Seed germination ecology of southeastern Australian rigid ryegrass (*Lolium rigidum*) populations

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Abstract

Herbicide resistance is an increasing issue in many weed species, including rigid ryegrass (*Lolium rigidum* Gaudin); a major weed of winter cropping systems in southern Australia. Recently, this weed has also been found in summer crops in the southeastern region of Australia. Effective control of this herbicide-resistant weed across southeastern Australia requires alternative management strategies. These strategies can be informed by analyses on the interaction of germinable seeds with their regional environments and by identifying the differences between populations of varying herbicide-resistance levels. In this study, we explore how various environmental factors differentially affect the seed germination and seedling emergence of three *L. rigidum* populations, including one glyphosate-resistant population (GR), one glyphosate-susceptible population (GS), and one population of unknown resistance status (CC04). Germination was greater than 90% for all populations at each temperature regime, except 15/5 C. Populations germinated at a lower rate under 15/5 C, ranging from 74% to 87% germination. Salt stress had a similar effect on the germination of all populations, with 0% germination occurring at 250 mM salt stress. Population GS had greater tolerance to osmotic stress, with 65% germination at −0.4 MPa compared with 47% and 43% germination for CC04 and GR, respectively; however, germination was inhibited at −0.8 and −1.6 MPa for all populations. All populations had lower germination when placed in complete darkness as opposed to alternating light/dark. Germination in darkness was lower for CC04 (69%) than GR (83%) and GS (83%). Seedling emergence declined with increasing burial depth with the lowest emergence occuring at 8 cm (37%) when averaged over the populations. These results indicate that *L. rigidum* can survive under a range of environmental variables and that the extent of survival differs based on population; however, there was no difference based on herbicide-resistance status.

Introduction

Herbicide resistance in weeds is putting increasing pressure on growers and researchers to find other methods of controlling infestations. While some populations of weeds develop resistance to only one type of herbicide, others may possess multiple herbicide resistance (Owen et al. 2007; Walsh et al. 2004). Furthermore, some types of resistance mechanisms may be able to resist herbicides that have not yet been developed (Powles and Yu 2010). Rigid ryegrass (*Lolium rigidum* Gaudin) is a winter annual weed species that is found in almost all states of Australia. Populations of this species have been found to possess resistance to a growing list of herbicides, such as glyphosate, paraquat, clethodim, and pyroxasulfone (Busi et al. 2018; Saini et al. 2017; Yu et al. 2007). Multiple herbicide resistance has evolved in some *L. rigidum* populations (Neve et al. 2004; Owen et al. 2007; Yu et al. 2007). This rise in herbicide-resistant *L. rigidum* is of great concern to infested winter cropping systems. Therefore, to reduce reliance on herbicides, it is crucial to further develop our understanding of the germination and seedling emergence of this weed in response to varying environmental conditions.

Germination is a crucial developmental step in a plant's life cycle, with germination under unfavorable conditions often being lethal for the seedling. To avoid germinating under these conditions, seeds can remain in a state of dormancy until the environment becomes more suitable for seedling growth (Baskin and Baskin 2004). Seed germination is affected by environmental conditions like temperature, light, soil salinity, soil osmotic potential, and burial depth (Chauhan et al. 2006a; Nandula et al. 2006; Tamado et al. 2002). Determining the range of each environmental variable that a weed can germinate in can help when developing future management strategies for that weed. Weeds that are able to germinate in darkness are suited to fields

where they are buried by tillage, while seeds that require light would be more suited to no-till cropping systems (Chauhan et al. 2006b). Australian farmers have moved more toward no-till systems to provide environmental and economic benefits (Dang et al. 2018), which is likely to favor weeds that are more suited to germinating in direct sunlight as opposed to those that require a period of darkness. The occurrence of seeds in or on the soil can also change other attributes of the environment, such as moisture and temperature, and lead to differences in germination.

