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Research Article

Cite this article: Mahajan G, Matloob A, Walsh M, Chauhan BS (2018) Germination Ecology of Two Australian Populations of African turnipweed (*Sisymbrium thellungii*). Weed Sci 66:752–757. doi: 10.1017/ wsc.2018.55

Received: 27 March 2018 Revised: 14 June 2018 Accepted: 9 July 2018

Key words:

Australia; seed germination; weed biology; weed ecology; weed spread; weed management strategies

Associate Editor: William Vencill, University of Georgia

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Germination Ecology of Two Australian Populations of African turnipweed (Sisymbrium thellungii)

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Abstract

African turnipweed (Sisymbrium thellungii O. E. Schulz) is an emerging problematic broadleaf weed of the northern grain region of Australia. Laboratory experiments were conducted to evaluate the effects of temperature, light, salinity, pH, seed burial depth, and the amount of wheat crop residue on germination and emergence of two Australian S. thellungii weed populations (population C, cropped area; population F, fence line). Both populations behaved similarly across different environmental conditions, except in the residue study. Although the seeds of both populations of S. thellungii could germinate under complete darkness, germination was best (~95%) under light/dark conditions at the 20/10 C temperature regime. Both populations of S. thellungii germinated over a wide range of day/ night temperatures (15/5, 20/10, 25/15, and 30/20 C). Osmotic stress had negative effects on germination, with 54% seeds (averaged over populations) able to germinate at -0.1 MPa. Complete germination inhibition for both populations was observed at -0.8 MPa osmotic potential. Both populations germinated at sodium chloride (NaCl) concentrations ranging from 50 to 100 mM, beyond which germination was completely inhibited. There were substantial reductions in seed germination, 32% (averaged over populations) under highly acidic conditions (pH 4.0) as compared with the control (water: pH 6.4). Seed germination of both populations on the soil surface was 77%, and no seedlings emerged from a burial depth of 1 cm. The addition of 6 Mg ha⁻¹ of wheat (*Triticum aestivum* L.) residue reduced the emergence of the C and F populations of S. thellungii by 75% and 64%, respectively, as compared with the control (no residue). Information gathered from this study provides a better understanding of the factors favorable for germination and emergence of S. thellungii, which will aid in developing management strategies in winter crops, especially wheat, barley (Hordeum vulgare L.), and chick pea (Cicer arietinum L.).

Introduction

African turnipweed (*Sisymbrium thellungii* O. E. Schulz) is an emerging problematic weed of the northern cropping region of Australia. Its chemical control is difficult in wheat (*Triticum aestivum* L.), as many populations are now resistant to Group 2 herbicides (Heap 2002). Although other herbicides are available for weed control in wheat, *S. thellungii* control ultimately will require alternative sustainable weed control measures. In oilseed and pulse crops, very limited options are available for the control of broadleaf weeds through herbicides, necessitating ecological approaches to weed control. There is no information on the competitive ability of *S. thellungii*. However, closely related *Brassica* spp. such as turnipweed [*Rapistrum rugosum* (L.) All.] have, when uncontrolled, reduced wheat and barley (*Hordeum vulgare* L.) yield by 24% and 11%, respectively (Robinson and Marley 1996). In the northern grain region of Australia, it is estimated that infestations of *R. rugosum* and *S. thellungii* result in revenue losses of about A\$10.3 million annually (Llewellyn et al. 2016).

Sisymbrium thellungii seedlings emerge and grow throughout winter and early spring (Warwick and Al-Shehbaz 2003). The life cycle of this weed is concurrent with important winter cereals and *Brassica* crops grown in Australia's northern cropping region, and therefore seeds of *S. thellungii* mature with these crops. Due to high seed production (6,000 seeds plant⁻¹; Gulshan Mahajan, personal observation), this weed has the potential to quickly establish and maintain a very large seedbank.

Germination ecology plays a key role in the establishment of weeds in any agroecosystem (Bajwa et al. 2015; Chauhan and Johnson 2010), as it influences the weed seedbank, weed competitive ability, and the decision-making processes for choosing management options. The

survival of weeds is highly influenced by their ability to germinate in response to environmental conditions available for growth, development, and subsequent maturation (Baskin and Baskin 1986). Despite the significance of the problems *S. thellungii* causes in the northern cropping region of Australia, there is only limited knowledge of its ecology. Seed germination is a critical event in the persistence of weeds in agroecosystems. Environmental factors such as temperature, pH, light, burial depth, and soil moisture play a key role in influencing and regulating germination (Chachalis and Reddy 2000; Koger et al. 2004; Taylorson 1987). A better understanding of germination and emergence behavior of different populations of *S. thellungii* in response to various environmental factors would be useful in developing weed control programs for this species.

