Weed Science

www.cambridge.org/wsc

Germination biology of four climatically varied populations of the invasive species African lovegrass (*Eragrostis curvula*)

Jason Roberts[1], Singarayer Florentine[2], Eddie van Etten[3] and Christopher Turville[4]

[1]BSc (Honors) Graduate, Centre for Environmental Management, School of Health and Life Science, Federation University, Mount Helen, Victoria, Australia; [2]Professor, Centre for Environmental Management, School of Health and Life Science, Federation University, Mount Helen, Victoria, Australia; [3]Senior Researcher, Centre for Ecosystem Management, School of Science, Edith Cowan University, Joondalup, Western Australia, Australia and [4]Senior Researcher, School of Engineering, Information Technology and Physical Sciences, Federation University, Mount Helen, Victoria, Australia

Research Article

Cite this article: Roberts J, Florentine S, van Etten E, Turville C (2021) Germination biology of four climatically varied populations of the invasive species African lovegrass (*Eragrostis curvula*). Weed Sci. **69**: 210–218. doi: 10.1017/wsc.2020.99

Received: 13 October 2020
Revised: 13 December 2020
Accepted: 21 December 2020
First published online: 12 January 2021

Associate Editor:
Nathan S. Boyd, Gulf Coast Research and Education Center

Keywords:
Invasive; management; seed germination; weeping lovegrass

Author for correspondence:
Singarayer Florentine, Centre for Environmental Management, School of Health and Life Science, Federation University, Mount Helen, Victoria 3350, Australia.
(Email: s.florentine@federation.edu.au)

Abstract

African lovegrass [*Eragrostis curvula* (Schrad.) Nees] is a highly invasive C_4 perennial grass that threatens global biodiversity. Appropriate management of this species has been hampered by a lack of knowledge concerning its seed ecology, resulting in significant economic and environmental impacts within various environments. Consequently, this study explored the effects of a selection of environmental factors (photoperiod, alternating temperature, pH, and salinity) by analyzing several measures of germination on four geographically distinct populations of *E. curvula* to assist in its extirpation from infested sites. Seeds were collected in Australia from Maffra and Shepparton, VIC; Tenterfield, NSW; and Midvale, WA. Key results showed that seeds from Maffra (54% vs. 79%), Tenterfield (38% vs. 61%), and Shepparton (34% vs. 71%) had significantly reduced germination in complete darkness compared with an alternating 12-h light and 12-h dark photoperiod, whereas Midvale had consistent germination (91% vs. 99%). Temperatures between 17/7 C reduced germination for Maffra (42% vs. 73%), Tenterfield (34% vs. 55%), and Shepparton (33% vs. 59%) compared with the mean of all other temperature combinations, whereas Midvale had consistent germination. Furthermore, germination for all populations was consistent between pH 4 and 9. For salinity, germination was significantly reduced at ≥100 mM for Maffra (29% vs. 67%), ≥150 mM for Tenterfield (29% vs. 94%) and Shepparton (39.5% vs. 81.5%), and 250 mM for Midvale (39% vs. 82%) compared with the mean of all other concentrations. Although each trial was conducted independently, the data can be used to generate species-targeted management. Such strategies include maintaining high levels of quarantine and hygiene programs to avoid future spread; where practical, applying light-limiting strategies (mulching, tilling, or scraping) for the Maffra, Tenterfield, and Shepparton populations; and maintaining management efforts year-round, as the species can germinate under a wide range of conditions.

Introduction

African lovegrass [*Eragrostis curvula* (Schrad.) Nees] is a highly invasive weed that is found within Australia and around the world, posing a serious threat to native biodiversity (Firn 2009). It has the capacity to quickly inundate landscapes and compete against native flora for resources such as light, nutrients, and soil moisture (Firn et al. 2018). Many animals (native and nonnative) avoid grazing this grass, which in addition to being unpalatable, provides little or no dietary benefit. This results in dense swards of *E. curvula* forming in grazed grass-dominated vegetation, altering the carrying capacity of an area, intensifying competition with other flora, and increasing fuel loads, which ultimately changes the frequency of fire throughout the landscape (Firn 2009; Firn et al. 2018). Although many management techniques have been explored, such as burning (Archibald et al. 2005), heavy grazing (Firn 2009), and herbicide application (Campbell et al. 1987; Firn et al. 2018), there has been little long-term success in controlling this weed.

To date, there has been no comprehensive study that investigates the seed ecology of *E. curvula* from multiple populations. However, it is known that investigating the seed ecology of any weed species can provide useful information for improved management by identifying what factors may inhibit or enhance its growth and future seed production (Ahmed et al. 2015; Schwartz et al. 2017). Many weed species can adapt remarkably well to different environmental conditions, an attribute often linked to genetic variability and ability to adapt to localized climatic and soil conditions (Geng et al. 2016; Hereford 2009; Seglias et al. 2018). This allows them to easily invade new areas across a wide area. Therefore, understanding the environmental

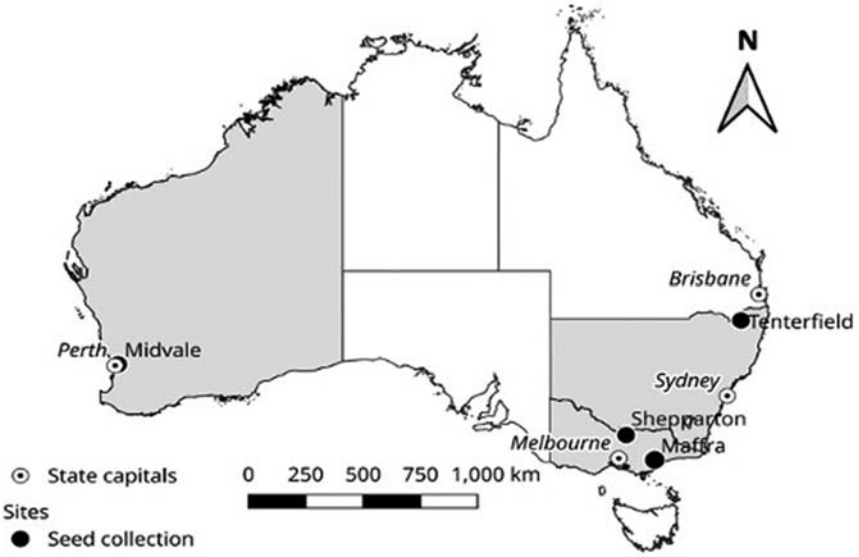

Figure 1. *Eragrostis curvula* seed collection sites, Australia.

factors that regulate seed germination is essential in formulating species-targeted management for greater efficacy in control (Chauhan et al. 2018; Mobli et al. 2020). Consequently, this study is innovative in that it investigated several environmental factors (photoperiod, alternating temperature, pH, and salinity) by analyzing several measures of seed germination on multiple climatically and spatially varied populations within Australia from Maffra (mild temperate climate) and Shepparton (hot dry summer–cool winter climate) VIC; Tenterfield, NSW (mild temperate climate); and Midvale, WA (warm temperate climate). Investigating which environmental factors influence this species' seed germination can provide information to be applied in a new holistic approach to managing this species at a landscape scale.