Tolerance of environmental constraints like water stress and high salinity can allow weeds to grow in environments that would otherwise be lethal to other species that lack such adaptations and compete with crops that have adapted to such environments. As such, different species have demonstrated varying abilities to germinate over a range of soil salinities (Li et al. 2011) and osmotic potentials (Mahajan et al. 2018; Ramirez et al. 2012), allowing them to occupy different areas. Some studies have tested the effect of these environmental factors on the germination efficiency of *L. rigidum* (Chauhan et al. 2006a; Rahman and Asaduzzaman 2019) but did not focus on differences between populations of varying herbicide-resistance status. *Lolium rigidum* is known to have high genetic diversity (Owen et al. 2007), which can lead to large variation in germination responses of populations. Factors such as temperature and soil salinity vary between and within different cropping regions across multiple states of Australia (Ivushkin et al. 2018). This would lead to varied germination responses across populations from different areas when germinating under multiple temperatures (Bhatt et al. 2020). Chauhan et al. (2006a) and Rahman and Asaduzzaman (2019) each used a single temperature regime to test salt and water stress, and germination of those populations at other temperatures is therefore unknown.

Chauhan et al. (2006a) and Rahman and Asaduzzaman (2019) showed slight differences in the percentage of seeds capable of germinating over the ranges of sodium chloride (NaCl) concentrations and osmotic potentials, which may suggest differences based on population that arise due to the high genetic diversity of *L. rigidum*. Both studies show an ability of *L. rigidum* to survive under moderate to high salinity levels, which may be an important factor for its ability to infest a wide area of Australia, given that more than 60% of cropping soils in Australia are sodic in nature (Rengasamy 2002). Climate varies across Australia (Peel et al. 2007); therefore, populations from one state may develop adaptations to different environments than populations from another state. This study analyzed populations collected from New South Wales and Victoria, which may differ in response to those from other regions, such as the South Australian populations investigated by Chauhan et al. (2006a).

This study incorporates a glyphosate-resistant population to compare against a glyphosate-susceptible population to identify whether the ability to survive herbicide application can help the seeds survive other stresses. Studies on other weed species have found differences in germination depending on herbicide-resistance status for certain variables, while other studies have observed no differences. Mutti et al. (2019) found that glyphosate-resistant populations of junglerice [*Echinochloa colona* (L.) Link] were more tolerant of high salinity than susceptible populations, while Shrestha et al. (2018) observed increased moisture stress tolerance in addition to salinity tolerance in the same species. However, these studies found no differences in germination in response to other variables, like temperature or light. Differences in the germination of *L. rigidum* populations resistant

to other herbicides (acetyl-CoA carboxylase [ACCase] and cytochrome P450 inhibitors) have also been observed, with ACCase inhibitor–resistant plants possessing lower germination at increasing burial depth as well as across a range of temperatures (Vila-Aiub et al. 2005).

It is important that researchers develop improved weed management systems due to the increasing prevalence of herbicide-resistant *L. rigidum* populations. To this end, we must develop a greater understanding of the germination ecology of *L. rigidum*, particularly as it relates to differences between populations both susceptible and resistant to herbicides. Therefore, this study aims to document the effect of five environmental conditions (temperature, salt, osmotic potential, light, and burial depth) on the germination of three different *L. rigidum* populations from New South Wales and Victoria, including a glyphosate-resistant population and a glyphosate-susceptible population.

Materials and Methods

Seed Populations

Seeds of three populations of *L. rigidum* were used throughout this study. Seeds from population CC04 were collected in November 2018 from a fallow field in Croppa Creek, NSW; its glyphosate resistance status was unknown. A glyphosate-resistant population (GR) was collected from Griffith, NSW, and a glyphosate-susceptible population (GS) was collected from Victoria. Populations GR and GS were grown in the same environment at the Gatton campus of the University of Queensland during the 2018 winter season to increase the number of seeds. Seeds produced from GR and GS grown in Gatton were used in the experiments of this study alongside seeds of CC04 collected from Croppa Creek. All experiments in this study took place at the Gatton Campus of the University of Queensland (27.5551°S, 152.3345°E for germination experiments; 27.5392°S, 152.3355°E for the burial depth experiment).

Germination Setup

The effects of five environmental conditions (temperature, salt concentration, osmotic potential, light, and burial depth) on the germination of three populations of *L. rigidum* were analyzed. Germination experiments analyzing temperature, salt stress, osmotic stress, and light were carried out in laboratory conditions using petri dishes. For each population in the laboratory experiments, 25 seeds were placed into a 9-cm-diameter petri dish on a double layer of moistened filter paper (Grade 615, Macherey-Nagel, Düren, Germany) and placed into plastic ziplock bags to prevent moisture loss, with each population consisting of three replicate petri dishes. For salt stress, osmotic stress, and light experiments, the bags containing petri dishes were placed into incubators at a day/night temperature of 25/15 C with a 12-h photoperiod. The temperature of 25/15 C was chosen based on results from the temperature experiments. The experiment analyzing burial depth was undertaken in pots inside a screen house.

