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Factors Affecting Seed Germination of Feather Fingergrass (*Chloris virgata*)

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Laboratory experiments were carried out to determine the effect of several environmental factors on seed germination of feather fingergrass, one of the most significant emerging weeds in warm regions of the world. Seed germination occurred over a broad range of temperatures (17/7, 25/10, and 30/20 C), but germination being highest at alternating temperatures of 30/20 C under both 12 h light/12 h dark and 24 h dark conditions. Although seed germination was favored by light, some seeds were capable of germinating in the dark. Increasing salt stress decreased seed germination until complete inhibition was reached at 250-mM sodium chloride. Germination decreased from 64 to 0.7% as osmotic potential decreased from 0 to –0.4 MPa, and was completely inhibited at –0.6 MPa. Higher seed germination (> 73%) was observed in the range of pH 6.4 to 8 than the other tested pH levels. Heat shock had a significant effect on seed germination. Germination of seeds placed at 130 C for 5 min was completely inhibited for both dry and presoaked seeds. The results of this study will help to develop protocols for managing feather fingergrass, and to thus avoid its establishment as a troublesome weed in economically important cropping regions.
Nomenclature: Feather fingergrass, *Chloris virgata* Sw.
Key words: Germination, heat, light, osmotic potential, salt stress, temperature, management protocols

Feather fingergrass (family: Poaceae) is a monocotyledonous, summer annual weed, which possesses C_4 photosynthesis mechanism. It has recently evolved from a minor roadside weed into one of the major concerns for cropping areas and in subtropical regions of Australia (Osten 2012). This emergent weed species has recently been identified as one of the top weeds in sorghum (*Sorghum* sp.) cropping systems in northern Australia. In addition, it is now recognized as a problematic weed in the vineyards and orchards in South Australia and in some parts of the Western Australian grain-growing regions (Osten 2012).

Feather fingergrass is distinguishable by its blue-green leaves and pale feathery panicles, together with its characteristic arrangement and furry appearance of the seeds (Osten 2012). Each mature feather fingergrass plant can produce up to 6,000 seeds, and because this can be done without cross pollination, it implies that a small population can grow and spread. The seeds are small, light in weight, with a triangular shape, and are easily shed from the heads making them good wind (animochory) and water (hydrochory) dispersers; they can be transported up to 13 m from mother plants by a moderate wind (Osten 2012). Because of the fine hairs on feather finger grass seeds, they have the ability to disperse in the agricultural systems by attaching to animal fur, agricultural equipment, and clothing. Furthermore, this invasive species has been identified as a host for aphids (Holman 2009), barley yellow dwarf and cereal yellow dwarf viruses (Hawkes and Jones 2005), and pathogenic nematodes, resulting in crop rotting and consequent reduced pasture yields (Holman 2009).

Seed germination is a key event in determining the success of a weed in an agroecosystem, and this process can be regulated by several environmental factors, such as temperature, photoperiod, soil salinity, pH, and moisture (Chachalis and Reddy 2000; Chauhan and Johnson 2008a). By understanding how environmental factors affect seed germination of feather fingergrass, better predictions can be made on its potential to spread, allowing the prioritization of best management practices. It is known that feather fingergrass is widely spread in grasslands in northern China, and mature plants are able to tolerate saline conditions (Zhang et al. 2012). This is an important observation, because salinity is considered to be a significant environmental issue in most cropping systems in the world. At this time, more than 60%

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of the 20 million ha cropping soils in Australia are sodic, and crops are generally grown on these soils under dryland conditions (Rengasamy 2010). Also, more than 80% of sodic soils in Australia have an alkaline pH (> 8.5) (Rengasamy 2002), but even though it has been reported that mature feather fingergrass plants can withstand high salinity and alkalinity (Li et al. 2009; Yang et al. 2009), the impacts of these conditions on seed germination are not well understood.

