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Pre- and post-harvest influences on physiological dormancy alleviation of an Australian *Asteraceae* species: *Actinobole uliginosum* (A. Gray) H. Eichler

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Abstract

The effects of maternal air temperature and soil moisture upon seed physiological dormancy (PD) alleviation of an Australian native *Asteraceae* were investigated. From the onset of flowering, *Actinobole uliginosum* plants growing *ex situ* were subjected to either a warm (mean 26°C) or cool (mean 17°C) temperature regime, with adequate or limited water availability. In the warm environment, the reproductive phase was accelerated, and plants yielded fewer seeds over a shorter, earlier harvest period, when compared to those in the cool environment. Initial germination of all seeds was low (<20% at 15°C) due to PD, which was gradually alleviated by a dry after-ripening (DAR) treatment (34/20°C, 40% relative humidity, in darkness). Seeds from plants grown in the warm environment were more responsive to DAR than seeds from the cool environment, but maternal plant water availability had little effect on dormancy status. Germination was higher at 15°C than at 25/15°C, reaching a plateau of *c.* 80% germination after 20 weeks DAR. Before DAR, application of GA_3 had little impact on seeds, which would consequently be classified as having deep PD if tested at the time of dispersal. However, DAR caused seeds to become increasingly responsive to GA_3, reaching 97% germination at 15°C following just 4 weeks of DAR, which would indicate non-deep PD if seeds were tested following a period of warm, dry storage. Maternal air temperature regulates PD status of *A. uliginosum*, such that seeds collected from a warmer environment are likely to be more responsive to DAR. Post-harvest storage in an environment suitable for DAR affects seed response to GA_3, which has implications for germination stimulation and dormancy classification.

Keywords: *Actinobole uliginosum*, after-ripening, *Asteraceae*, Australia, climate, dormancy, seeds

Introduction

There is a growing need to include native understorey species in land revegetation. However, it is often difficult to predict and maximize germination of native herbaceous species. For example, many seeds from the *Asteraceae* family are difficult to germinate without first alleviating physiological dormancy (PD) (Bunker, 1994; Plummer and Bell, 1995; Schutz *et al.*, 2002; Hoyle *et al.*, 2008a). *Actinobole uliginosum* (A. Gray) H. Eichler (*Asteraceae*) is an Australian native, annual, prostrate daisy that flowers between July and October (late winter to spring). Seeds dispersed in late spring in south-western Queensland have PD that enables seeds to avoid seedling establishment during hot summer months by post-poning germination until the following autumn (Hoyle *et al.*, 2008a). Widespread across all mainland Australian states in a variety of inland habitats, including sandy/granitic soils and those in which perennial pasture is sparse (FloraBase, 2008; Plant-NET, 2008), *A. uliginosum* has great potential for inclusion in land revegetation projects, particularly if we have increased knowledge of factors affecting dormancy status in this species.

Seed PD status is reportedly regulated through interactions with the environment during the period in which seeds develop on the parent plant (Fenner, 1991; Wulff, 1995). In the field, different phenological schedules or geographical locations may cause

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individual plants of the same species to experience different environmental conditions. As a result, seeds from a single species can vary greatly in dormancy status, depending on where and when they are collected. Air temperature and soil moisture are particularly influential environmental factors during seed development (for reviews, see Fenner, 1991; Wulff, 1995; Gutterman, 2000), the effect of which will depend on the type of dormancy exhibited by seeds (Baskin and Baskin, 2001).

Numerous studies have investigated the effect of the maternal environment upon seeds of crop and weed species and report that, on the whole, warmer temperatures during seed development and maturation result in decreased PD status, while cooler temperatures have the opposite effect. Invasive weeds within Australia are no exception. For example, high maternal day/night temperatures (30/25°C) during the production of *Lolium rigidum* (*Poaceae*) seeds reduced dormancy (Steadman *et al.*, 2004), and *Onopordum acanthium* (*Asteraceae*) seeds achieved higher germination after parent plants were grown in warm compared to cool environments (Qaderi *et al.*, 2006). Limited soil moisture during seed development also causes variation in seed dormancy status, depending on the species and the dormancy mechanism(s) involved (Fenner, 1991). It is often observed that maternal drought reduces PD, as seen in the herbaceous weed *Sinapis arvensis* (*Brassicaceae*) (Luzuriaga *et al.*, 2005) and *Avena fatua* (*Poaceae*) (Peters, 1982).

With increased understanding of the environmental determinants of variation in dormancy status, it may be possible to make predictions about PD status and germination requirements of any particular seed lot. Whether Australian native seeds conform to the general trends in response to maternal air temperature and soil moisture is not known. Furthermore, as duration of seed maturation is often undefined for wild species, differences in dormancy status of seeds produced early in the reproductive phase compared to those produced later may be relevant. Therefore, *A. uliginosum* seeds, collected in south-western Queensland, were used to investigate whether manipulating maternal air temperature and soil water availability, from the onset of flowering through seed maturation, regulate the PD status/PD alleviation of F_1 seeds, as determined by the response of seeds to dry after-ripening (DAR).