Salinity has become a major issue in Australia, and it has been estimated that about 60% of the 20-million-hectare Australian cropping region has sodic soils (Rengasamy 2010). High accumulation of alkaline salts has resulted in increased pH of many soils (as high as 9.0; de Caritat et al. 2011). The germination response of weeds to these factors (pH and salinity) may determine invasive potential and pattern of spread.

Therefore, from an ecological perspective, it is important to evaluate the impact of environmental factors on germination and emergence of *S. thellungii*. There are differing responses to germination cues in species closely related to *S. thellungii*. Seed germination is stimulated by light in oriental mustard (*Sisymbrium orientale* L.) (Chauhan et al. 2006), but inhibited by light in *R. rugosum* (Cousens et al. 1994). Considering these differences in germination reponses in closely related species (*S. orientale* and *R. rugosum*), it is pertinent to evaluate the germination behavior of *S. thellungii* in response to different environmental factors.

The temperature response of *S. thellungii* appears to be similar to that of many winter *Brassicaceae* weed species, with maximum germination at 10 to 25 C (Cousens et al. 1994). In several weed species, seed burial depth has an important role in inducing depth-mediated dormancy, especially in small-sized weed seeds (Benvenuti et al. 2001). In general, weed seedbanks are heterogenous, with genetic diversity both within and between populations (Forcella et al. 1997). Therefore, it is pertinent to evaluate the germination behavior of different populations of *S. thellungii*.

It has been reported that emergence of many weeds species is reduced with the addition of crop residue (Chauhan and Johnson 2009; Opeña et al. 2014). Therefore, better understanding is needed of any crop residue factor that could affect the germination of *S. thellungii* under zero-tillage systems. Such knowledge will help in developing effective cultural management practices for this problematic weed. The primary objective of this study was to determine the germination behavior of *S. thellungii* as influenced by temperature, light, salt, osmotic stress, pH, crop residue, and burial depth under controlled conditions. A secondary objective was to investigate the influence of agricultural environment (fence line vs. cropped area) on the germination ecology of this weed species.

Materials and Methods

Seed Description

Laboratory experiments were conducted in 2017 at the Gatton Campus of the University of Queensland, Australia, using *S. thellungii* seeds collected from mature plants in farmers' fields in the Saint George region of Queensland in October 2016. The soil in the Saint George region is a vertisol, and it cracks on drying. The long-term average (average from 1997 to 2017) maximum and minimum temperatures in the region were 34.9 and 5.2 C, respectively. *Sisymbrium thellungii* plants were collected from fallow wheat fields and their fence lines, and seeds were collected by shaking weed plants over trays. The collected seed samples were dried under properly ventilated, dry conditions to prevent microbial contamination and physiological deterioration and then stored in plastic containers in a shade house for 4 mo under identical ambient conditions. The temperature conditions in the shade house were similar to the outside environment, but seeds were not exposed to rain. Seeds of *S. thellungii* were considered mature when the plants were completely senesced. The 100-seed weight of *S. thellungii* was 0.040 and 0.046 g for populations C and F, respectively.

The coordinates were 28.007°S, 148.538°E for population C (wheat crop field) and 28.026°S, 148.536°E for population F (fence line). The weed populations were selected from different sites (cropped and fence line) under the assumption that management practices would influence the maternal environment during weed seed development and, potentially, weed germination ecology.

General Germination Test Protocol

Sisymbrium thellungii germination was evaluated by evenly distributing 25 seeds from each population in a 9-cm-diameter petri dish that had a double layer of Whatman No. 1 filter paper moistened with 5 ml of distilled water or a treatment solution. Petri dishes were placed in sealed plastic bags to avoid water loss due to evaporation and were then placed in an incubator.

The experiments evaluating the effect of osmotic stress, salt stress, pH, burial depth, and amount of residue on germination were conducted in an incubator set at 20/10 C day/night temperature, with a 12-h photoperiod matched with the thermoperiod. These conditions were best for germination during the initial run of the first experiment evaluating the effect of light and temperature on germination of *S. thellungii*. Fluorescent lamps with a light intensity of 85 mol m⁻² s⁻¹ were used as the source of light in the incubators.