Methods and Materials

Seed Collection, Site Description, and Seed Storage

Mature *E. curvula* seeds were collected using haphazard sampling methods between January and March 2019 from >100 individual plants from each of four localities across Australia: Maffra (37.925°S, 146.996°E) and Shepparton (36.348°S, 145.368°E) in Victoria; Tenterfield (29.093°S, 152.003°E) in New South Wales; and Midvale (31.872°S, 116.033°E) in Western Australia (Figure 1). These localities were chosen to represent genetic diversity of the species from several spatially varied climatic regions in Australia (Table 1). Studying multiple populations allows for a greater understanding of the species' seed ecology and can also identify any potential adaptations as a result of a difference in climatic conditions or selective pressures (Gamba and Muchhala 2020). Seeds collected from all localities were carefully sealed within labeled paper bags and transported to Federation University's seed ecology lab where all trials took place. Seeds were then air-dried for a week, cleaned, and stored in airtight containers at room temperature until trials began in late March 2019.

Seed Germination Protocol

All seed germination trials were conducted at Federation University's seed ecology laboratory using the following protocol. To remove the influence of any pathogens, all seeds were carefully surfaced sterilized using 1% sodium hypochlorite for 2 min and then thoroughly rinsed with sterilized reverse-osmosis water (RO water). For each individual treatment, three replicates (repeated twice) of 20 seeds (per population) were placed evenly into a 9-cm petri dish lined with sterilized filter paper (Whatman® No.10). Seeds for each replicate were randomly selected from the batch of seeds collected from their corresponding location. Each filter was then moistened with approximately 10 ml of RO water or another specified solution. Petri dishes were then wrapped in transparent Parafilm® and placed randomly into temperature and light incubators (Thermoline Scientific and Humidity Cabinet, TRISLH-495-1-SD, Volume 240, Wetherill Park, NSW, Australia) fit with white fluorescent lamps with a photosynthetic photon flux of 40 μmol m^{-2} s^{-1}. Seeds were monitored for 30 d and were considered germinated when the radicle had emerged and reached approximately 2 mm in length (Ferrari and Parera 2015). All nongerminated seeds were then assessed for viability using the triphenyl tetrazolium chloride test (Waes and Debergh 1986). For the 24-h dark trials, petri dishes were wrapped with a double layer of aluminum foil and only examined under a green safe light to avoid any potential photoreaction with the seeds.

Effect of Photoperiod and Alternating Temperature

Seeds from all populations were exposed to two photoperiod regimes (12-h light/12-h dark, and 24h darkness) and incubated under four alternating temperature combinations on 12-h cycles (35/25, 30/20, 25/15, and 17/7 C). These combinations were selected as they correspond approximately with the climatic conditions that occur during the growing season of the species within southern Australia between spring to summer (Borger et al. 2019). The most efficient combination for germination identified within this study was under the alternating 12-h light and 12-h dark photoperiod at 30/20 C. Therefore, all subsequent trials were conducted under this temperature and photoperiod combination.

Effect of pH

The effect of pH on germination was investigated by exposing seeds to a range of pH buffer solutions between 4 to 10, which are representative of average Australian soils (De Caritat et al.

https://doi.org/10.1017/wsc.2020.99 Published online by Cambridge University Press

Table 1. Site description of the seed locations of *Eragrostis curvula* seeds from Australia.

Site location:	Maffra (Victoria)	Tenterfield (New South Wales)	Shepparton (Victoria)	Midvale (Western Australia)
Bioregion:	Gippsland Plains	New England Tableland	Victoria Riverina	Swan Coastal Plain
Climate:	Mild temperate	Mild temperate	Hot dry summer with cool winter	Warm temperate
Land use and description:	Heavily grazed, open plains woodland with current and previous grazing	Open disturbed, grassy woodland with current and previous grazing	Disturbed, open plains woodland with previous grazing, although not presently being grazed	Disturbed, open woodland with previous grazing, although not presently being grazed
Vegetation at the site:	Dense understory of *Eragrostis curvula* with several *Eucalyptus* species of different ages such as yellow stringybark (*Eucalyptus muelleriana* A.W. Howitt) and red box (*Eucalyptus polyanthemos* Schauer)	Dense understory of *Eragrostis curvula* with scattered native grasses including kangaroo grass (*Themeda triandra* Forssk.) and barbed wire grass [*Cymbopogon refractus* (R. Br.) A. Camus]	Scattered understory of *Eragrostis curvula* with several native grasses including wallaby grass (*Rytidosperma caespitosum* [Gaudich] Connor & Edgar) and spear grass [*Heteropogon contortus* (L.) P. Beauv. ex Roem. & Schult.]	Moderately dense understory of *Eragrostis curvula* with several native tree species including Marri [*Corymbia calophylla* (Lindl.) K.D. Hill & L.A.S. Johnson] and flooded gum (*Eucalyptus rudis* Endl.)
Soil type:	Alluvial trace deposits (sandy gravels) with good drainage	Granite intrusions and sandy gravels with good drainage	Gravels, clays, and sandy soils with good drainage	Yellow duplex soils with sandy loam topsoil with good drainage
Average yearly rainfall (mm):	529 (Figure 2A)	847 (Figure 2B)	492 (Figure 2C)	781 (Figure 2D)
Average yearly temperature (C):	17–23 (Figure 2A)	19–25 (Figure 2B)	17–25 (Figure 2C)	21–28 (Figure 2D)

2011). Solutions were prepared and used to moisten the filter paper according to the procedures outlined by Chachalis and Reddy (2000): for pH 4, 2 mM of potassium hydrogen phthalate solution was prepared and adjusted using 1 N hydrogen chloride; for pH 5 and 6, 2 mM of 2-(*N*-morpholino) ethanesulfonic acid was adjusted using 1 N of sodium hydroxide (NaOH) solution; for pH 7 and 8, 2 mM of HEPES [*N*-(2-hydroxymethly) piperazine-*N*-(2-ethanesulfonic acid)] solution was adjusted using 1 N NaOH; and for pH 9 and 10, tricine [*N*-Tris (hydroxymethyl) methylglycine] was adjusted using 1 N of NaOH.