Temperature

The effect of five different alternating day/night (12-h/12-h) temperature regimes on the germination of three *L. rigidum* populations was analyzed following the same germination setup. The five different temperature regimes were 15/5 C, 20/10 C, 25/15 C,

30/20 C, and 35/25 C, with one incubator for each temperature. Germination was counted every 7 d over a period of 28 d.

Salt and Osmotic Potential

The same germination setup was used to evaluate the effects of salt and osmotic stress on germination, except filter paper was moistened with 5 ml of different concentrations of either sodium chloride (NaCl) for the salt stress treatment or polyethylene glycol 8000 for the osmotic stress treatment. For the salt treatment, concentrations of 0, 25, 50, 100, 150, 200, and 250 mM NaCl were used. Osmotic stress treatments were prepared as solutions with osmotic potentials of 0, −0.1, −0.2, −0.4, −0.8, and −1.6 MPa by dissolving 94.9, 138.2, 189.9, 268.5, and 379.7 g of polyethylene glycol 8000 in 1 L of water, respectively (Michel 1983).

Germination was counted every 7 d over a period of 28 d. At the end of the 28 d, the seeds of treatments with the highest concentration of NaCl (250 mM) and the highest osmotic potential (−1.6 MPa) were rinsed with water and placed into fresh petri dishes on a double layer of filter paper moistened with 5 ml of water. The petri dishes were again incubated at 25/15 C, and seeds were checked for further germination at 7 and 14 d after rinsing.

Light

This trial compared the effect of alternating light/dark to complete darkness on the germination of three *L. rigidum* populations. The alternating light/dark–treated seeds were placed in incubators, as described earlier, with a 12-h photoperiod. The dark-treated seeds were wrapped in a double layer of aluminum foil to block out all light and placed in the same incubators as the light/dark treatment. Germination was counted every 7 d for seeds exposed to alternating light/dark over a period of 28 d. For seeds kept in darkness, germination was counted only at the end of the 28 d. The seeds from the dark treatment were then kept in alternating light/dark conditions for a further 14 d and counted every 7 d.

Burial Depth

A pot experiment was established in 11-cm-diameter opaque pots (12-cm height) to test the effect of multiple seed burial depths on the emergence of each *L. rigidum* population. Pots were filled with soil from a field at the Crop Research Unit at the Gatton Campus of the University of Queensland. Clay loam soil with 2.7% total organic matter was used in each pot. For each population, 50 seeds were placed on the surface (0 cm) or buried at 1-, 2-, 4-, 8-, and 10-cm depths in the soil. Each depth was set up with three replicate pots per population. Pots were watered with an automatic irrigation system. Emergence was counted every 7 d across 4 wk, and seedlings were marked as emerged once the seedling was visible above the soil surface. Control pots, in which no seeds were spread, were set up to determine the *L. rigidum* seedbank of the soil. No *L. rigidum* emerged from these pots. Seed weight was taken to identify potential differences in energy reserves of the seeds from each population and was calculated by weighing 500 seeds per population and taking the average.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics v. 25 (IBM, Armonk, NY, USA). Each experiment was conducted twice with runs at approximately two 3-mo intervals. No significant differences were found between the two runs of any treatment, so all runs were pooled together for each respective treatment.
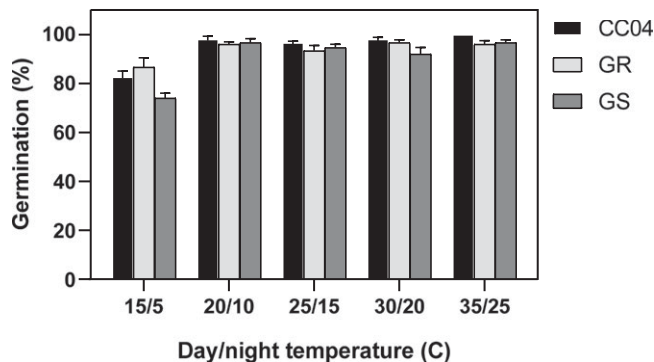

Figure 1. Effect of alternating day/night temperature on the germination of three populations of *Lolium rigidum* incubated under alternating light/dark for 28 d. Data are the means averaged from both experimental runs. Vertical bars represent the SE. GR, glyphosate-resistant population; GS, glyphosate-susceptible population; CC04, population of unknown resistance status.