To test germination of *S. thellungii* in complete darkness, petri dishes were wrapped in three layers of aluminum foil and only opened once for a germination count after 21 d. Seeds were considered germinated when the radical was at least 2-mm long. Seed germination percentages were calculated for each replicate based on the aggregated germination count after 21 d. The viability of the nongerminated seeds was assessed by pressing the seeds with a pair of forceps, because the tetrazolium test was difficult to conduct on this small-seeded species (Chauhan et al. 2006). Firm or hard seeds were considered viable, and soft seeds that lacked structural integrity were considered nonviable.

Effect of Temperature and Light on Germination

To determine favorable temperature and light conditions for germination of *S. thellungii*, seeds of C and F populations were placed in incubators under alternating day/night temperatures (15/5, 20/10, 25/15, and 30/20 C) in light/dark and dark conditions. These temperature regimes were selected to simulate temperature fluctuations during autumn and winter in the northern cropping region of Australia.

Effect of Salt and Osmotic Stress on Germination

Germination of *S. thellungii* in relation to salt stress was studied using sodium chloride (NaCl) solutions of varied concentrations: 0, 50, 100, 150, 200, and 250 mM. This range represents soil salinity levels in many salt-affected cropping regions of Australia (Rengasamy 2002, 2010). To examine the effect of osmotic stress, water solutions with osmotic potentials of 0, -0.1, -0.2, -0.4, -0.6, -0.8, and -1.0 MPa were prepared by dissolving polyethylene glycol 8000 in 1 L of distilled water at quantities of 0, 91.6, 129.5, 183.1, 224.2, 258.9, and 289.8 g (Michel 1983).

Effect of pH on Germination

To study the effect of pH on seed germination of *S. thellungii*, buffer solutions of pH 4 to 10 were used and prepared according to the method described by Chachalis and Reddy (2000). The pH levels were selected based on the documented pH range of Australian soils, from 4.0 to 10.0 (de Caritat et al. 2011). Buffer solutions were created as follows: pH 4, a 2-mM potassium hydrogen phthalate buffer solution was adjusted with 1 N HCl; pH 5 or 6, a 2-mM solution of MES [2-(*N*-morpholino) ethanesulfonic acid] was adjusted with 1 N HCl or NaOH; pH 7 or 8, a 2-mM solution of HEPES [*N*-(2-hydroxymethyl)piperazine-*N'*-(2-ethanesulfonic acid)] was adjusted with 1 N NaOH; pH 9 or 10, 2 mM tricine [*N*-Tris(hydroxymethyl)methylglycine] was adjusted with 1 N NaOH. Distilled water (pH 6.4) was used as a control.

Effect of Wheat Residue Amount on Seedling Emergence

Fifty seeds each from the C and F populations of *S. thellungii* were placed on the surface of soil in 10-cm-diameter plastic pots, and finely chopped (2- to 3-cm) wheat straw (leaves and stems) ('Spitfire') was spread evenly on the soil surface. The soil used for this experiment was a clay loam with 2.7% total organic matter. Crop residue at rates equivalent to 0, 1, 2, 3,4, 5, and 6 Mg ha⁻¹, were spread on the soil surface, and the pots were then placed in an incubator. All the pots were placed in plastic trays, and water was applied every other day to keep the soil moist. The emergence pattern was recorded at 1-wk intervals for 4 wk.

Effect of Seed Burial Depth on Seedling Emergence

The effect of seed burial depth on seedling emergence of *S. thellungii* was conducted in an incubator. For this, 50 seeds each from the C and F populations of *S. thellungii* were planted at soil depths of 0, 1, 2, 3, 4, and 5 cm in 10-cm-diameter plastic pots filled with soil. The soil used for this pot experiment was a clay loam with 2.7% total organic matter. The criteria for emergence was when two cotyledons appeared, and the experiment continued until 28 d after planting.

Statistical Analyses

A complete randomized design with three replications was used in all the experimental runs. Each experiment was repeated after the termination of the first run. The data represent the average of the runs, as there was no time by treatment interaction as determined by ANOVA. ANOVAs were used to identify any significant treatment and interaction effects (P < 0.05). Where treatments were significant (P < 0.05), means were separated using Fisher's protected LSD test at P = 0.05. Nonlinear regression analysis was used to determine relationships between germination and residue amounts. These data were described with a functional three-parameter sigmoid model using SigmaPlot v. 13.0:

$$G(\%) = G_{\max} / \left[1 + (x / x_{50})^{Grate} \right]$$
[1]

where G is the total germination (%) at residue amount x, G_{max} is the maximum germination (%), x_{50} is residue amount for 50% inhibition of the maximum germination, and G_{rate} indicates the slope.