Effect of Salinity

The effect of salinity on seed germination was determined using the following sodium chloride (NaCl) solutions: 0 (control), 25, 50, 100, 150, 200, and 250 mM. Solutions were used to moisten filter paper and were made by using the following concentrations of analytical reagent grade NaCl dissolved in 250 ml of RO water: 0, 0.360, 0.731, 1.461, 2.192, 2.922, and 3.653 g, respectively. The range of NaCl solutions was chosen to represent the conditions of Australian soils where this species has the potential to occur (Cook and Dias 2006).

Statistical Analysis

Statistical tests to measure the effects of various environmental factors (photoperiod, light, pH, and salinity) on *E. curvula* seed germination were conducted using SPSS statistical software (v. 23, IBM, Armonk, NY, USA). The following measures were used to compare any differences in the means of each treatment and population if they occurred: final germination percentage (FGP), mean germination time (MGT), germination index (GI), time to reach 50% germination (T_{50}), and time to start germination (T_{SG}) (Kader 2005). The differences in the levels of each factor, population, and their interactions were compared using ANOVAs and relevant post hoc tests (e.g., Tukey's test). Significant interactions from the ANOVAs were analyzed by investigating the simple main effects with Bonferroni adjustments. For each test constructed, the assumption of normality of the residuals were investigated. To address the large number of ANOVAs constructed for the various measures and treatments, only results with a $P < 0.001$ were used to identify any significant difference. This cutoff value was chosen to address some slight departures from the assumptions while also addressing any concerns over inflated type I errors. To determine the optimal growing conditions of the species, a three-way ANOVA with interactions was conducted by comparing the final germination percentage of each population across all photoperiod and temperature regimes examined.

The following formulae were used to determine various measures as shown below:

$$\text{Final germination percentage} = \frac{\text{NSG}}{\text{TNS}} \times 100 \quad [1]$$

where NSG is the final number of seeds germinated and TNS is the total number of seeds before germination.

$$\text{Mean germination time} = \left(\frac{\sum Dn}{\sum n}\right) \quad [2]$$

where n is the seeds germinated on day D and D_n is the total number of days from the beginning of the trial.

$$\text{Germination index} = \left(\frac{n}{\text{day of first count}}\right) + (\ldots) + \left(\frac{n}{\text{day of final count}}\right) \quad [3]$$

where GI is the final germination index, and n is the number of germinated seeds.

https://doi.org/10.1017/wsc.2020.99 Published online by Cambridge University Press

$$\text{Time taken to reach 50\% germination} = t_i \frac{(\frac{n}{2} - n_i)(t_j - t_i)}{(n_j - n_i)} \quad [4]$$

where n is the total number of seeds germinated, n_j and n_i are the collective number of seeds germinated at the counts at days t_j and t_i.

Time to start germination (T_{SG}) was calculated by averaging the days taken for seeds to start germinating.

Results and Discussion

Effect of Photoperiod and Alternating Temperature

Collectively, key results from this study show that *E. curvula* seed germination was greatest under the 12-h light and 12-h dark photoperiod at 30/20 C. For the effect of photoperiod, there was a significant interaction between the population and photoperiod for the FGP and GI (Table 2). Relevant post hoc tests indicated that germination was significantly lower for Maffra, Tenterfield, and Shepparton under the 24-h dark photoperiod compared with the 12-h light and 12-h dark photoperiod for the FGP (52% vs. 79%, 38% vs. 61%, 34% vs. 71%, respectively), and GI (1.3 vs. 2.4, 0.6 vs. 1.4, 0.8 vs. 2.1, respectively). However, Midvale had a consistent FGP (91% vs. 99%) and GI (3.6 vs. 3.8) for both photoperiods. Furthermore, the effect of photoperiod was not significant for the MGT, T_{50}, and T_{SG}.

With alternating growth temperatures, there was a significant interaction between the population and temperature for all measures (Table 2). Relevant post hoc tests indicated that Maffra, Tenterfield, and Shepparton had significantly lower or longer germination at 17/7 C compared with the means of all other temperature combinations for the FGP (43% vs. 73%, 34% vs. 55%, 33% vs. 59%, respectively), GI (0.7 vs. 2.2, 0.3 vs. 1.3, 0.4 vs. 1.8, respectively), MGT (15.4 d vs. 6.6 d, 15.1 d vs. 5.4 d, 12.4 d vs. 6.8 d, respectively), T_{50} (14.6 d vs. 4.1 d, 14.2 d vs. 4.7 d, 11.2 d vs. 5.6 d, respectively), and T_{SG} (12.8 d vs. 4.8 d, 14.5 d vs. 4.6 d, 11.1 d vs. 5.1 d, respectively). However, the germination for Midvale was consistent for all measures across all temperature combinations.

There was only a single photoperiod and temperature interaction, seen for the FGP (Table 2). Post hoc tests indicated that across all populations, the mean FGP was significantly lower under the 24-h dark photoperiod compared with the 12-h light and 12-h dark photoperiod (55% vs. 77%). It also showed that the mean FGP (56% vs. 84%) under the 12-h light and 12-h dark photoperiod was significantly lower at 17/7 C compared with all other temperature combinations. However, under the 24-h dark photoperiod, the mean FGP was significantly higher at 30/20 C (64%) compared with 25/15 (49%) and 17/7 C (46%).

Light is an important factor that regulates seed germination, and it can be mediated by the position of a seed within the soil and any surrounding shadow competition (Batlla and Benech-Arnold 2014). Results from this study identify seeds from Maffra, Tenterfield, and Shepparton as photoblastic. Seeds often require greater amounts of energy and resources when buried within soil, which prevents germination until they are moved closer to the surface or when resources are widely available (Milberg and Lamont 1995). This pattern of germination has also been observed in several small-seeded species such as South African lovegrass (*Eragrostis plana* Nees) (Bittencourt et al. 2017), feather lovegrass [Eragrostis tenella (L.) P. Beauv. ex Roem. & Schult.] (Chauhan 2013), and goosegrass [Eleusine indica (L.) Gaertn.] (Chauhan and Johnson 2008). In contrast, seed germination can also occur in complete darkness by the seed utilizing a switch from photomorphogenetic to skotomorphogenic development (Josse and Halliday 2008). This results in the allocation of resources to hypocotyl elongation, cotyledon growth, and root development, all aimed at seeking light at an accelerated rate (Josse and Halliday 2008). It is clear from this study that seeds from Midvale did not have reduced germination in complete darkness. This may be a result of localized adaptation. This population was sourced from a diverse open woodland with several larger species of different ages (Table 1). Therefore, increased competition and ground cover may have resulted in the population evolving to produce seeds better adapted to light-limited conditions. However, seeds from the other populations were sourced from an open disturbed grassland with less competition, so this selective pressure may not be as significant.