A two-way ANOVA was performed using the General Linear Model function in SPSS, with treatment and population as the independent factors and germination/seedling emergence as the dependent factor. Homogeneity of variance was confirmed using Levene's test, and no transformation of the data was required. A three-parameter logistic model was fit to the germination data for salt stress and burial depth using the equation:

$$y = \frac{a}{1 + \left(\frac{x}{x_0}\right)^b} \qquad [1]$$

A three-parameter sigmoid model was fit to the germination data for osmotic stress using the equation:

$$y = \frac{a}{1 + e^{-\left(\frac{x - x_0}{b}\right)}} \qquad [2]$$

where a is the maximum germination, b is the slope, and x_0 is the stress level or burial depth required to reduce germination or emergence by 50%.

Results and Discussion

Effect of Temperature on Germination

A two-way ANOVA identified a significant interaction effect on germination between population and temperature ($P = 0.012$). Population had a significant effect on germination ($P = 0.002$); however, only population GS was significantly different ($P < 0.015$) (Figure 1), and this was not observed over all temperatures. Population GS was significantly different from CC04 at temperature regimes of 15/5 ($P = 0.049$), 30/20 ($P = 0.033$), and 35/25 C ($P = 0.049$), and significantly different from GR at 15/5 C ($P = 0.007$).

Temperature also significantly affected germination ($P < 0.001$), with the lowest germination seen at the coldest temperature regime of 15/5 C (82.7% for CC04, 86.7% for GR, and 74.0% for GS) (Figure 1). Germination at 15/5 C was significantly different than at all other temperatures for populations CC04 and GS ($P < 0.001$), and different than at temperatures 20/10, 30/20, and 35/25 C for population GR ($P < 0.05$). No other effect of temperature was observed. The only difference observed between the GR and GS populations occurred at 15/5 C.

Germination percentages were greater than 90% for all temperatures above 15/5 C (Figure 1). This similar level of germination among temperatures indicates there was no optimal germination temperature regime. The authors have not encountered reports of similarly high germination at 35/25 C compared with lower temperature regimes. Previous studies have used temperatures between 20/10 and 35/25 C to study germination of *L. rigidum*, for example, 20/12 C in Chauhan et al. (2006a) and 25/20 C in Rahman and Asaduzzaman (2019). Additionally, Gramshaw (1976) also found that *L. rigidum* germinated under a range of temperatures, with the optimum day temperature falling between 24 and 29 C, and the night temperature falling between 9 and 14 C. We decided to perform the remaining experiments in this study under 25/15 C.

Seeds of all three populations in this study were able to germinate under all five temperature regimes (minimum of 74% germination), demonstrating the potential for this species to germinate in field conditions in Queensland and New South Wales throughout the year. As this species is typically a winter annual species, germinating out of season will increase the difficulty of managing this weed.

Effect of Salt Stress on Germination

Population did not have a significant effect on the germination response to varying NaCl concentrations. Therefore, data were averaged across population for further analysis. The effect of NaCl concentration on germination was significant (P < 0.001). Germination of *L. rigidum* was similar at 0 (control), 25, and 50 mM NaCl, but was lower at higher concentrations (i.e., 100, 150, 200, and 200 mM) (Figure 2). The NaCl concentration at which germination declined to 50% occurred at 131 mM NaCl (Figure 2). All populations dropped to approximately 10% germination around 200 mM NaCl before complete inhibition occurred at 250 mM NaCl. No difference between the GS and GR populations was observed.

In previous studies by Chauhan et al. (2006a) and Rahman and Asaduzzaman (2019), germination began to decline at 25 mM and was completely inhibited beyond 160 mM (Chauhan et al. 2006a) or at 200 mM (Rahman and Asaduzzaman 2019). The populations in our study retained a high percent of germinability until >100 mM NaCl and retained some germination at 200 mM NaCl. These differences could be due to differences in the populations and may indicate an adaptation to high soil salinity in the seeds of this study's population. This could be due to different environmental conditions of the regions where each population was collected. However, other factors may also be responsible, such as maternal environment, afterripening conditions, or the temperature at which the experiments were conducted. Temperature is a likely factor, as it can affect germination of seeds exposed to different NaCl concentrations (Khan and Gulzar 2003). Chauhan et al. (2006a) used a lower temperature (20/12 C) than the current study (25/15 C), while Rahman and Asaduzzaman (2019) used a higher dark temperature with the same light temperature (25/20 C).