Results and Discussion

Effects of Light and Temperature

An interaction between light conditions and temperature regimes was observed for germination of *S. thellungii* (Table 1). At 15/5 C, germination between light/dark and dark conditions was not different (78% to 81%). However, at 20/10 and 25/15 C, the germination was higher in light/dark (85% to 97%) compared with dark conditions (19% to 65%). Germination was very low (2.7%) in alternating light/dark conditions at 30/20 C, and no germination occurred at this temperature regime when seeds were kept in constant darkness. Exposure to alternating light/dark conditions increased germination to >90% at 20/10 C (optimal temperature conditions).

Higher germination of both biotypes under fluctuating light/ dark conditions (as compared with complete darkness) is consistent with reports that S. thellungii germination is improved when exposed to light, which possibly triggers dormancy release (Chauhan et al. 2006), suggesting that this species is photoblastic. Stimulation of germination by alternating light and dark conditions (favorable conditions) at favorable temperature conditions is a positive indication that seeds of S. thellungii are photoblastic. Many Brassicaceae and broadleaf small-seeded weeds exhibit photoblasticity (Chauhan et al. 2006; Widderick et al. 2004); however, light is not necessary for germination, and germination in the dark does occur. Our results indicate that germination conditions in the field will be conducive when seeds are at or near the soil surface. Thus, this species is adapted to conservation cropping systems, where a large proportion of weed seeds remain at the soil surface.

Effect of Osmotic Stress

Averaged over population, seed germination of *S. thellungii* was affected by osmotic potential (Table 2). Germination was highest

 Table 1. Effect of alternating day/night temperatures (15/5 to 30/20 C) on the germination of Sisymbrium thellungii seeds (incubated for 21 d) under light/ dark (12-h photoperiod) and complete dark (24-h photoperiod).

	Germination —%—	
Alternating temperature regimes (day/night)	Light/dark	Dark
15/5 C	81.0	77.7
20/10 C	97.3	65.3
25/15 C	84.7	19.0
30/20 C	2.7	0.0
LSD (0.05)	6.8	

(94%) in the control treatment, and reduced to 54% at an osmotic potential of -0.1 MPa germination. Little germination (7%) occurred at -0.6 MPa, and no seeds germinated at osmotic potentials of -0.8 MPa and -1.0 MPa.

These results indicate that germination of S. thellungii requires relatively high levels of soil moisture. Sisymbrium thellungii was extremely sensitive to low water potential, and under extreme water-stress conditions, S. thellungii may not germinate. Water stress is a limiting factor for germination of many weed seeds, because water absorption and tissue hydration are essential in the sequence of metabolic events that lead to embryo development and seedling growth (Marcos-Filho 2005). Osmotic stress negatively affecting germination has been reported for many weeds (e.g., Ngo et al. 2017; Reddy and Singh 1992). However, water stress tolerance during germination has also been reported for some closely related weed species. For example, only 8% of R. rugosum seeds germinated at an osmotic potential of -1.0 MPa (Chauhan et al. 2006). Similarly, 52% of London rocket (Sisymbrium irio L.) seeds germinated at an osmotic potential of -1.2 MPa (Ray et al. 2005). Inhibition of germination under low water potential may enhance the survival mechanism of weeds by ensuring seed dormancy until favorable moisture conditions are available for successful germination (Fernando et al. 2016).

Effect of Salt Stress

Averaged over population, germination was highest (94%) in the control treatment (Table 3). *Sisymbrium thellungii* was even able to germinate at a high NaCl concentration of 100 mM; however, beyond this concentration, there was no germination.

It is clear that *S. thellungii* has the ability to germinate under high salt concentrations. However, germination was reduced at concentrations of 100 mM NaCl, which might be due to physiological disruption of metabolism caused by ion toxicity or high osmotic stress (Farooq et al. 2015). Soils having salt concentrations higher than 20 mM NaCl can be categorized as salt-affected soils (Abrol et al. 1988). Chauhan et al. (2006) reported a similar decline in seed germination of *S. orientale* under saline conditions. The concentration required to inhibit 50% germination of *S. orientale* was 68 mM NaCl, and no germination occurred at 160 mM NaCl. Given the increasing problem of salinity in arid environments of Australia, *S. thellungii* could become a more serious problem in the future. Under such a high-salinity scenario, *S. thellungii* may compete with crops by having greater

Table 2. Effect of osmotic potential on germination of *Sisymbrium thellungii* seeds (incubated for 21 d) at alternating day/night temperatures of 20/10 C under a 12-h photoperiod.