High rainfall has been reported to be associated with feather fingergrass population outbreaks (Pezzani and Montana 2006), suggesting that water is an important factor for seed germination. Feather fingergrass possesses C_4 photosynthesis mechanism; it has better water use efficiency than grasses with C_3 photosynthesis mechanism. Nevertheless, it is unknown how low soil moisture levels can affect seed germination of feather fingergrass. Feather fingergrass possesses C_4 photosynthesis mechanism; it has better water-use efficiency than grasses with C_3 photosynthesis mechanism. Also, as a subtropical species, feather fingergrass grows best at warmer temperatures between 20 and 30 C, with the optimal temperature being 25 C (Halvorson and Guertin 2003). Heat also plays a prime role in germination and acts as a signal triggering germination of seeds in several grass species (Ghebrehiwot et al. 2012). For example, heat caused by low-intensity fire greatly enhances the germination rates of C_4 subtropical grass species, such as kangaroo grass (*Themeda triandra* Forssk.), by breaking seed dormancy via seed scarification (Ghebrehiwot et al. 2012; Pezzani and Montana 2006). However, the effect of heat shock caused by low- and/or high-intensity fires on feather fingergrass seed germination has not been specifically studied.

Despite the increasing problem of feather fingergrass in Australian agroecosystems, until recently little was known about seed biology and the effects of selected environmental factors on seed germination. Understanding the seed ecology of this aggressive and newly emerging weed species is therefore essential to provide insights into developing integrated weed management plans to reduce invasion and impact on crops. In addition, better understanding of seed germination and emergence behavior of feather fingergrass would contribute to our ability to predict the potential of this species of spreading into new areas due to changes of climate and farming systems. Therefore, to contribute to better management strategies, the objectives of this study were (1) to determine the effect of light, temperature, salt and osmotic stress, and pH on germination of feather fingergrass seeds, and (2) to determine the effect of heat shock on germination of dry and presoaked seeds of feather fingergrass.

Materials and Methods

Seed Collection and Storage. Seeds of feather fingergrass were collected at maturity from approximately 50 plants on December 18 and 19, 2014 from several fields near Dalby-Jandowae road (27.090°S and 151.140°E), Queensland, Australia. Seeds were placed in a labeled paper bag and transported to the Seed Ecology lab of Federation University Australia, Mt. Helen, Australia. After seeds were dried at room temperature (25 C) for 1 wk, they were separated from chaff manually. Viable (dark colored) seeds were separated from unviable (pale colored) seeds and placed in a labeled dark airtight bottle and stored at room temperature (20 C) until needed (Zhang et al. 2012).

General Seed Germination Test Protocol. All experiments were done in 2015 at the lab in incubators (Thermoline Scientific and Humidity Cabinet, TRISLH-495-1-SD, Vol. 240, Australia) fitted with cool white fluorescent lamps that produced a photosynthetic photon-flux density of 100 μmol m^{-2} s^{-1}. Seeds were surface sterilized by soaking in 1% v/v sodium hypochlorite for 1 min, and then rinsed quickly in sterilized reverse osmosis (RO) water before the start of each germination trial, to avoid fungal growth on seeds. Seed germination was evaluated by placing 25 seeds evenly in a 9-cm diameter Petri dish containing a layer of Whatman No. 10 sterilized filter paper. They were then moistened with sterilized RO water or a treatment solution, and the filter papers were moistened every 2 d with relevant treatment solution. Petri dishes were wrapped with transparent parafilm to reduce evaporation. The temperature of the incubator was set at 30/20 C, with a 12-h photoperiod coinciding with the high temperature period unless otherwise specified. This 30/20 C temperature regime was found optimum in our initial study on the temperature regime and photoperiods for germination of feather fingergrass (Fernando, unpublished data). All the seeds were prepared under green safe light and to simulate complete dark period of 24 h; the Petri dishes were wrapped in two layers of aluminum foil. Also, all the seed germination counts were done under green safe light and sterilized forceps used to handle the

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seeds. Seeds were considered germinated when the radical was approximately 2 mm long and cotyledons had emerged from the seed coat (Ferrari and Parera 2015). The counting of the number of germinated seeds was started on the second day of the experiment, and then every other day for 45 d. Except the heat-shock experiment, all the other experiments were arranged in a randomized complete block design. Each experiment was conducted two times with three replicates. Each replication was arranged on different shelves in the cabinets and considered as a block. Heat-shock experiment was a factorial experimental design with two fixed factors (two seed treatments and six heat-shock levels) and experiments were conducted two times with three replicates. At the conclusion of the germination test, nongerminated seeds were checked for viability using the 2,3,5-triphenyltetrazolium chloride (TTC) test (Saatkamp et al. 2011; Waes and Debergh 1986). Seed germination percentages were calculated for each replicate, and reported germination percentages are presented as viability adjusted seed germination.

