Factors affecting seed germination and emergence of *Gomphrena perennis*

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Summary

Controlled growth chamber experiments were conducted to determine factors affecting seed germination and emergence of the troublesome weed *Gomphrena perennis*. The objective of this research was to examine the effects of temperature, light, moist chilling, osmotic potential, dry storage and depth of seed burial on *G. perennis* germination and emergence. The optimum temperature for germination was around 15–20°C. Seeds showed germination rates above 90% under 20/10 and 25/15°C temperature regimes. The minimum exposure to light needed to stimulate germination was 1 min. However, the light requirement was reduced after a long storage period. Furthermore, germination was high (>90%) in all moist-chilling treatments tested. Germination was highly sensitive to increasing osmotic stress. The highest germination percentage (94%) was achieved at 0 MPa, and decreasing osmotic potential from 0 to −0.3 MPa reduced germination to 11%. The highest seedling emergence occurred for seeds placed from 0 to 1 cm deep, and no seedlings emerged from a 5-cm burial depth. *Gomphrena perennis* has a suitable environment in a no-till soybean field, where seeds remaining on the surface have the required temperature, light and depth needed for germination.

Keywords: light, moist chilling, osmotic potential, temperature, weed seed.

Introduction

*Gomphrena perennis* L. (Amaranthaceae) is an important perennial weed with a C₄ photosynthetic pathway. Native of tropical and subtropical regions of South America, it has spread as a weed throughout several countries, including Argentina and Brazil (Pedersen, 1987). In Argentina, *G. perennis* has extended into 13 states localised in northern and central regions (Pedersen, 1987) and is associated with no-till glyphosate-resistant soybean (*Glycine max* (L.) Merr.) production (Puricelli et al., 2008).

Soybean is a major crop in Argentina, and a high proportion of the crop is under no-tillage cropping systems with glyphosate-resistant (GR) soybean varieties (SIIA, 2011). In Argentina, weed population shifts have been observed in the last two decades as a consequence of the increasing use of the no-tillage system, characterised by the lack of soil inversion and the presence of large quantities of crop residue. Although some of the population shifts could be attributed to environmental changes that may influence weed seed dynamics (Facchi & Vitta, 2005), current farming practices, such as no-tillage cropping systems...
that rely heavily on glyphosate, have contributed to its increase as a troublesome weed. *Gomphrena perennis* can be difficult to control with glyphosate and has been reported as glyphosate-tolerant (Puricelli et al., 2008). In the vegetative stage, conventional glyphosate treatment with 1.4 kg a.i. ha$^{-1}$ provided only 53% control, and an application of 4.8 kg a.i. ha$^{-1}$ was needed for adequate control of 88% (Nisensohn et al., 2007).

Environmental concern and the increasing public pressure for more sustainable crop production methods have led to a growing interest in integrated weed control strategies. The development of effective integrated weed management systems, however, depends on a thorough understanding of seed biology (Chauhan & Johnson, 2000). Seed germination and seedling emergence are key events in determining the success of a weed in an agroecosystem (Forcella et al., 2000). The presence of *G. perennis* seedlings in spring and autumn might indicate several emergence periods throughout the year (JM Acosta, unpubl. obs.). *Gomphrena perennis* does not have any type of vegetative reproduction, so its populations are spread only through seeds (Pedersen, 1987). For this reason, knowledge about the environmental requirements for germination is essential to implement a rational strategy for weed management. Information concerning several factors affecting seed germination has been cited for related species, such as *Amaranthus retroflexus* L. (Ghorbani et al., 1999), *Amaranthus spinosus* L. and *Amaranthus viridis* L. (Chauhan & Johnson, 2009) and *Amaranthus albus* L. (Chadouef-Hannel & Taylorson, 1985). However, factors affecting seed germination and emergence of *G. perennis* are unknown.

Information on the biology and the reasons for the success of this species as a weed is scarce. The aim of this research was to determine (i) the effect of temperature, light, osmotic potential, moist chilling and dry storage on seed germination and (ii) the effect of burial depth on *G. perennis* seedling emergence.