Osmotic potential —MPa—	Germination —%—
0.0	93.7
-0.1	54.3
-0.2	28.7
-0.4	17.0
-0.6	7.3
-0.8	0.0
- 1.0	0.0
LSD (0.05)	11.9

Table 3. Effect of sodium chloride (NaCl) concentration on germination of *Sisymbrium thellungii* seeds (incubated for 21 d) at alternating day/night temperatures of 20/10 C under a 12-h photoperiod.

NaCl concentration —mM—	Germination —%—
0	93.7
50	87.3
100	11.0
150	0.0
200	0.0
250	0.0
LSD (0.05)	6.1

adaptability to that environment, thus reducing winter crop [e.g., wheat, barley, chick pea (*Cicer arietinum* L.)] yields.

Effect of pH

Averaged over population, germination of *S. thellungii* was influenced by pH range (Table 4). Germination was highest (92%) in the control (distilled water) treatment. Seed germination was more than 78%, even at pH levels ranging from 8.0 to 10.0, but lower than the control (Table 4). Seed germination was largely reduced at lower pH levels, and it was reduced by 32% at pH 4.0 as compared with the control.

The results show that *S. thellungii* can germinate at a wide range of pH levels. In fact, these pH regimes represent most of the soil types in Australia (Rengasamy 2006). Therefore, pH is not a limitation for *S. thellungii* germination. This attribute is common to other important *Brassicaceae* weed species, such as *S. orientale* (Chauhan et al. 2006). *Sisymbrium orientale* germination was greater than 50% over a pH range of 4 to 10; however, optimal germination (60% to 71%) occurred from pH levels of 7 to 9.

Effect of Residue Amount

A sigmoidal curve described the emergence pattern of *S. thellungii* as influenced by increasing amounts of wheat residue (Figure 1). Seedling emergence of both populations of *S. thellungii* decreased with an increasing rate of wheat residue. With no added residue, germination was more than 80% in both populations, and

Table 4. Effect of pH on germination of *Sisymbrium thellungii* seeds (incubated for 21 d) at alternating day/night temperatures of 20/10 C under a 12-h photoperiod.

рН	Germination —%—
4.0	62.7
5.0	72.7
6.0	73.7
6.4 (control)	92.0
7.0	81.0
8.0	82.0
9.0	79.0
10.0	78.7
LSD (0.05)	7.3

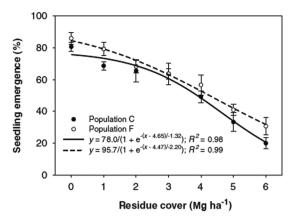


Figure 1. Seedling emergence of two populations (population C, cropped area; population F, fence line) of *Sisymbrium thellungii* in response to residue amount (Mg ha⁻¹) when grown in an incubator at alternating day/night temperatures of 20/10 C under a 12-h photoperiod. The lines represent a sigmoid model fit to the data. Vertical bars represent the ±standard errors of the mean (n = 6).

emergence decreased sharply (~50%) with the addition of 4 Mg ha⁻¹ or more of residue. The residue amount required to reduce 50% emergence of *S. thellungii* weed was estimated in the range of 4.5 to 4.7 Mg ha⁻¹. The seedling emergence for population F (fence line) was higher than for population C, suggesting that the fence-line populations have adapted to grow up through greater residue.

Emergence of many weed species has been reported to decrease with the addition of crop residue. With the addition of 6 Mg ha^{-1} of residue, the emergence of population F was reduced by 59% and the emergence of population C was reduced by 64% as compared with the control treatment (no residue). The residue load of >4 Mg ha⁻¹ might have provided a shading effect that reduced seed germination or might have physically obstructed emergence of weed seedlings (Crutcffield et al. 1985; Faceli and Pickett 1991; Opeña et al. 2014). It was evident from the temperature and light experiment that germination of S. thellungii was reduced under the dark environment condition (Table 1). Therefore, a residue load of 4 Mg ha⁻¹ may help in reducing the germination of S. thellungii by shading it in the field. Weed suppression could also be attributed to the chemical effect of mulches on seed germination, which includes allelopathy and toxic microbial products (Chauhan and Johnson 2010); however, these effects were not studied. Low production systems (2 to 3 Mg ha⁻¹) are common in Australia, and there was less weed suppression at these residue amounts. The position and uniformity of crop residue could also affect the degree of weed suppression. In the present study, loose and chopped residue was spread evenly on the soil surface. Overall, the results suggest that crop residue retention as part of conservation tillage practices could help growers in reducing infestations of S. thellungii. However, there is a need to integrate mulch retention with other management options (e.g., herbicide use).