Effects of Temperature and Light. To determine the effects of temperature and light on seed germination, dishes containing 25 seeds were exposed to three alternating temperatures (17/7, 25/10, and 30/20 C) under two different periods of light conditions: complete dark (24 h dark) and alternating light and dark (12 h light/12 h dark). Other environmental conditions were the same as described in the general germination test.

Effect of Salt Stress on Germination. The effect of salinity on seed germination was determined by using sodium chloride (NaCl) solutions of 0, 25, 50, 100, 150, 200, and 250 mM. Sterilized filter papers (autoclaved at 121 C for 15 min) were dampened with relevant salt solutions, and the germination test proceeded at alternating day and night temperature of 30/20 C under 12 h light/12 h dark and 24 h dark.

Effect of Osmotic Stress on Germination. To examine the effect of osmotic stress on seed germination, aqueous solutions of osmotic potentials of 0, −0.1, −0.2, −0.4, −0.6, −0.8, −1.0, and −1.6 MPa were prepared with polyethylene glycol 8000 (Sigma-Aldrich Co., St. Louis, MO 63103) as described by Michel (1983). Sterilized filter papers were dampened with relevant solutions and the germination test was conducted at alternating day (12 h) and night (12 h) temperatures of 30/20 C.

Effect of pH on Germination. Buffer solutions with pH values of 4 to 10 were prepared according to the method described by Chachalis and Reddy (2000). A 2-mM solution of MES [2-(N-morpholino) ethanesulfonic acid] was adjusted to pH 4, 5, and 6 with 0.1 N hydrogen chloride (HCl) or sodium hydroxide (NaOH). A 2-mM solution of HEPES [N-(2-hydroxymethyl) piperaziine–N –(2-ethane sulfonic acid)] was adjusted to pH 7 and 8 with 0.1 N NaOH. Buffer solutions of pH 9 and 10 were prepared with 2-mM tricine [N-Tris (hydroxymethyl) methyl glycine] and adjusted with 0.1 N NaOH. Unbuffered deionized water (pH 6.4) was used as the control. Filter papers were dampened with relevant solutions and germination test was carried out at alternating day (12 h) and night (12 h) temperatures of 30/20 C.

Effect of Heat-Shock on Germination. The effect of heat shock on seed germination was tested in a factorial experimental design with two seed treatments and six heat-shock levels. Seeds were either used as dry or presoaked for 24 h in sterilized water before being used for the experiment. Twenty-five seeds were placed in each mesh bag and placed into a container and covered with pre-heated soil of 20, 40, 70, 100, 130, and 160 C. Then the containers with seeds were placed in heat chambers with relevant temperatures for 5 min and seed bags were removed from the soil. Seeds were then placed in Petri dishes with filter papers dampened with sterilized water and placed into the incubator at 30/20 C set temperature with 12-h photoperiod.

Statistical Analyses. Except for the heat-shock experiment, all the other experiments were arranged in a randomized complete block design, and were conducted two times with three replicates. The experimental design for heat-shock experiment was a factorial experimental design with two fixed factors (two seed treatments and six heat-shock levels). Replicates were considered as a random factor in both ANOVA analyses. According to Bartlett's test, homogeneity of variance was not improved by transformation of data; thus ANOVA was performed on nontransformed percent germination values (MINITAB 16 statistical software). Therefore, data from the repeated experiments were subjected to ANOVA and there were no significant time-by-treatment interaction ($P > 0.05$), so data were pooled for analysis. Means were separated using Fisher's protected LSD test at $P = 0.05$.

