed germination and emergence of *Gomphrena perennis*. *Weed Research* **53**, 69–75.

Baskin CC & Baskin JM (1998) *Seeds: Ecology, Biogeography and Evolution of Dormancy and Germination*. Academic Press, New York.

Baskin CC, Thompson K & Baskin JM (2006) Mistakes in germination ecology and how to avoid them. *Seed Science Research* **16**, 165–168.

Bedi S & Basra AS (1993) Chilling injury in germinating seeds: basic mechanisms and agricultural implications. *Seed Science Research* **3**, 219–229.

Chauhan BS & Johnson DE (2009) Germination ecology of spiny (*Amaranthus spinosus*) and slender amaranth (*A. viridis*): troublesome weeds of direct-seeded rice. *Weed Science* **57**, 379–385.

Cristaudo A, Gresta F, Luciani F & Restuccia A (2007) Effects of after-harvest period and environmental factors on seed dormancy of *Amaranthus* species. *Weed Research* **47**, 327–334.

Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M & Robledo CW (2011) *InfoStat*.. Universidad Nacional de Córdoba, Córdoba, Argentina.

Faccini D & Vitta JI (2005) Germination characteristics of *Amaranthus quitensis* as affected by seed production date and duration of burial. *Weed Research* **45**, 371–378.

Ferrari G & Leguizamón ES (2006) Requerimientos germinativos y modelización de la emergencia de plantulas de *Portulaca oleraceae* L. (Verdolaga). Revista de Investigaciones de la Facultad de Ciencias Agrarias 9. Available at: http://www.fcagr.unr.edu.ar/Investigacion/revista/rev9/2.htm (last accessed 20 February 2014).

Forcella F, Benech AR, Sanchez R & Ghersa CM (2000) Modeling seedling emergence. *Field Crops Research* **67**, 123–139.

Ghersa CM & Martínez-Ghersa MA (2000) Ecological correlates of weed seed size and persistence in the soil under different tilling systems: implications for weed management. *Field Crops Research* **67**, 141–148.

Guillemin JP, Gardarin A, Granger S, Reibel C, Munier-Jolain N & Colbach N (2013) Assessing potential germination period of weeds with base temperatures and base water potentials. *Weed Research* **53**, 76–87.

Johnson MD & Lowery B (1985) Effect of three conservation tillage practices on soil temperature and thermal properties. *Soil Science Society of American Journal* **49**, 1547–1552.

Kellman M (1980) Geographic patterning in tropical weed communities and early secondary successions. *Biotropica* **12**, 34–39.

Mas MT, Verdú AMC, Kruk BC, De AD, Guglielmini AC & Satorre EH (2010) Weed communities of transgenic glyphosate-tolerant soyabean crops in ex-pasture land in the southern Mesopotamic Pampas of Argentina. *Weed Research* **50**, 320–330.

Mennan H & Ngouajio M (2006) Seasonal cycles in germination and seedling emergence of summer and winter populations of catchweed bedstraw (*Galium aparine*) and wild mustard (*Brassica kaber*). *Weed Science* **54**, 114–120.

Michel BE (1983) Evaluation of the water potentials of solutions of polyethylene glycol 8000 both in the absence and presence of other solutes. *Plant Physiology* **72**, 66–70.

Mitskas MB, Tsolis CE, Eleftherohorinos IG & Damalas CA (2003) Interference between corn and johnsongrass (*Sorghum halepense*) from seed or rhizomes. *Weed Science* **51**, 540–545.

Pedersen TM (1987) Amaranthaceae. In: *Flora Ilustrada de Entre Ríos (Parte IV)*, **Vol. 6**. (ed. A Burkart), 160–203. INTA, Buenos Aires.

Puricelli E & Tuesca D (2005) Weed density and diversity under glyphosate-resistant crop sequences. *Crop Protection* **24**, 533–542.

Robertson KR (1981) The genera of Amaranthaceae in the southeastern United States. *Journal of the Arnold Arboretum* **62**, 267–314.

Sanchez Del Pino I, Flores Olvera H & Valdés J (1999) La familia Amaranthaceae en la flora halófila y gipsófila de México. *Anales del Instituto de Biología Universidad Nacional Autónoma de México, Serie Botánica* **70**, 29–135.

Schütz W, Milberg P & Lamont BB (2002) Seed dormancy, after-ripening and light requirements of four annual Asteraceae in south-western Australia. *Annals of Botany* **90**, 707–714.

Scursoni JA & Satorre EH (2010) Glyphosate management strategies, weed diversity and soybean yield in Argentina. *Crop Protection* **29**, 957–962.

SIIA (2013) Estimaciones Agrícolas: Soja. MAGyP, Ministerio de Agricultura, Ganadería y Pesca de la Nación

Argentina. Available at: http://www.siia.gov.ar/index.php/series-por-tema/agricultura (last accessed 10 March 2013).

Toole EH, Toole VK, Borthwick HA & Hendricks SB (1955) Interaction of temperature and light in germination of seeds. *Plant Physiology* **30**, 473–478.

Ungar IA (1996) Effect of salinity on seed germination, growth and ion accumulation of *Atriplex patula* (Chenopodiaceae). *American Journal of Botany* **83**, 604–607.

Vitta JI, Tuesca D & Puricelli E (2004) Widespread use of glyphosate tolerant soybean and weed community richness in Argentina. *Agriculture, Ecosystems & Environment* **103**, 621–624.

Zambrano-Navea C, Bastida F & Gonzalez-Andujar JL (2013) A hydrothermal seedling emergence model for *Conyza bonariensis*. *Weed Research* **53**, 213–220.