When nongerminated seeds from the 250 mM NaCl treatment were rinsed with water and placed on fresh moistened filter paper (0 mM NaCl), many of the seeds of all three populations began to germinate within 14 d (Table 1). Debez et al. (2018) similarly found that seeds of sea rocket (*Cakile maritima* Scop.) had suppressed germination under high salinity but were able to germinate once the salinity was alleviated and the seeds placed in distilled water.

Table 1. Germination percentage of seeds germinated in 250 mM NaCl media after 28 d and 42 d (after 28 d the seeds were rinsed with water and germinated for a further 14 d at 0 mM NaCl).[a]

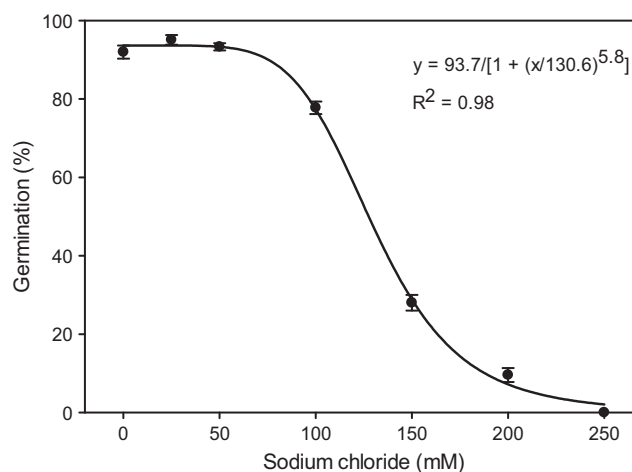| Population	Germination	
	28 d	42 d
	%	
CC04	0	82.7 ± 3.5
GR	0	85.3 ± 2.5
GS	0	82.0 ± 1.4

[a]Data are shown as the mean ± SE.

Figure 2. Effect of sodium chloride (NaCl) on the germination of three populations of *Lolium rigidum* incubated under alternating light/dark for 28 d at 25/15 C. Data are the means averaged from both experimental runs and for all populations. Vertical bars represent the SE.

They suggested that the seeds move into a state of dormancy in soils of high salinity that is broken when rain lowers the salinity, thus allowing germination to occur. In our study, germination after rinsing with water was not as high as the germination of the control treatment, with some seeds either remaining dormant or having decayed. Salt-affected soil is a widespread problem in cropping regions of Australia. It has been estimated that water table–induced salinity affects 16% of dryland cropping areas and that transient salinity could affect as much as 67% of dryland cropping areas (Rengasamy 2002). Therefore, the recoverability of *L. rigidum* seeds after alleviation of high salt conditions suggests great potential for this species to germinate over large Australian agricultural areas given sufficient rain. However, this study tested the recoverability of seeds after 28 d of salt exposure, which may not reflect the reality of long-term exposure.

Effect of Osmotic Stress on Germination

Analysis by two-way ANOVA identified a significant interaction effect between osmotic potential and population (P < 0.001). Germination of seeds for each population was highest at 0 MPa and decreased with increasing osmotic stress until complete inhibition at both −0.8 and −1.6 MPa (Figure 3). Germination was reduced to 50% at −0.4 MPa for all populations. Seeds from population CC04 showed a greater initial decline in germination at −0.2 MPa than GS and GR. At −0.4 MPa, population GS showed greater resistance to osmotic stress. This is unexpected, given the

Table 2. Germination percentage of seeds germinated in media of −1.6 MPa osmotic potential after 28 d and 42 d (after 28 d the seeds were rinsed with water and germinated for a further 14 d at 0 MPa).[a]

Population	Germination	
	28 d	42 d
	%	
CC04	0	60.0 ± 4.8
GR	0	72.7 ± 4.7
GS	0	68.7 ± 4.6

[a]Data are shown as the mean ± SE.