**Materials and methods**

*Gomphrena perennis* seeds were harvested in May 2009 from populations located in several GR soybean fields of Santa Fe province, Argentina (31.26°S, 60.56°W). Inflorescences collected from many randomly selected plants were bulked. Seeds were cleaned and stored at room temperature (18–20°C) and low relative humidity (≤15%) in sealed opaque containers until used in the experiment. The 1000-seed weight was 315 ± 27.5 mg. All the experiments were conducted 3–5 months after harvest, except for the dry storage experiment, which started immediately after the seeds were harvested from the same population in May 2009 and was finished 2 years later.

**Test protocol general information**

Unless otherwise stated, germination was determined by placing 50 seeds evenly in a 9-cm Petri dish containing two pieces of Qualy® filter paper (J.Prolab, Av. Rocha Pomba 2114, São José dos Pinhais, Paraguay) soaked in 5 mL of distilled water (or the solution appropriate for the experiment). Seed viability was 92% according to a tetrazolium chloride test (ISTA, 1985). Distilled water (or test solution) was added on a regular basis to maintain appropriate humidity. Dishes were incubated at 25/15°C alternating temperatures. The photoperiod was set at 12 h to coincide with the high-temperature period. White fluorescent lamps were used to produce a photosynthetic photon flux density of 150 μmol m$^{-2}$ s$^{-1}$. Germination was monitored for 3 weeks. Germination percentages were based on the total number of seeds placed in the Petri dish. A seed was considered germinated when a visible radicle could be discerned and, at that time, it was removed from the Petri dish. The viability of non-germinated seeds was tested with a 0.4% tetrazolium chloride solution. Seeds showing a pink to reddish colour after 4 h were considered viable.

**Temperature**

Constant temperature effects were examined by using a modified aluminium thermal plate, as described by Chaterton and Kadish (1969) to generate a thermal gradient. The temperature ranged from 5 to 32.5°C. One hundred seeds were put on tissue paper for each one of the twelve tested temperatures, including 5, 7.5, 10, 12.5, 15, 17.5, 20, 22.5, 25, 27.5, 30 and 32.5°C. This experiment was conducted in a completely dark room, and the light regime was 5–10 min to prevent possible changes in temperature due to light exposure. Alternating temperature effects on germination were determined in growth chambers under fluctuating day/night temperature regimes (15/5, 20/10, 25/15, and 30/20°C) in light/dark (12/12 h) periods. Temperature regimes were selected to reflect the temperature variation in regional areas of field infestation at different times of the year.

**Light exposure**

The influence of the time of light exposure was determined by four different treatments: 0 (complete darkness), 1, 10 and 720-min light incidence. The source of light was as mentioned above. After light exposure, the Petri dishes were wrapped in a double layer of aluminium foil to maintain complete darkness. Germination count was performed, and water was supplied in a dark room lit by an indirect green safety light (Cavadevices, Bolivia 1340, Buenos Aires, Argentina).
Osmotic stress

Six treatments were conducted to determine the effect of osmotic stress on germination. Seeds were incubated in aqueous solutions with osmotic potentials of 0, –0.3, –0.4, –0.6, –0.9 and –1.3 MPa, which were prepared by dissolving 0, 154, 191, 230, 297 and 350 g of polyethylene glycol (PEG) 6000 (Biopack, Ruta 9 Km 105, Buenos Aires, Argentina) in 1 L of distilled water respectively. The equation of Michel (1983) was used to determine the required amount of PEG 6000.

Moist chilling

The effect of moist chilling was determined by placing seeds in dishes containing 5 mL of distilled water and exposing them to either 0 (non-chilled control), 4, 8, 12 or 16 weeks of moist chilling in darkness in a refrigerator at 4°C. All moist-chilling treatments were started at the same time. Following each respective moist chilling storage period, seeds were incubated according to the methodology described above.

Dry storage

Fifty seeds were placed in each of 60 paper bags and stored at room temperature at 20°C (±2°C) for 0, 90, 180, 360 and 720 days after harvest. The 0 day treatment seeds were incubated immediately after they were collected and cleaned. Germination was determined in growth chambers under fluctuating day/night temperatures (25/15°C) in light/dark (12/12 h) and continuous dark (24 h) periods. For germination in complete darkness, dishes were wrapped in a double layer of aluminium foil and checked as described under the light exposure experiment.