Temperature is also a significant factor regulating seed germination (Baskin and Baskin 2014). Seasonal changes in temperature and light availability can influence the rate of germination by altering the availability of resources (nutrients, and soil moisture) and increasing seed deterioration, all factors that may have dormancy-breaking effects on the seed (Kebreab and Murdoch 1999). Results from the present study highlight that seed germination was lower and took longer at temperatures between 17/7 C for Maffra, Tenterfield, and Shepparton. This pattern of germination is also observed in two other *Eragrostis* species, *E. plana* (Bittencourt et al. 2017) and *E. tenella* (Chauhan 2013). In contrast, many species can also germinate across a wide range of temperatures, allowing them to invade a greater range of environments (Burke et al. 2003). Seeds from Midvale were not directly influenced by temperature; this suggests a potential localized adaptation or difference in the original seed source. The climate in this region is warmer, and most of its rainfall occurs in winter, in contrast to Maffra, Tenterfield, and Shepparton, which have cooler temperatures and rainfall distributed more evenly throughout the year (Table 1; Figure 2). Therefore, seeds from Midvale may have adapted to respond to such conditions, or germination could be strongly associated with other environmental factors, such as soil moisture.

Effect of pH

Key results from this study show that *E. curvula* seeds from all populations investigated within this study can germinate successfully between pH 4 to 9. For the effect of pH, there was a significant interaction between the population and pH for most measures (Table 3). Only the Maffra population showed significant variation in its germination, and post hoc tests indicated that germination was significantly lower at pH 10 compared with the means of all other pH values for the FGP (59% vs. 85%) and GI (1.4 vs. 2.8). Further, Maffra also had a significantly longer mean MGT (10.5 d vs. 5.5 d) at pH 8 to 10 and mean T_{50} (8.9 d vs. 4.8 d) at pH 9 to 10 compared with all other pH values. For all populations, the effect of pH did not influence the time to start germination (T_{SG}). For the Tenterfield, Shepparton, and Midvale populations, the effect of pH did not significantly influence germination or germination time.

For many weed species, being able to tolerate a wide range of soil pH is beneficial, as it allows them to exploit a wider range of soil types (Humpheries et al. 2018). This trait allows weeds to invade a greater range of environments and has been described in several other weed species, including American sloughgrass [*Beckmannia syzigachne* (Steud.) Fernald] (Rao et al. 2008) and serrated tussock [*Nassella trichotoma* (Nees) Hack.] (Humpheries et al. 2018). In contrast, a higher pH can inhibit germination, as it can indicate a change in soil conditions and potentially a change in the available

https://doi.org/10.1017/wsc.2020.99 Published online by Cambridge University Press

Table 2. The effect of photoperiod and alternating temperature on *Eragrostis curvula* seed germination.[a]

Measure	Photoperiod	Temperature —C—	Population				Significant interactions
			Maffra	Tenterfield	Shepparton	Midvale	
Final germination percentage (FGP)	24-h dark	35/25	66.2 aAC	37.3 aB	42.7 aAB	79.0 aC	Population*temperature (df = 9, $F = 6.6$, $P < 0.001$)
		30/20	62.3 aA	49.3 aA	43.0 aA	94.8 aB	
		25/15	46.7 abA	35.8 aA	22.3 bA	93.8 aB	
		17/7	32.3 bA	29.2 aA	26.8 bA	96.3 aB	
	12-h light/12-h dark	35/25	85.8 aAB	64.7 aB	79.3 aB	100.0 aA	
		30/20	94.8 aA	74.0 aA	86.7 aA	100.0 aA	
		25/15	85.8 aAB	64.7 aA	77.0 aAB	99.1 aB	
		17/7	50.8 bB	38.3 bB	39.8 bA	98.3 aB	
	24-h dark		51.9 aA	37.9 aB	33.7 aB	91.0 aC	Population*photoperiod (df = 3, $F = 13.3$, $P < 0.001$)
	12-h light/12-h dark		79.3 bA	60.4 bB	70.7 bAB	99.4 aC	

Temperature	Photoperiod		Significant interactions
	24-h dark	12-h light/12-h dark	
35/25	56.3 abA	82.5 aB	Photoperiod* temperature (df = 3, $F = 7.8$, $P < 0.001$)
30/20	62.4 bA	88.9 aB	
25/15	49.7 aA	81.2 aB	
17/7	46.2 aA	56.3 bB	

Treatments	Photoperiod	Temperature —C—	Population				Significant interactions
			Maffra	Tenterfield	Shepparton	Midvale	
Germination index (GI)	24-h dark	35/25	2.2 aA	0.82 aB	1.4 aAB	3.6 aC	Population*temperature (df = 9, $F = 2.4$, $P < 0.001$)
		30/20	1.8 aA	0.80 aA	1.1 aA	4.1 aB	
		25/15	0.90 abA	0.62 aA	0.34 aA	3.7 aB	
		17/7	0.40 bA	0.31 aA	0.33 aA	2.9 aB	
	12-h light/12-h dark	35/25	3.4 aA	1.8 aB	3.5 aA	4.8 aC	
		30/20	2.9 aAB	1.9 aA	2.4 abA	3.8 abB	
		25/15	2.5 aAB	1.5 abA	1.8 bcA	3.5 abB	
		17/7	0.96 bA	0.29 bA	0.53 cA	2.8 bB	
	24-h dark		1.3 aA	0.64 aB	0.82 aAB	3.6 aC	Population*photoperiod (df = 3, $F = 8.4$, $P < 0.001$)
	12-h light/12-h dark		2.4 bA	1.4 bB	2.1 bA	3.8 aC	
Mean germination time (MGT) (d)	24-h dark	35/25	5.3 aA	5.8 aA	4.7 aA	4.2 aA	Population*temperature (df = 9, $F = 3.9$, $P < 0.001$)
		30/20	6.2 aA	6.0 aA	7.6 abA	4.9 aA	
		25/15	9.1 aA	6.6 aA	10.1 abA	5.3 aA	
		17/7	15.7 bA	18.2 bA	11.1 bA	6.5 aB	
	12-h light/12-h dark	35/25	5.4 aA	4.2 aA	3.9 aA	4.5 aA	
		30/20	6.1 aA	4.5 aA	6.7 abA	5.3 aA	
		25/15	7.5 aA	5.4 aA	8.3 abA	5.6 aA	
		17/7	15.2 bA	12.0 bA	13.6 bA	7.4 aB	
Time to reach 50% germination (T_{50}) (d)	24-h dark	35/25	4.5 aA	4.7 aA	2.8 aA	3.5 aA	Population*temperature (df = 9, $F = 4.2$, $P < 0.001$)
		30/20	4.9 aA	5.4 aA	5.1 abA	4.9 aA	
		25/15	9.1 aA	6.6 aA	10.1 bcA	5.3 aA	
		17/7	14.2 bA	17.6 bA	10.7 cA	5.2 aB	
	12-h light/12-h dark	35/25	4.3 aA	2.7 aA	3.4 aA	3.3 aA	
		30/20	4.9 aA	4.1 aA	5.6 abA	5.1 aA	
		25/15	5.7 aA	4.8 aA	5.9 bcA	5.2 aA	
		17/7	14.9 bA	10.7 bA	11.7 cA	6.4 aB	
Time to start germination (T_{SG}) (d)	24-h dark	35/25	4.0 aA	5.2 aA	3.0 aA	3.0 aA	Population*temperature (df = 9, $F = 4.1$, $P < 0.001$)
		30/20	4.0 aA	5.3 aA	4.5 aA	3.0 aA	
		25/15	6.8 aA	5.8 aA	9.2 abA	3.3 aA	
		17/7	14 bA	18.0 bA	10.6 bA	5.0 aB	
	12-h light/12-h dark	35/25	4.0 aA	3.0 aA	3.0 aA	3.0 aA	
		30/20	4.0 aA	3.5 aA	5.0 aA	3.0 aA	
		25/15	5.7 aA	4.8 aA	6.0 abA	4.5 aA	
		17/7	11.5 bA	11.0 bA	11.5 bA	5.0 aB	