Table 3. Germination percentage of seeds germinated in complete darkness after 28 d and 42 d (after 28 d the seeds were exposed to alternating light/dark conditions for a further 14 d).[a]

Population	Germination	
	28 d	42 d
	%	
CC04	69.3 ± 2.7	94.0 ± 2.0
GR	82.7 ± 2.7	95.3 ± 1.9
GS	83.3 ± 3.5	96.7 ± 1.9

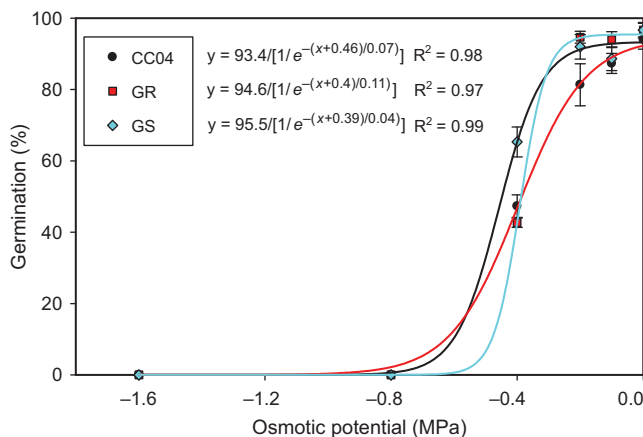[a]Data are shown as the mean ± SE.

Figure 3. Effect of osmotic potential on the germination of three populations of *Lolium rigidum* incubated under alternating light/dark for 28 d at 25/15 C. Data are the means averaged from both experimental runs. Vertical bars represent the SE. GR, glyphosate-resistant population; GS, glyphosate-susceptible population; CC04, population of unknown resistance status.

Figure 4. Effect of light on the germination of three populations of *Lolium rigidum* incubated under for 28 d at 25/15 C. Data are the means averaged from both experimental runs. Vertical bars represent the SE. GR, glyphosate-resistant population; GS, glyphosate-susceptible population; CC04, population of unknown resistance status.

from New South Wales and Victoria. Other grass species have shown levels of water stress tolerance similar to those see in this study; for example, *E. colona* (Mutti et al. 2019) and goosegrass [*Eleusine indica* (L.) Gaertn.] (Chauhan and Johnson 2008), which both possessed germination up to −0.8 MPa. Windmill grass (*Chloris truncata* R. Br.), however, still possessed low germination at −0.8 MPa (Chauhan et al. 2018). Shrestha et al. (2018) observed higher water stress tolerance in *E. colona* as well as differences between glyphosate-resistant and glyphosate-susceptible populations, with germination reduced to 50% at −1.5 and −2.3 MPa for susceptible and resistant populations, respectively, and both populations exhibiting low germination at −5.5 MPa.

In this study, rinsing the −1.6 MPa–treated seeds with water caused some seeds to germinate the following week (Table 2). This suggests that the seeds enter a state of dormancy that can be relieved once the water stress is alleviated, as in the salt experiment. This is reflected in the species' natural germination cycle: seeds are typically dormant in dry summer months with low rainfall (high water stress) and germinate once rain becomes more common in late autumn. Therefore, when taken together with the results from the temperature experiment, which demonstrates *L. rigidum* can germinate over a range of temperatures, water availability has a greater impact on germination than temperature. While temperature influences the rate at which seeds lose dormancy (Steadman et al. 2003), nondormant seeds require sufficient water to initiate germination (Bewley 1997).

Effect of Light on Germination

A significant interaction was found between population and light treatment (P = 0.013). Germination percentage exceeded 90% for all populations when grown under light/dark conditions. This was significantly higher than the germination of seeds germinated in complete darkness for 28 d (P < 0.001) (Figure 4). Population CC04 had significantly lower germination compared with both GR (P = 0.019) and GS (P = 0.010) under dark conditions, but there was no difference between the GR and GS populations.

Exposing the seeds of the darkness-only treatment to alternating light/darkness for 14 d increased total germination for each population (Table 3), resulting in similar germination percentages compared with the light/dark-only treatment. This indicates that light plays a role in breaking dormancy of seeds and that varied dormancy levels exist within a population. However, germination

similar germination percentages of each population under salt stress, which is also a form of osmotic stress. The method that this population uses to resist the effects of high salt concentrations may not be linked with osmotic potential. Additionally, the chemical used to simulate osmotic stress (polyethylene glycol 8000) is a different type of compound than NaCl and is different in size, which may also affect the way in which seeds imbibe water in the presence of these different compounds.