Seed burial depth

Fifty seeds were placed on the soil surface or buried at 0.5, 1, 2, 5 and 10-cm deep in 15 cm diameter 1 L plastic pots. The soil was a silt loam that had been collected from soybean fields. Soil characteristics were 2.1% organic matter and 6.3 pH, which is representative of the area of study. The soil was autoclaved and sieved through a 2-mm mesh screen. Soil fractions were subsequently put in plastic pots with a hydraulic press to reach a uniform bulk density of 1.0 g cm⁻³ in all the pots. A compaction pressure of 2.0 MPa was determined suitable to reach the desired bulk density. Seeds were exposed to light for at least 15 min (as described in the light exposure time experiment), prior to burial at appropriate depths to remove their light requirement. Seeds were placed in the soil after pressing. Pots were placed randomly inside the growth chamber under fluctuating day/night temperature conditions (25/15°C) in a light/dark (12/12 h) period. Pots were watered daily to maintain field capacity. Seedlings were checked daily and were considered to have emerged when cotyledons were visible at the soil surface. Once a seedling reached this stage, it was removed from the pot.

Statistical analysis

Laboratory experiments were conducted in a completely randomised design, except for the dry storage experiment, where a split-plot design was used, with storage time as a principal factor and light/dark or complete darkness as a subfactor. Treatments of each experiment were replicated three times, and each experiment was conducted twice. Data were combined for analyses when there was no time by treatment interaction. Each replicate for each treatment was placed randomly inside the growth chamber and rearranged daily. In the alternating temperature experiment, each Petri dish was placed randomly in different growth chambers with their respective temperature regimes. Data were checked to confirm normality and homogeneity of variance. ANOVA and regression analyses were performed on the non-transformed percentage of germination. In the alternating temperature, light exposure, moist chilling and dry storage experiments, the germination percentages were analysed using ANOVA. The analysis was performed using InfoStat (InfoStat® Professional Version 2009; Statistical Software. Grupo InfoStat FCA, Universidad Nacional de Córdoba, Argentina). Means were separated by using the LSD test at \( P = 0.01 \).

In the experiments of seed burial depth and osmotic stress, data were analysed by means of regression analysis using the R software (R Statistical Software 2.13; R Development Core Team). An exponential decay curve was fitted to the germination (%) obtained at different osmotic potentials (Chauhan & Johnson, 2009) of the form

\[
G = G_{\text{max}} \times \exp\left(-G_{\text{rate}} \times x\right)
\]

where, \( G \) represents cumulative germination (%) at osmotic potential \( x \), \( G_{\text{max}} \) is the maximum germination and \( G_{\text{rate}} \) determines the steepness of the decay. The seedling emergence (%) values obtained at different burial depths were fitted to a functional three-parameter logistic model (Eslami, 2011) of the form

\[
E = E_{\text{max}} / \left[1 + \left(\frac{x}{x_{50}}\right)^{E_{\text{rat}}}ight]
\]

where, \( E \) is the total seedling emergence (%) at burial depth \( x \), \( E_{\text{max}} \) is the maximum seedling emergence (%), \( x_{50} \) is the depth to reach 50% of maximum seedling
emergence and $E_{rate}$ indicates the slope. In the case of non-linear regression, an $F$-test for ‘lack-of-fit’ was used to verify whether the equation showed a good fit with the experimental data (Ritz & Striebig, 2008; Onofri et al., 2010). Because of operational problems with the thermogradient plate, the constant temperature experiment only had two replications in time.

**Results**

**Effect of temperature**

Constant temperature affected seed germination of *G. perennis* appreciably ($F = 94; \ P < 0.01$). Inhibited, elevated and intermediate germination responses are represented in Fig. 1 (LSD = 15). While inhibition of germination ($\leq 1\%$) was in a range from 5 to 7.5°C, elevated germination ($\geq 75\%$) was measured in a range from 10 to 22.5°C. Intermediate germination occurred at temperatures ranging from 25 to 32.5°C, with germination gradually decaying from 55% to 37% as the temperature increased up to 32.5°C.