[a]For each measure, values (means) that run down each column (within that population) and have the same lower-case letter are not significantly different at $P < 0.001$. Also, for each measure, values (means) that run across each row and have the same upper-case letter are not significantly different at $P < 0.001$.

https://doi.org/10.1017/wsc.2020.99 Published online by Cambridge University Press

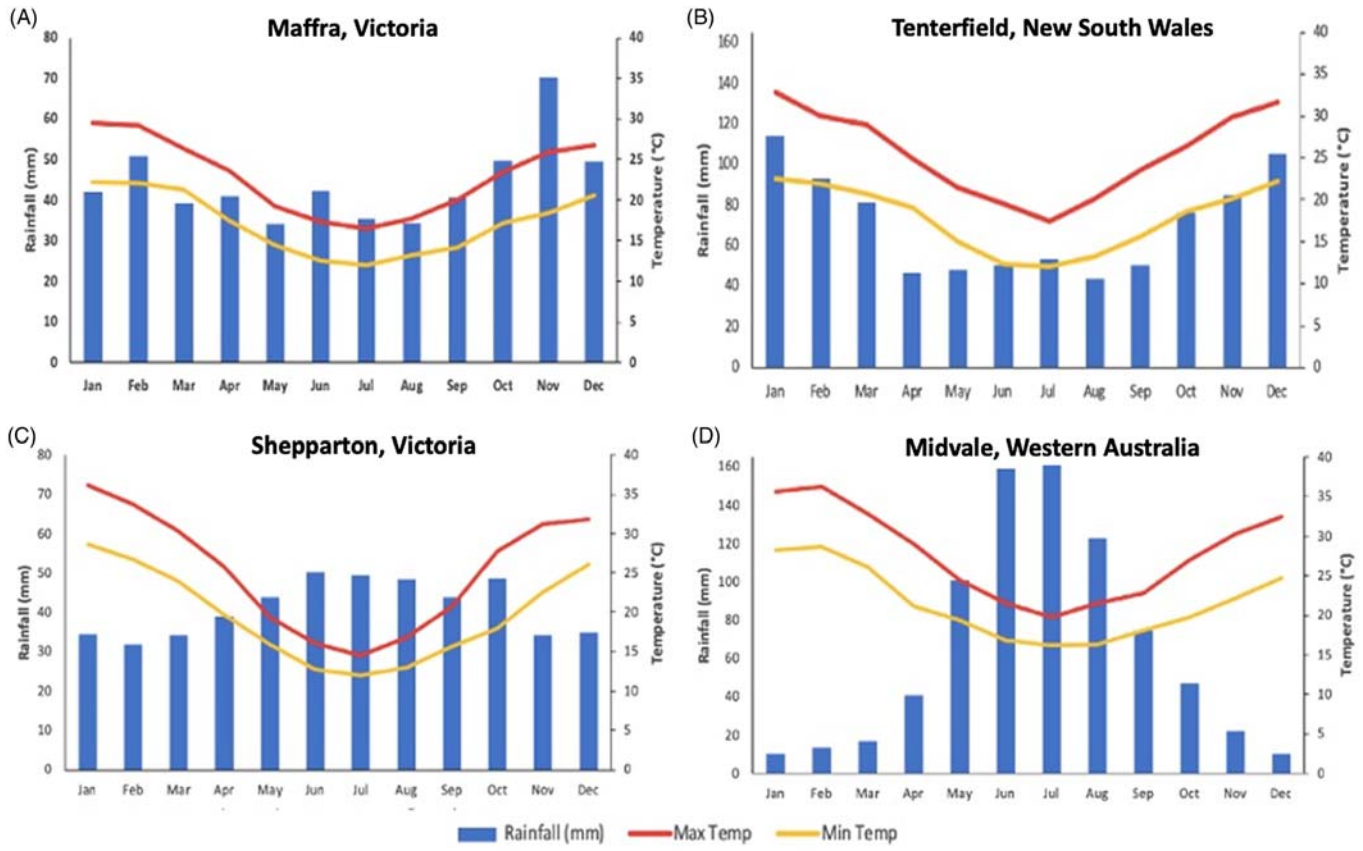

Figure 2. (A) The average climatic conditions for Maffra, VIC. The average monthly rainfall data (1993 to 2019) were obtained from the Maffra weather station (Station number 85297), approximately 1.4 km from the site. Average temperature data (1945 to 2019) were obtained from the East Sale weather station (Station number 85072), approximately 21.7 km from the site (Bureau of Meteorology 2020). (B) The average climatic conditions for Tenterfield, NSW. The average monthly rainfall data and temperature data (1888 to 2019) were obtained from the Tenterfield (Federation Park) weather station (Station number 56032), approximately 5.9 km from the site (Bureau of Meteorology 2020). (C) The average climatic conditions for Shepparton, VIC. The average monthly rainfall data (1885 to 2019) were obtained from the Mooroopna weather station (Station number 81032), approximately 5.3 km from the site. Average temperature data (1996 to 2019) were obtained from the Shepparton airport weather station (Station number 81125), approximately 5.4 km from the site (Bureau of Meteorology 2020). (D) The average climatic conditions for Midvale, WA. The average monthly rainfall data (1914 to 2019) were obtained from the Midland weather station (Station number 9025), approximately 2.6 km from the site. Average temperature data (1944 to 2019) were obtained from the Perth Airport weather station (Station number 9021), approximately 6.5 km from the site (Bureau of Meteorology 2020).

resources, such as soil nutrients (Fenner and Thompson 2005). Results show that only Maffra had reduced and slower germination at the higher pH levels, which may be due to localized adaptation to soil composition or other environmental factors. This pattern of germination has also been described in crowfootgrass [*Dactyloctenium aegyptium* (L.) Willd.] (Burke et al. 2003) and Crofton weed (*Eupatorium adenophorum* Spreng.) (Lu and Ma 2006), for which higher soil pH reduced germination. Most Australian soils fall within the range of pH 4 to 9, which suggests that *E. curvula* has the potential to exploit a range of environments and become problematic in many regions across Australia (Rengasamy 2006).