The South Australian populations from Chauhan et al. (2006a) and the population (unknown location) from Rahman and Asaduzzaman (2019) retained the ability to germinate at −0.8 MPa, albeit at a low percentage. Chauhan et al. (2006a) found *L. rigidum* still germinated in 8% of seeds at −1.0 MPa. As such, it appears that some *L. rigidum* populations, like those from South Australia, can germinate under moderate water stress, while others have a more limited range, such as the populations in this study
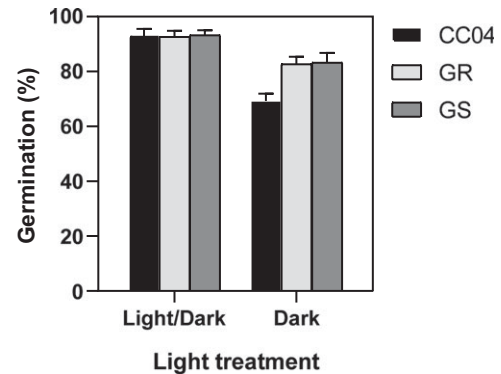
$$y = 85.8/[1 + (x/7.7)^{-1.3}]$$
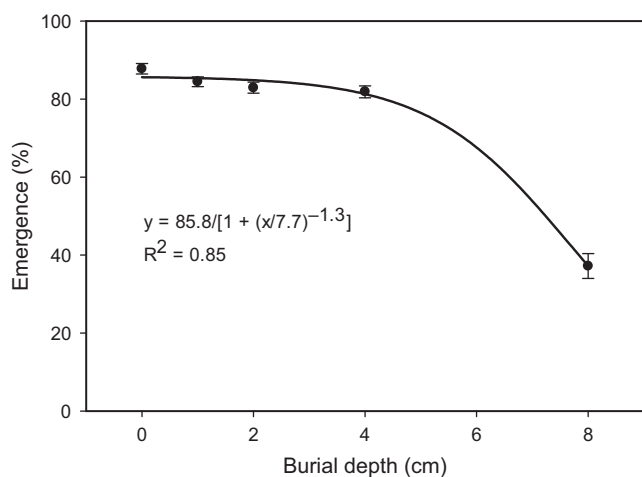$$R^2 = 0.85$$

Figure 5. Effect of burial depth on the seedling emergence of three populations of *Lolium rigidum* in a shadehouse. Data points are the means averaged from both experimental runs and for all populations. Vertical bars represent the SE.

percentages ranged from 69% to 83% in complete darkness, demonstrating that not all seeds of *L. rigidum* need light to germinate.

Light exposure is an important factor in the germination of seeds, but not all species have the same light requirements. Some species prefer to germinate in the presence of light, others in complete darkness, and yet other species may show no preference (Baskin and Baskin 1998). Our results indicate that *L. rigidum* prefers light but can still germinate in darkness. Chauhan et al. (2018) found that temperature played a role in the germination response of *C. truncata* to light and dark conditions. In this study, our light experiment was conducted at 25/15 C, and so these results may be different if tested at a lower or higher temperature. Light requirement may also change depending on the dormancy status of the seeds (Baskin and Baskin 1998). Germination in this study was tested on seeds from three populations in which most seeds had lost dormancy. As such, these results may only hold true for non-dormant seeds of these populations. In this study, germination of the GR population in response to light or darkness was no different from that of the GS population.

Effect of Burial Depth on Emergence

Burial depth had a significant effect on the emergence percent of all three populations ($P < 0.001$) (Figure 5). The highest emergence was recorded for all populations at 0 cm (soil surface). There was no significant difference observed among populations, as emergence was similar across each burial depth. The burial depth at which emergence was reduced to 50% was calculated as 7.7 cm when the populations were pooled together. Of the three populations, GS had the largest seeds with an average seed weight of 1.87 mg compared with 1.70 and 1.40 mg for CC04 and GR, respectively.

Our results for the burial depth experiment align with those of the light/dark experiment. Light was not essential for germination/seedling emergence, although fewer seeds germinated or emerged in dark conditions. Exposure to light may be the reason for greater germination on the soil surface. In the burial depth experiment, the seeds that did not emerge when buried at 8 cm were not placed in the light to check for emergence after light stimulation. Therefore, no comparison can be made with the transferred seeds from dark to light/dark in the laboratory experiment.