Alternating temperatures also influenced seed germination significantly ($F = 9; \ P < 0.01$; Fig. 2). Seeds showed higher germination rates in the two intermediate temperature regimes 20/10°C (93%) and 25/15°C (92%) than in the lowest 15/5°C (79%) and highest 30/20°C (80%) temperature regime treatments used in the laboratory (LSD = 10).

**Effect of light exposure**

*Gomphrena perennis* seed germination was influenced by light exposure ($F = 25; \ P < 0.01$). Germination was higher in light exposure treatments than in total darkness (0 min). While the percentages of germination were 93%, 92%, 95% in light exposure treatments of 1, 10, 720 min respectively, only 20% of seeds germinated in total darkness (LSD = 8). Thus, a minimal exposure to light is required to stimulate germination in *G. perennis* seeds.

**Effect of moist chilling**

Differences in seed germination could not be detected ($F = 0.6; \ P = 0.4$) among varying durations of cold/moist chilling. Germination ranged from 94 to 96% for 0 to 16 weeks in moist-chilling treatments respectively.

**Effect of osmotic stress**

Decreasing osmotic potentials of the solution reduced the germination percentage of *G. perennis* seeds (Fig. 3). The highest seed germination percentage (94%) was achieved at 0 MPa (distilled water), and a decreasing osmotic potential from 0 to −0.3 MPa caused an 80% reduction in germinability (11% germination). Germination ceased at −0.6 MPa. The osmotic potential required for 50% inhibition of maximum germination ($x_{50}$) determined from the fitted model (Eqn (1); $F = 0.2; \ P = 0.95$) was −0.1 MPa (±0.01).

**Effect of dry storage**

In this study, seeds of *G. perennis* showed high viability at dispersal (>90%), suggesting a nearly complete lack of seed dormancy in this species. After-ripened seeds for up to 720 days (2 years) in the laboratory showed high viability too (>90%). However, the interaction between
Dry storage and light/dark treatment on seed germination was significant ($F = 34; P < 0.01$; Fig. 4). In the light, differences could not be detected in the dry storage periods tested ($F = 0.5; P = 0.72; \text{LSD} = 7$), while in complete darkness, *G. perennis* seed germination was lower for the dry storage periods from 0 to 360 days (<20%) than for the 720-day period (61%) ($F = 50; P < 0.01; \text{LSD} = 11$).

**Effect of burial depth on seedling emergence**

Seed burial depth greatly influenced seedling emergence of *G. perennis* seeds (Fig. 5). Seedling emergence was higher (290%) for seeds placed in the soil surface up to 1-cm deep. As estimated from the fitted model (Eqn (2); $F = 0.4; P = 0.77$), 50% inhibition of maximum emergence ($x_{50}$) occurred at 2.1 cm ($\pm 0.12$). Emergence was totally inhibited at planting depths of 5 and 10 cm (0%).

**Discussion**

*Gomphrena perennis* seeds germinated over the range of fluctuating temperatures tested, suggesting its ability to germinate throughout the year at low altitudes in subtropical countries. However, germination rates of *G. perennis* seeds were higher at the two intermediate fluctuating temperatures than at the warmer (30/20°C) and at the colder temperature regimes (15/5°C). The optimum temperature for germination is around 15–20°C, and the minimum temperature is around 7.5–10°C. In *Amaranthus quitensis* Kunth., a weed found in similar regions to those of *G. perennis*, the maximum percentage of germination was recorded at 35/25°C (Faccini & Vitta, 2005). Due to the intermediate range of temperatures over which *G. perennis* seeds have shown higher germination rates, this weed might germinate especially well in spring and autumn. Such intermediate temperature regimes are more likely to occur in tropical and subtropical regions. The intermediate range of temperatures could be a possible competitive advantage over other weed species, because *G. perennis* seeds might germinate earlier in the season than seeds of weeds with optimum germination rates at warmer regimes.