Viability adjusted germination (VAG), which is the total percentage of viable seeds that germinated

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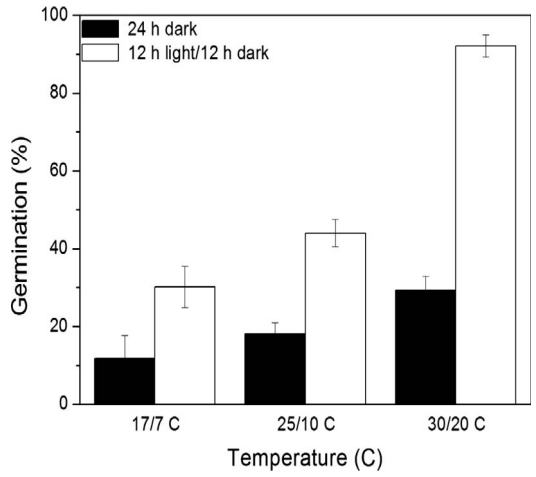

Figure 1. Effect of temperature (17/7 C, 25/10 C, and 30/20 C) on germination of feather fingergrass seeds under alternating light and dark (12 h light/12 h dark) and complete dark (24 h dark). Seeds were incubated for 45 d. Data represent the mean ± standard errors of the mean ($n = 6$).

per replicate, was calculated using the following formula:

$$\text{VAG} = \frac{N_{\text{seed\_germ}}}{(N_{\text{seed\_germ}} + N_{\text{viable\_non\_germ}})} \times 100, \quad [1]$$

where $N_{\text{seed\_germ}}$ = number of seeds germinated and $N_{\text{viable\_non\_germ}}$ = number of seeds that did not germinate, but were viable.

VAG percentage values at different NaCl concentrations were fitted to a two-parameter exponential decay model using SigmaPlot (Version 13).

The model fitted was

$$G(\%) = a \times e^{(-bx)} \quad [2]$$

where G is the total viability adjusted germination (%) at concentration x mM.

VAG percentage values at different osmotic potentials were fitted to a three-parameter sigmoid model with the use of SigmaPlot (Version 13). The model fitted was

$$G(\%) = a/\left[1 + e^{(-(x-x0)/b)}\right], \quad [3]$$

where G is the total viability adjusted germination (%) at osmotic potential of x MPa.

VAG percentage values for both heat-shock treatments (dried seed exposed to heat-shock, and pre-soaked seeds exposed to heat-shock) were fitted to three-parameter sigmoid model using SigmaPlot (Version 13). The model fitted was

$$G(\%) = a/\left[1 + e^{(-(x-x0)/b)}\right], \quad [4]$$

where G is the total viability adjusted germination (%) at temperature x C. The R^2 values were used to determine the goodness of fit to all selected models (Koger et al. 2004).

Results and Discussion

Effects of Temperature and Light on Germination. The interactive effect of light (light/dark and dark) and temperature (17/7, 25/10, and 30/20 C) was significant for seed germination of feather fingergrass. Seed germination in the light/dark regime was highest (92%) at the day/night temperature of 30/20 C (Figure 1). Similarly, seed germination in 24 h dark was highest (29%) at day/night temperature 30/20 C (Figure 1). Lowest seed germination was recorded at 17/7 C under both 12 h light/12 h dark and 24 h dark conditions. Germination was stimulated by presence of light at all the three temperature levels tested. It has been reported that the photoperiod regulates dormancy termination and the subsequent germination in many weed species (Batlla and Luis 2014). For seeds in the soil, the photoperiod represents important signals carrying essential information, cueing germination in the proper environmental situation. This photoperiod carries such information through its spectral composition and irradiance. These results suggest that feather fingergrass seed germination and subsequent emergence in the field will be favored by the presence of seeds at or near the soil surface. Similar responses to light were observed in junglerice [*Echinochloa colona* (L.) Link] (Chauhan and Johnson 2009) and Chinese sprangletop [*Leptochloa chinensis* (L.) Nees.] (Chauhan and Johnson 2008b). The previous studies reported that light strongly stimulated germination in both species and greater emergence of these species would be expected in no-till systems as most seeds remain on the soil surface after crop planting. In contrast, it has been reported that germination of seeds in the soil surface can also be inhibited by direct solar radiation (Bewley and Black 1994; Górski and Górska 1979). This inhibitory effect is supposed to be mediated by the high irradiance response, and it has been hypothesized that it might provide a mechanism for reducing seedling death due to the extreme high temperatures and dry soil conditions that are likely to occur at the soil surface (Górski and Górska 1979; Pons 2000). This type of response has been reported for many weed species; for example, common lambsquarters (*Chenopodium album* L.), catchweed bedstraw (*Galium aparine* L.),