Effect of Salinity (NaCl)

Key results from this study show that *E. curvula* seeds from all populations were influenced by increasing salinity, with Midvale being the most salt tolerant and Maffra the least. For the effect of salinity (NaCl), there was a significant interaction between the population and NaCl for all measures (Table 4). Post hoc tests indicated that the mean FGP was significantly lower for Maffra (29% vs. 67%) at ≥100 mM, Tenterfield (29.8% vs. 94%) and Shepparton (39.5% vs. 81.5%) at ≥150 mM, and Midvale (39.5% vs. 81.5%) at 250 mM compared with the mean of all other concentrations. Further, the mean GI was also significantly lower for Maffra (0.8 vs. 2.5) and Tenterfield (1.9 vs. 8.4) at ≥100 mM, Shepparton (1.2 vs. 3.8), at ≥150 mM, and Midvale (3.2 vs. 6.9) at ≥50 mM compared with the mean of all other concentrations. Regarding the MGT, only the Tenterfield population (14.1 d vs. 3.9 d) had a significantly longer MGT at 250 mM compared with the mean of all other concentrations. For the mean T_{50}, germination was significantly longer for Maffra (8.9 d vs. 3.3 d) and Tenterfield (5.6 d vs. 2.6 d) at ≥200 mM, Shepparton (13 d vs. 3 d) at 250 mM, and Midvale (6 d vs. 2 d) at ≥50 mM compared with the mean of all other concentrations. Regarding the T_{SG}, only Maffra (7.6 d vs. 3.2 d) had a significantly longer mean T_{SG} at 250 mM compared with all other concentrations.

Salinity influences germination through (1) ion toxicity and (2) osmotic stress (Bliss et al. 1986). Studies of ion toxicity have shown that an increase in Na^+ and Cl^- ions can disrupt seed development by limiting cellular activity, energy production, and the essential uptake of macronutrients (Gupta and Huang 2014; Maathuis et al. 2014). In addition, osmotic stress reduces uptake of water and important nutrients (potassium, calcium, and magnesium) and alters the hormonal and enzymatic processes within a seed (Thiam et al. 2013). Therefore, germination is reduced or slowed

https://doi.org/10.1017/wsc.2020.99 Published online by Cambridge University Press

Table 3. The effect of pH on *Eragrostis curvula* seed germination.[a]

Measure	pH Value	Population				Significant interactions
		Maffra	Tenterfield	Shepparton	Midvale	
Final germination percentage (FGP)	4	89.2 aA	95.3 aA	84.0 aA	94.0 aA	Population*pH
	5	91.2 aA	98.2 aA	82.2 aA	89.5 aA	(df = 18, $F = 3.1$, $P < 0.001$)
	6	88.7 aA	99.0 aA	78.7 aB	86.2 aAB	
	7	82.0 aA	98.3 aA	81.3 aA	80.1 aA	
	8	78.0 abA	95.7 aA	84.3 aA	86.0 aA	
	9	77.0 abA	99.2 aB	86.3 aAB	86.5 aAB	
	10	59.8 bA	96.3 aB	87.3 aBC	76.7 aAC	
Germination index (GI)	4	3.1 abAB	4.8 aB	3.5 aAB	2.6 aA	Population*pH
	5	3.2 abA	5.9 aB	3.4 aA	2.3 aA	(df = 18, $F = 5.4$, $P < 0.001$)
	6	3.8 bA	6.2 aB	3.0 aA	2.1 aA	
	7	3.2 abA	6.2 aB	3.1 aA	1.9 aA	
	8	1.9 acA	5.8 aB	4.3 aB	2.3 aA	
	9	2.1 abcA	6.4 aC	3.9 aB	2.5 aAB	
	10	1.4 cA	6.0 aB	4.3 aC	1.9 aA	
Mean germination time (MGT) (d)	4	6.3 acAB	3.9 aA	4.4 aA	8.1 aB	Population*pH
	5	6.0 acAB	3.6 aA	4.3 aA	8.4 aB	(df = 18, $F = 12.9$, $P < 0.001$)
	6	5.4 aAB	3.2 aA	4.8 aA	7.7 aB	
	7	4.6 aA	3.1 aA	4.6 aA	7.8 aB	
	8	8.6 cA	3.4 aB	4.8 aB	7.2 aA	
	9	8.1 cA	3.1 aB	4.7 aBC	6.7 aAC	
	10	14.9 bA	3.1 aB	4.3 aB	7.8 aC	
Time taken to reach 50% germination (T_{50}) (d)	4	4.5 acAB	3.2 aA	3.9 aA	5.9 aB	Population*pH
	5	4.7 acAB	2.8 aA	4.2 aAB	6.2 aB	(df = 18, $F = 7.8$, $P < 0.001$)
	6	4.1 aA	2.5 aA	4.4 aAB	6.4 aB	
	7	4.3 aAB	2.5 aA	4.3 aAB	5.9 aB	
	8	6.2 cdA	2.8 aB	4.3 aB	6.4 aA	
	9	8.1 bdA	3.1 aB	4.7 aB	6.7 aA	
	10	9.8 bA	2.5 aB	4.2 aB	6.5 aC	
Time to start germination (T_{SG}) (d)	4	4.0 aAB	3.0 aA	2.0 aA	5.6 aB	Nil
	5	3.6 aAB	3.0 aA	2.5 aA	5.6 aB	(df = 18, $F = 1.5$, $P < 0.001$)
	6	3.8 aAB	3.0 aA	2.5 aA	6.0 aB	
	7	3.3 aAB	3.0 aA	3.5 aAB	5.6 aB	
	8	5.3 aAC	3.0 aAB	2.0 aB	6.0 aC	
	9	4.6 aA	3.0 aAB	2.0 aB	5.0 aA	
	10	5.1 aAC	3.0 aAB	2.5 aB	4.6 aC	

[a]For each measure, values (means) that run down each column (within that population) and have the same lower-case letter are not significantly different at $P < 0.001$. Also, for each measure, values (means) that run across each row and have the same upper-case letter are not significantly different at $P < 0.001$.

with increasing salinity, which was observed within this study. This pattern of germination has also been observed in several *Eragrostis* species, such as *E. plana* (Bittencourt et al. 2017), *E. tenella* (Chauhan 2013), and teff [*Eragrostis tef* (Zuccagni) Trotter] (Papastylianou et al. 2019). In contrast, some plants can develop over time to produce seeds that withstand a greater salt tolerance as a result of a difference in genetic variation or adaption to localized climatic conditions (Dehnavi et al. 2020). In this study, the Midvale population had a higher salt tolerance compared with Maffra, Tenterfield, and Shepparton, but it also had a slightly longer germination time. This could be due to localized adaption to the climatic conditions or a difference in the original seed source. Midvale receives most of its yearly rainfall in winter (Figure 2D), therefore, outside these months, the soil salt concentration could be higher, resulting in seeds needing to be capable of germinating higher saline conditions. However, Maffra, Tenterfield, and Shepparton have evenly distributed rainfall throughout the year (Figures 2A–C), so saline conditions might be less likely to develop. Therefore, our results suggest that *E. curvula* may have evolved at a local scale to produce seeds that withstand different environmental constraints.