Light seems to be critical for germination in *G. perennis*. Similar light requirements for germination were found in related weed species (Faccini & Vitta, 2005; Chauhan & Johnson, 2009). Batlla *et al.* (2000) found that only 2 min of light exposure is enough to stimulate germination of *A. quitensis*. The light requirements have been suggested as effective mechanisms for detecting the presence of dense canopies and avoiding futile germination when the probability of successful seedling establishment is low (Washtitani, 1985). The
light requirement for germination is also related to a seminal translucent cover (Pons, 2000). In *G. perennis*, the seed cover has been characterised as clear enough to allow light to pass (Pedersen, 1987). As *G. perennis* germination was stimulated strongly by light, it may act as an adaptive strategy of its seeds to detect exposure to light. For *A. retroflexus* seeds, Gallagher and Cardina (1998) indicated that the germination response to light and temperature can vary throughout the growing season. In *G. perennis*, higher germination rates are likely to be restricted to the surface soil and, therefore, in no-till systems, high levels of crop residues and crop canopy could play an important role in its germination dynamics through the year. Thus, to determine seasonal periodicity of germination requirements in *G. perennis* seeds, additional studies are necessary.

Moist-chilling treatments have induced secondary dormancy in other weeds, such as the case of *Hesperis matronalis* L. seeds (Susko & Hussein, 2008). In addition, 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 and reduction in yield (Bedi & Basra, 1993). However, *G. perennis* seeds did not show either decreased viability or significantly lower germination rates after moist-chilling treatments. So, seeds could remain viable during the winter and germinate at a high percentage as temperatures increase in the following spring.

*Gomphrena perennis* seeds were very sensitive to low osmotic potential. These results were similar to those reported for other related species such as *A. viridis* (Chauhan & Johnson, 2009) and *A. retroflexus* (Habib & Morton, 1987). An osmotic potential of −0.2 MPa reduced germination of slender amaranth by 86% compared with the control (Chauhan & Johnson, 2009). By contrast, in other weed species reported as rather tolerant to low osmotic potentials, such as *Solanum rostratum* Dunal, germination exceeded 95% at −0.3 MPa (Wei et al., 2009). Osmotic stress could be a key factor affecting germination time in *G. perennis*, and seedling emergence in fields could be restricted to rainy seasons. These results suggest that its germination and establishment could remain high in poorly drained soil conditions, but that it could not have a competitive advantage over other weed species under water stress conditions or in drought-prone soils.

Viability of *G. perennis* seeds remained high until the end of the experiment. This fact indicates that *G. perennis* seeds were not recalcitrant. Our results showed that germination in the light was high after harvest and did not decrease with the dry storage. 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; the main emergence flush occurs during the following spring (Faccini & Vitta, 2005). Our results suggest that high germination rates are possible in *G. perennis* seeds at dispersal time, if light exposure occurs, so an important emergence flush in autumn is possible. In addition, in some species, drying can cause seeds to enter dormancy (Baskin et al., 2006). With *G. perennis* seeds, 2 years of dry storage was responsible for reducing the light dormancy of the seed and making it less sensitive to light.

Reduced emergence of several weed species due to increased burial depth has been reported (Susko & Hussein, 2008; Chauhan & Johnson, 2009). In general, small-seeded species might have limited carbohydrate reserves restricting them to shallow emergence (Webb et al., 1987; Baskin & Baskin, 1998). Therefore, in addition to the need for light, decreased seedling emergence because of increased seed burial depth also could be related to the seed size of *G. perennis*. In no-till soybean practices, *G. perennis* seeds remain near the soil surface; consequently, seedling emergence would be enhanced, since the depth and light requirements for seed germination would be met. *Gomphrena perennis* seedling emergence was inhibited totally at depths greater than 5 cm. So, tillage operations that bury seeds below this depth could be an option to limit the germination of this weed species.

**Conclusion**

This study has shown that *G. perennis* is adapted to germinate at dispersal time under a wide range of environmental conditions commonly found in the tropics and subtropics, which partially explains its successful infestation in Argentinean soybean fields. Intermediate alternating temperature regimes provided better conditions for germination than colder and warmer regimes tested. Because of the effect of ‘osmotic stress’, germination could be mainly related to the times of the year when humidity is high, namely spring and autumn, at which times intermediate alternating temperature regimes occur. Because *G. perennis* seeds could germinate very early in the season, competitive advantages over other weed species in similar regions are possible.

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