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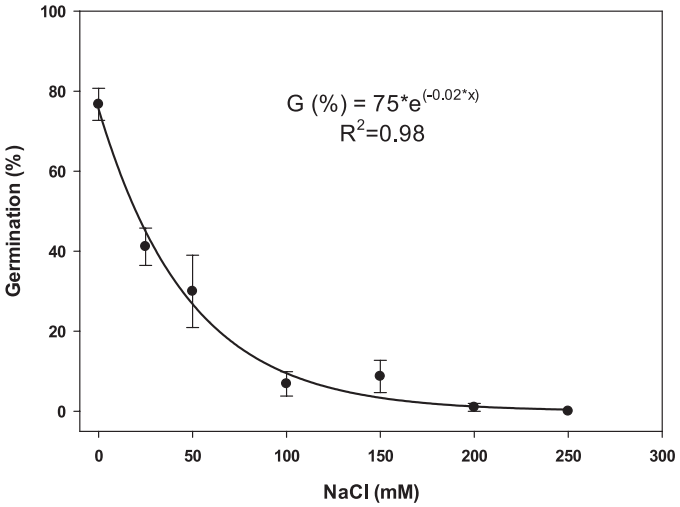

Figure 2. Effect of sodium chloride (NaCl) concentration on germination of feather fingergrass seeds incubated at 30/20 C day/night temperatures in 12-h photoperiod for 45 d. Line represents the two-parameter exponential decay model ($G[\%] = a \times e^{(-bx)}$) fitted to the data. Data represent the mean ± standard errors of the mean ($n = 6$).

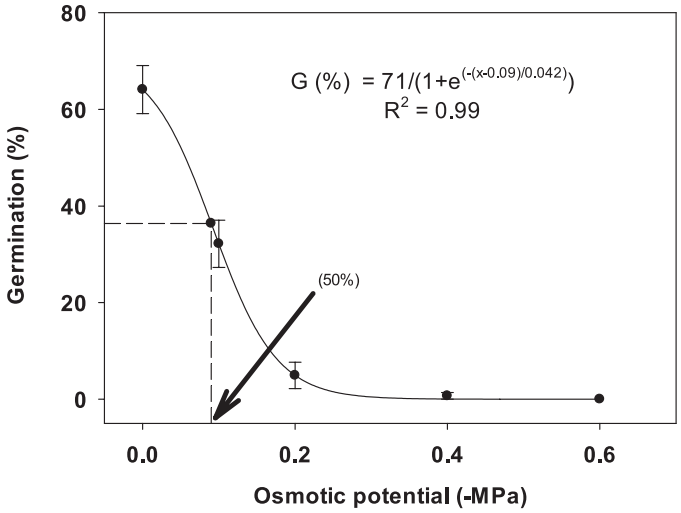

Figure 3. Effect of osmotic potential on germination of feather fingergrass seeds incubated at 30/20 C day/night temperatures in 12-h photoperiod for 45 d. Line represents the three-parameter sigmoid model $G(\%) = a/[1 + e^{(-(x - x0)/b)}]$ fitted to the data. The osmotic potential for 50% inhibition of the maximum germination, estimated from the fitted model, is indicated by the arrow. Data represent the mean ± standard errors of the mean ($n = 6$).

and foxtail amaranthus (*Amaranthus caudatus* L.) (Batlla and Luis 2014). Our study suggested that feather fingergrass has an increased ability to germinate at higher temperatures (30/20 C) when compared to lower temperature regimes. Consequently, a major reason behind the wide spread of feather fingergrass in the above warm areas in Australia may be their higher germination capacity under high temperatures with the presence of light.