Effect of Seed Burial Depth on Seedling Emergence

For both populations, seedling emergence was only observed for seeds placed on the soil surface (77.5%). Emergence of both populations was completely inhibited with burial. Even at the burial depth of 1 cm, seedlings of *S. thellungii* did not emerge. These results suggest that light plays a key role in stimulating germination of *S. thellungii*. Various studies have reported that seeds buried at depths of more than 2 mm below the soil surface

receive little solar radiation, usually less than 1% of incident light (Egley 1986; Woolley and Stoller 1978). However, in our temperature and light/dark experiment, it was observed that at the lowest temperature regimes (15/5 C), >70% of seeds germinated in the dark. The observed difference between germination in the dark and seedling emergence only from the soil surface could be related to the very small seed size of S. thellungii, which may make it difficult to emerge from a burial depth of even 1 cm. In contrast to S. thellungii, the seedling emergence of S. irio was from deeper in the soil profile, and greater emergence of S. irio was observed when seeds were buried at shallow depths (Ray et al. 2005). Similar to S. thellungii, seedlings of S. orientale did not emerge from 1-cm burial depth (Chauhan et al. 2006). Our results suggest that under field conditions, S. thellungii, with its potential for greatest emergence from the soil surface, would be favored by zero-tillage or conservation tillage practices. Under such scenarios, a large proportion of the seedbank remains on the soil surface after crop-planting operations (Chauhan et al. 2006), and such conditions could favor germination and infestation of this positively photoblastic weed.

In summary, S. thellungii weed germinates over a wide range of environmental conditions. Seed germination of S. thellungii was affected by osmotic stress, suggesting that retaining a small amount of crop residue on the soil surface may stimulate emergence of this weed by conserving soil moisture. As S. thellungii is tolerant to a wide range of pH levels, it may compete with crops in problematic soils. Crop residue retention on the soil surface reduced the emergence of S. thellungii, so integrating this practice in the current production system could be used to reduce the germination of S. thellungii. Emergence of S. thellungii was greatest from the soil surface, and seedlings did not emerge from seeds placed at 1 cm or greater depths. If a large weed seedbank accumulates on the soil surface under zero-tillage systems, a shallow cultivation could be used strategically (once every 5 to 10 yr) to reduce the seedbank. This cultivation would bury most seeds below the maximum depth of emergence.

Although seeds were collected from the same field (cropped area and fence line), the slight differences in populations for germination in residue study suggest that populations may have undergone differential adaptations. However, we are not sure whether this is attributable to maternal environment or genetic diversity, because this species is cross-pollinated. In one study, Gioria and Pyšek (2017) suggested that differences between populations may arise either due to maternal environment or adaptation to climate change or genetic diversity. Therefore, genetic variation, maternal effects, and prevailing microclimatic conditions could be the potential drivers of the observed variations for population in residue study. However, there is no evidence to support this inference, and a possible genetic basis requires further investigation.

Various models for predicting future climate change in Australia have revealed that frequent droughts with rising temperatures are predicted for the arid regions of eastern Australia (Nguyen et al. 2017). As a consequence of climate change, weed populations will adapt and shift, which may impact management strategies (Mahajan et al. 2012). Although *S. thellungii* is a winter weed, our study revealed that even at a temperature of 25/15 C under light/dark conditions, it demonstrated greater than 80% germination. It was also observed that *S. thellungii* weed could even germinate at an osmotic potential of -0.6 MPa, suggesting that this weed may have drought adaptability. Therefore, it is expected that *S. thellungii* has a tendency to adapt to futuristic

climate change and may spread to larger areas in Australia in the wake of climate change.

Acknowledgments. The authors would like to thank the Grains Research and Development Corporation (GRDC) for investing in this research under Project US00084. No conflicts of interest have been declared. We would also like to thank William Vencill and two anonymous reviewers for providing critical comments on the manuscript.

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