This study highlights several similarities and differences in the seed germination of *E. curvula* across Australia. Seeds from Midvale can germinate across a wide range of conditions from complete darkness, temperatures between 7 to 35 C, soil pH between 4 to 10, and saline conditions ≤200 mM. Seeds from this population may be more conductive in growing across a range of climatic regions, and preventative strategies to reduce seed spread should be of high priority. This can be achieved by maintaining a high level of quarantine and hygiene (cleaning vehicles and equipment), managing and limiting the movement of livestock, and reducing soil disturbance around *E. curvula* infestations. For Maffra, Tenterfield, and Shepparton, germination was reduced in complete darkness; therefore, when practical, the use of light-limiting strategies (mulching, scraping, or tilling) would be beneficial in reducing the germination of new seeds in these areas. Furthermore, the germination of seed from these localities was more prevalent at temperatures between 15 to 35 C, in soil pH between 4 to 9, and in saline conditions <150 mM, which should help local managers identify conditions that promote spread of the species. Although germination may not be as vigorous as for the Midvale population, this study suggests that *E. curvula* seeds can adapt to changing climatic conditions. Therefore, the preventative strategies mentioned should also be applied in all localities, and management should be conducted year-round, as the species is likely to germinate across various environments. Although the factors in this study were investigated individually, they are interlaced and may interact with each other; however, interactions were not specifically investigated here, and we recommend that they be considered in future studies. Further,

https://doi.org/10.1017/wsc.2020.99 Published online by Cambridge University Press

Table 4. The effect of salinity (NaCl) on *Eragrostis curvula* seed germination.[a]

Measure	NaCl value —mM—	Population				Significant interactions
		Maffra	Tenterfield	Shepparton	Midvale	
Final germination percentage (FGP)	0	71.8 aA	96.5 aAB	82.6 aAB	100 aB	Population*NaCl
	25	62.6 abA	95.6 aBC	85.6 aAC	100 aBC	(df = 18, $F = 6.1$, $P < 0.001$)
	50	66.5 abA	96.3 aBC	81.8 abAC	98.3 aBC	
	100	39.5 bcA	89.7 abB	75.8 abB	98.3 aB	
	150	41.6 cA	62.1 bA	54.8 bA	91.6 aB	
	200	20.1 cA	26.2 cAB	53.1 bB	83.0 aC	
	250	14.1 cA	0.8 cA	10.8 cA	26.1 bA	
Germination index (GI)	0	3.0 aA	9.0 aB	3.9 aA	7.7 aB	Population*NaCl
	25	1.7 abA	8.5 aB	4.2 aC	6.1 abcD	(df = 18, $F = 18.4$, $P < 0.001$)
	50	2.8 aA	7.8 aB	3.6 abAC	4.8 bceC	
	100	1.1 abA	5.2 bB	3.4 abB	4.6 bdB	
	150	1.2 abA	1.8 cAB	2.0 bcAB	3.6 cdeB	
	200	.58 bA	.75 cA	1.6 cA	2.4 eA	
	250	.23 bA	.03 cA	.13 cA	.77 fA	
Mean germination time (MGT) (d)	0	3.3 aA	2.0 aA	3.2 aA	3.4 aA	Population*NaCl
	25	4.8 aA	2.2 aA	3.6 aA	4.3 aA	(df = 18, $F = 10.1$, $P < 0.001$)
	50	3.7 aA	2.6 aA	3.5 aA	5.1 aA	
	100	5.2 aA	4.5 aA	3.9 aA	5.2 aA	
	150	4.2 aA	6.0 aA	3.7 aA	5.6 aA	
	200	3.2 aA	6.6 aA	5.1 aA	7.0 aA	
	250	5.5 aA	14.1 bB	6.1 aA	7.1 aA	
Time taken to reach 50% germination (T_{50}) (d)	0	2.5 aA	1.5 aA	2.5 aA	1.8 aA	Population*NaCl
	25	3.6 aA	1.6 aA	2.6 aA	2.2 aA	(df = 18, $F = 11.2$, $P < 0.001$)
	50	2.2 aA	1.6 aA	2.6 aAB	5.3 bB	
	100	3.9 aA	3.8 abA	2.7 aA	5.4 bA	
	150	4.3 abA	4.7 abA	2.7 aA	5.5 bA	
	200	7.2 bA	6.6 bA	5.1 aA	7.0 bA	
	250	10.6 cA	4.5 abB	13.0 bA	6.8 bB	
Time to start germination (T_{SG}) (d)	0	2.5 aA	2.0 aA	3.0 aA	2.0 aA	Population*NaCl
	25	4.0 aA	2.0 aA	3.0 aA	2.0 aA	(df = 18, $F = 7.4$ $P < 0.001$)
	50	2.8 aA	2.0 aA	3.0 aA	2.0 aA	
	100	4.0 aA	2.0 aA	3.0 aA	2.0 aA	
	150	3.1 aA	5.0 aA	3.0 aA	2.0 aA	
	200	2.5 aA	5.5 aA	3.3 aA	5.3 aA	
	250	7.6 bA	5.0 aA	4.8 aA	6.3 aA	

[a]For each measure, values (means) that run down each column (within that population) and have the same lower-case letter are not significantly different at $P < 0.001$. Also, for each measure, values (means) that run across each row and have the same upper-case letter are not significantly different at $P < 0.001$.

management of *E. curvula* should be conducted at the landscape scale, as localized adaptation and variation in seed germination was identified in this study.

Acknowledgments. The authors thank Michael McBain for creating Figure 1. This research received no specific grant from any funding agency or the commercial or not-for-profit sectors. No conflicts of interest have been declared.