Studies on other weed species also found varying effects of burial depth on seedling emergence. Sweet signalgrass [*Brachiaria eruciformis* (Sm.) Griseb.] had 63% germination on the soil surface, which rose to 70% at 0.5 cm, before rapidly declining to 10% at 4 cm and no emergence at 8 cm (Mobli et al. 2020). Another weed, red sprangletop [*Dinebra panicea* (Retz.) P.M. Peterson & N. Snow], requires light to a far greater degree than *L. rigidum*. Weller et al. (2019) observed that this weed did not germinate if buried beyond 0.5 cm, much shallower than the 8 cm that *L. rigidum* emerged from in the present study.

South Australian populations from Chauhan et al. (2006a) demonstrated their greatest emergence at 1-cm burial depth (49%), while only 16% of seeds germinated on the soil surface. This is a large difference compared with the current study, which ranged from 87% to 89% germination on the soil surface and 84% to 85% at 1 cm, further suggesting population differences between those from South Australia and New South Wales or Victoria. It is important to note that the populations used in this study had greater germination overall than the population used in Chauhan et al. (2006a), for burial depth as well as salt and osmotic stress, although the seeds of that study could germinate over a wider range of osmotic potentials. This indicates the differences are population based and cannot solely be explained by variation in dormancy.

The effect that burial depth has on seedling emergence is also modified by soil factors like texture and compaction (Benvenuti and Mazzoncini 2019). Soil compaction, particularly due to clay particles in the soil, appears to induce a secondary dormancy that prevents seeds from germination in unfavorable conditions (Benvenuti and Mazzoncini 2019). As such, soil compaction, which may also affect the level of light that makes it through the soil, can play a role in determining seedling emergence. Tillage may help to break up the compaction or bring the seeds to the surface, breaking dormancy. Our results demonstrate the potential of this weed to germinate in the dark under field conditions such as under crop canopies or residues as well as being buried in the soil through tillage.

This study demonstrated the capacity for *L. rigidum* populations collected from New South Wales and Victoria to germinate over a wide range of alternating day/night temperatures. This indicates the potential of these populations to germinate throughout the year in areas of Australia such as Queensland, New South Wales, and Victoria, with all populations possessing high germination under 35/25 C. As such, temperature does not appear to restrict *L. rigidum* germination to the cooler seasons of autumn or winter. Results from this study also demonstrated that these populations can survive in high-salinity and water-stressed conditions and that some seeds can delay germination until more favorable conditions arrive, such as the alleviation of salt and water stress after rainfall. Thus, these populations could be highly competitive with crops in high-saline conditions. Water availability has a greater impact on germination than temperature. In this study, seeds germinated over a range of temperatures but were more sensitive to changes in salinity or osmotic stress, with germination improving in seeds incubated in 250 mM NaCl or −1.6 MPa when placed in 0 NaCl or 0 MPa solutions. Therefore, management strategies should be based on *L. rigidum* emerging after rainfall events regardless of temperature. Germination and emergence were the highest under alternating light/darkness; however, this study's populations could also germinate and emerge in dark conditions, as evidenced by the ability of the seeds to germinate in darkness in laboratory conditions and emerge from burial depths

of up to 8 cm in soil. Our results indicate that fewer seedlings will emerge after tillage; however, seedlings will still emerge. This study demonstrates that management strategies are required that consider the adaptability and widespread potential of these populations, which can survive and emerge under a range of environmental stresses. Therefore, more information is needed on the growth of this weed in different cropping situations, such as crop time of sowing or row spacing, as well as the effect of different cultivars on weed competitiveness. Due to increasing cases of herbicide resistance, herbicides cannot be relied upon as the main form of control. This study's results differed from those of previous studies on *L. rigidum*, which suggests differences in germination response between populations to different environmental variables. As such, management strategies may need to be targeted at specific populations. There was no indication that herbicide-resistance status caused different responses; however, more populations with known resistance need to be studied, particularly those from similar regions. If herbicide resistance confers no change in fitness, the management strategy should be targeted to the population rather than based on resistance status, with an emphasis on integrated weed management to reduce the selection pressure from herbicide use.

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