Effect of Salt Stress on Germination. A two-parameter exponential decay model was best fitted to the seed germination percentage at different salt concentrations. The highest germination was 76.7% in the control (no salt stress), with seed germination being reduced with increasing salt concentration (Figure 2). Some seeds germinated at 150-mM NaCl (9%) and 200-mM NaCl (1%), but germination was completely inhibited at 250-mM NaCl (Figure 2). These results suggest that a small proportion of feather fingergrass may germinate under high soil salinity, and such soil types are common in many parts of Australia (Rengasamy 2002).

Salinity has the ability to inhibit seed germination, even under favorable climatic conditions (Kim and Park 2008). Salinity can reduce the biosynthesis of the hormone gibberellic acid, which is essential for breaking dormancy and controlling the growth of the seedling (Kim and Park 2008). Although the seeds did not germinate under high-salinity stress, the tetrazolium test demonstrated that the seeds did not lose their viability. This suggests that feather fingergrass seeds, like other halophyte species, could still germinate if the conditions returned to a more preferable state (Rasheed et al. 2015).

In a study conducted in China, feather fingergrass seed germination decreased with increasing salinity stress; however, the germination percentages were higher than the current study (Zhang et al. 2012). In the previous study, seed germination at 250-mM NaCl was around 70% at a constant temperature of 30 C. The discrepancy of higher salinity resistance of feather fingergrass grown in China may be due to constant experimental temperature (30 C), in addition to their genetic adaptability to the salinity conditions. However, our results suggest that feather fingergrass in Australia may not tolerate higher salinity levels like feather fingergrass grown in China, which has the ability to grow under high-salinity conditions.

Effect of Osmotic Stress on Germination. A three-parameter sigmoid model was fitted to the seed germination at different osmotic stress. Seed germination decreased from 64 to 0.7% as osmotic potential decreased from 0 to –0.4 MPa (Figure 3). Germination was completely inhibited at an osmotic potential of –0.6 MPa or lower (Figure 3). Our study shows that osmotic concentration required for 50% reduction in germination was –0.09 MPa.

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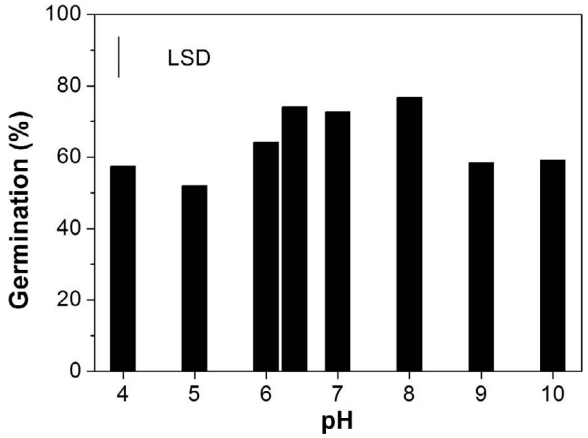

Figure 4. Effect of buffered pH solution on germination of feather fingergrass seeds incubated at 30/20 C day/night temperatures in 12-h photoperiod for 45 d.

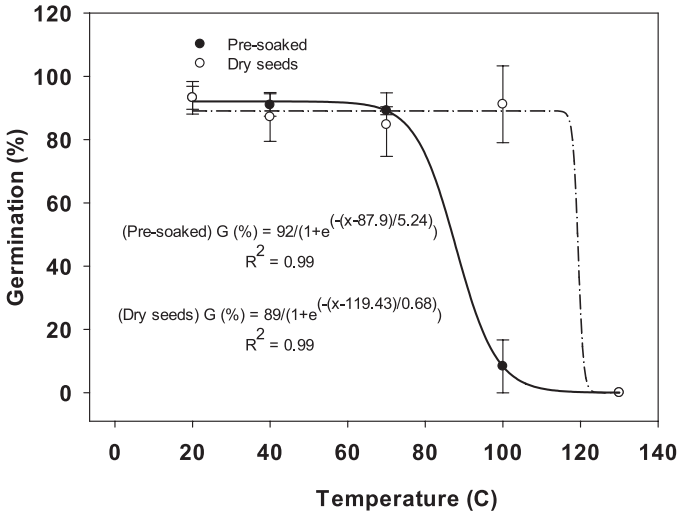

Figure 5. Effect of heat-shock on germination of feather fingergrass seeds incubated at 30/20 C day/night temperatures in 12-h photoperiod for 45 d. Continuous line with closed circles represents the three-parameter sigmoid model $G(\%) = a/(1 + e^{(-(x - x0)/b)})$ fitted to the germination percentage data for presoaked seeds. Dotted line with open circles represents the three-parameter sigmoid model $G(\%) = a/(1 + e^{(-(x - x0)/b)})$ fitted to the germination percentage data for seeds without presoaking. Data represent the mean ± standard errors of the mean ($n = 6$).