References

Ahmed S, Opeña JL, Chauhan BS (2015) Seed germination ecology of doveweed (*Murdannia nudiflora*) and its implication for management in dry-seeded rice. Weed Sci 63:491–501

Archibald S, Bond WJ, Stock WD, Fairbanks DHK (2005) Shaping the landscape: fire-grazer interactions in an African savanna. Ecol Appl 15:96–109

Baskin CC, Baskin JM (2014) Seeds: Ecology, Biogeography and Evolution of Dormancy and Germination. San Diego: Elsevier Science. 1600 p

Batlla D, Benech-Arnold RL (2014) Weed seed germination and the light environment: implications for weed management. Weed Biol Manag 14:77–87

Bittencourt HVH, Bonome LTS, Trezzi MM, Vidal RS, Lana MA (2017) Seed germination ecology of *Eragrostis plana*, an invasive weed of South American pasture lands. S Afr J Bot 109:246–252

Bliss RD, Plattaloia KA, Thomson WW (1986) Osmotic sensitivity in relation to salt sensitivity in germinating barley seeds. Plant Cell Environ 9:721–725

Borger C, Hashem A, D'Antuono (2019) Summer weed species incidence in Western Australia varies between seasons. Weed Sci 67:589–594

Bureau of Meteorology (2020) Climate Data online. http://www.bom.gov.au/climate/data. Accessed: May 20, 2020

Burke IC, Thomas WE, Spears JF, Wilcut JW (2003) Influence of environmental factors on after-ripened crowfoot grass (*Dactyloctenium aegyptium*) seed germination. Weed Sci 51:342–347

Campbell MH, Kemp HW, Murson RD, Dellow JJ, Ridings H (1987) Use of herbicides for selective removal of *Eragrostis curvula* (Schrad.) Nees from a *Pennisetum clandestinum* pasture. Aust J Exp Agric 27:359–365

Chachalis D, Reddy KN (2000) Factors affecting *Campsis radicans* seed germination and seedling emergence. Weed Sci 48:212–216

Chauhan BS (2013) Seed germination ecology of feather lovegrass [*Eragrostis tenella* (L.) Beauv. Ex Roemer & J.A. Schultes]. PLoS ONE 8:e79398

Chauhan BS, Johnson DE (2008) Germination ecology of goosegrass (*Eleusine indica*): an important grass weed of rainfed rice. Weed Sci 56: 699–706

Chauhan SC, Manalil S, Florentine S, Jha P (2018) Germination ecology of *Chloris truncate* and its implications for weed management. PLoS ONE 13:e0199949

Cook GD, Dias L (2006) It was no accident: deliberate plant introductions by Australian government agencies during the 20th century. Aust J Bot 54:601–625

De Caritat P, Cooper M, Wilford J (2011) The pH of Australian soils: field results from a national survey. Soil Res 49:173–182

Dehnavi AR, Zahedi M, Ludwiczak A, Perez SC, Piernik A (2020) Effect of salinity on seed germination and seedling development of sorghum (*Sorghum bicolor* (L.) Moench) genotypes. Agron 10:859–874

Fenner M, Thompson K (2005) The Ecology of Seeds. Cambridge, UK: Cambridge University Press. 260 p

Ferrari FN, Parera CA (2015) Germination of six native perennial grasses that can be used as potential soil cover in drip-irrigated vineyards in semiarid environs of Argentina. J Arid Environ 113:1–5

Firn J (2009) African lovegrass in Australia: a valuable pasture species or embarrassing invader? Trop Grassl 43:86–97

Firn J, Ladouceur E, Dorrough J (2018) Integrating local knowledge and research to refine the management of an invasive non-native grass in critically endangered grassy woodlands. J Appl Ecol 55:321–330

Gamba D, Muchhala N (2020) Global patterns of population genetic differentiation in seed plants. Mol Ecol 29:3412–3428

Geng Y, Van Klinken RD, Sosa A, Li B, Chen J, Xu CY (2016) The relative importance of genetic diversity and phenotypic plasticity in determining invasion success of a clonal weed in the USA and China. Front Plant Sci 7:213–226

Gupta B, Huang B (2014) Mechanism of salinity tolerance in plants: physiological, biochemical and molecular characterization. Int J Genomics 39:1–18

Hereford J (2009) A quantitative survey of local adaptation and fitness tradeoffs. Am Nat 173:579–88

Humpheries T, Chauhan BS, Florentine SK (2018) Environmental factors effecting the germination and seedling emergence of two populations of an aggressive agricultural weed; *Nassella trichotoma*. PLoS ONE 13: e0199491

Josse EM, Halliday KJ (2008) Skotomorphogenesis: the dark side of light signalling. Cell Press 18:1144–1146

Kader MA (2005) A comparison of seed germination calculation formulae and associated interpretation of resulting data. J Proc Roy Soc NSW 138:66–75

Kebreab E, Murdoch AJ (1999) A model of the effects of a wide range of constant and altering temperatures on seed germination of four *Orobanche* species. Ann Bot 84:549–558

Lu Z, Ma K (2006) Spread of the exotic Crofton weed (*Eupatorium adenophorum*) across southwest China along roads and streams. Weed Sci 56:1068–1072

Maathuis FJM, Ahmad I, Patishtan J (2014) Regulation of Na^+ fluxes in plants. Front Plant Sci 124:451–467

Milberg P, Lamont BB (1995) Fire enhances invasion of roadside vegetation in southwestern Australia. Biol Conserv 73:45–49

Mobli A, Mollaee M, Manalil S, Chauhan BS (2020) Germination Ecology of *Brachiaria euciformis* in Australia and its implications for weed management. Agron J 10:1–11

Papastylianou P, Travlos I, Roussis I, Bilalis D (2019) Sensitivity of seed germination to salt stress in Teff [*Eragrostis tef* (Zucc.) Trotter]. Bull UASVM Horticulture 76:91–95

Rao N, Dong L, Li J, Zhang H (2008) Influence of environmental factors on seed germination and emergence of American sloughgrass (*Beckmannia syzigachne*). Weed Sci 55:529–533

Rengasamy P (2006) World salinilization with emphasis on Australia. J Exp Bot 57:1017–1023

Schwartz LM, Gibson DJ, Gage KL, Matthews JL, Jordan DL, Owen MDK, Shaw DR, Weller SC, Wilson RG, Young BG (2017) Seedbank and field emergence of weeds in glyphosate-resistant cropping systems in the United States. Weed Sci 63:425–439

Seglias AE, Williams E, Bilge A, Kramer AT (2018) Phylogeny and source climate impact seed dormancy and germination of restoration-relevant forb species. PLoS ONE 13:e0191931

Thiam M, Champion A, Diouf D, Ourye M (2013) NaCl effects on in vitro germination and growth of some Senegalese cowpea (*Vigna unguiculata* (L) Walp.) cultivars. Biotechnol J 2013:1–11

Waes JM, Debergh PC (1986) Adaptation of the tetrazolium method for testing the seed viability, and scanning electron microscopy study of some western European orchids. Plant Physiol 66:435–442

https://doi.org/10.1017/wsc.2020.99 Published online by Cambridge University Press