The results indicate that feather fingergrass may not germinate under drought stress, because it is a weed favored by a moist environment. The tetrazolium test, performed on the nongerminated seeds in the osmotic potential experiments (data not shown), demonstrated that the seeds remained viable at all the osmotic potential levels tested. These indicate that feather fingergrass seeds can remain dormant during drought periods, and potentially germinate with the next high rainfall event contributing to the development of high population densities in arid zones.

Effect of pH on Germination. Feather fingergrass seed germination averaged 64% in the range of pH 6.4 to 8, with the highest germination rate (76%) being recorded at pH 8 (Figure 4). Further results indicated that germination rates declined as the pH decreased from 5 to 4, and as the pH increased from 9 to 10 (Figure 4). This observation of high seed germination potential over a broad range of pH indicates that pH should not be a limiting factor for seed germination in most soil conditions. Feather fingergrass is known as a naturally alkali-tolerant halophyte, which naturally occurs on highly alkaline soils of pH >10, and can even colonize bare alkaline patches as a pioneer species in degraded semiarid grasslands in China (Li et al. 2009).

Effect of Heat-Shock on Germination. Seed germination was significantly affected when presoaked seeds were exposed to heat shock ($P < 0.001$) (Figure 5). Whereas seed germination of presoaked seeds when exposed to 40 and 70 C was not significantly different to the control (Figure 5), when the temperature was increased to 100 C, seed germination was sharply reduced to 8% and completely inhibited at 130 C (Figure 5). Similarly, seed germination was significantly affected when dry feather fingergrass seeds were exposed to the heat-shock treatment ($P < 0.001$) (Figure 5). The temperature for 50% inhibition of the maximum germination, estimated from the fitted models (Equation 3) were 87.9 C for the presoaked seeds and 119.4 C for the dry seeds. Seed germination of dry seeds exposed to 40, 70, and 100 C were not different than the control treatment and showed germination above 85% (Figure 5). Similar to presoaked seeds, dry seed germination was also completely inhibited when exposed to 130 C, which indicates that if the soil surface temperature is increased 130 C for a short time (minimum 5 min), the seed viability of feather fingergrass will be completely lost. By comparison, studies have shown that to guarantee the death of rigid ryegrass (*Lolium rigidum* Gaud.) seeds (one of the most troublesome weeds in the world) requires temperatures in excess of 400 C for at least 10 s (Walsh and Newman 2007). A narrow windrow burning system is currently the most widely adopted harvest weed seed control system in Australia, as it is a simple and effective mechanism (Walsh et al. 2013). As our study demonstrated that germination of feather fingergrass seeds is completely inhibited at 130 C, seeds on the

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soil surface should be easily destroyed by a wildfire and/or narrow windrow burning.

In conclusion, among the factors tested on the germination success of feather fingergrass seeds, light, salinity, osmotic potential appeared as the critical factors. As light stimulates the feather fingergrass seed germination in each temperature regime tested compared to dark, practices such as seed burial by tillage and/or crop residue as mulch might be helpful for successful management of feather fingergrass. Of particular interest is the observation that seeds remain viable under osmotic and salt stress conditions, and are potentially able to germinate if favorable conditions are returned. However, these seeds did not remain viable at temperatures over 130 C, which suggests that fire and/or narrow windrow burning with enough stubble to increase the top soil temperature higher than 130 C can be an effective mechanism for soil seed removal. As feather fingergrass has become a significant weed in agricultural systems worldwide, and especially in Northern Australia and recently in South and Western Australia, these findings will help in the implementation of management strategies for feather fingergrass.

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