Materials and methods

Seed material

Seeds of *A. uliginosum* (*Asteraceae*) were collected from south-western Queensland (28°04′07″S, 145°45′50″E), on 22 October 2004. At least 10,000 seeds were collected from >50 individual plants within one population. Only mature seeds were collected, i.e. seeds were brown in colour and dispersing naturally. In the laboratory, seeds were processed (non-seed material was removed by gently pushing flowers and seeds through a sieve using a hand-held rubber stopper, and then separating filled seeds from empty seeds and chaff using an aspirator), and stored at 15 ± 2°C and 15–20% relative humidity (RH) until experimentation began in April 2006.

Plant growth

Application of gibberellic acid (GA_3) stimulates germination of *A. uliginosum* (Hoyle *et al.*, 2008a). Therefore, to obtain seedlings for subsequent glasshouse experiments, multiple replicates of 20 seeds were sown into 9 cm diameter plastic Petri dishes containing GA_3 (200 mg l^{-1}) in a 1% agar–water medium. Dishes were sealed in transparent plastic bags to maintain hydration and placed into incubators at 25/15°C, 12/12 h photoperiod. Light was provided by white fluorescent tubes (*c.* 50 μmol m^{-2} s^{-1}). To avoid seedling abnormalities often caused by GA_3 (e.g. Daws *et al.*, 2007), seeds were moved to plain agar as soon as they germinated (i.e. visible radicle emergence of at least 1 mm). When seedlings had fully emerged from the seed coat and were >5 mm in length (*c.* 5–10 d after germination), seedlings were transplanted into multiple-celled trays containing a University of California potting mix (UC Mix B), which is 1:1 (v/v) river sand and peat with 4 kg of stock fertilizer per 0.50 m^3 of mix; 1 kg of stock fertilizer contained blood and bone (185 g), potassium nitrate (30 g), potassium sulphate (15 g), superphosphate (185 g), dolomite (310 g), hydrate lime (185 g) and gypsum (90 g). Trays were placed on benches in ambient glasshouse conditions [12–31°C (mean 18°C) and 21–94% RH (mean 68%)] with natural illumination, and seedlings were watered daily for the first 10 d and every 2 d thereafter. Between 48 and 50 d later, plants were transplanted into 15 cm diameter pots containing 1.7 kg UC Mix B. Additional fertilizer was applied to pots at this stage, and after flowering had begun, at a rate of 1 g l^{-1} of Aquasol™ (Yates Ltd; www.yates.com.au) water-soluble fertilizer dissolved in distilled water, with 50 ml of the solution added to each pot. Plants were watered every 2–4 d.

Temperature and soil moisture treatments

When individual plants exhibited more than five yellow flowers that were 75–100% open, and more than five buds, they were randomly assigned to 'wet' or 'dry' soil moisture treatments (described below)

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and moved to either a 'warm' (minimum 21°C, maximum 39°C, mean 25.8 ± 0.1°C) or 'cool' (minimum 13°C, maximum 26°C, mean 16.8 ± 0.1°C) controlled temperature glasshouse. Air RH was between 30 and 94% (mean 69.5 ± 0.2%) in the warm glasshouse and between 47 and 93% (mean 79.6 ± 0.2%) in the cool glasshouse. Air temperature and RH were recorded using T-TEC data loggers (Temperature Technology, Adelaide, Australia). As it was not possible to replicate glasshouses, the experimental design consisted of two soil treatments nested within two temperature environments (four treatments in total), in a randomized block design. All plants were moved into one of the four treatments within a 14 d period and distributed evenly between the three replicates per glasshouse, each replicate containing 12 plants. Just before transfer to the four treatment conditions, the mean length of the longest and shortest plant branch was 13.2 ± 0.2 and 7.8 ± 0.1 cm, respectively, and plants had a mean estimated percentage soil coverage of 75 ± 1%.

A soil moisture retention curve for the UC Mix B was determined previously using the pressure plate method (O'Donnell and Adkins, 2001). The percentage available water content of the UC Mix B was established from mean fresh soil weight and mean oven-dry weight {[(Fresh soil weight − oven-dry weight)/oven-dry weight] × 100}. Three soil samples were taken at the beginning, middle and end of the two-day potting-up session, and closed tins were weighed (fresh soil weight). Lids were then removed, and tins placed at 105°C for at least 24 h before lids were replaced and tins re-weighed (oven-dry weight).

A soil moisture deficit treatment was applied when plants had been moved to the controlled temperature glasshouses, and an open watering system was adopted. 'Wet' plants were maintained at an average of 80% field capacity (FC) or 24.7% soil moisture content (−0.01 MPa), and 'dry' plants were allowed to dry down to 8.5% soil moisture content (−1 MPa) before being watered back up to 80% FC. This equated to 'wet' plants being watered daily (warm) or every 2 d (cool), and the 'dry' plants were watered every 5 d (warm) or every 7 d (cool). 'Dry' plant pots were placed in trays to allow dry soil to take up water. While in treatment conditions, plants were regularly examined for general health and development. The cool–dry treatment was expected to mimic most closely the conditions that *A. uliginosum* would experience during the reproductive phase *in situ*.

Seed harvest and storage

Fans in the glasshouses provided a breeze to enable plants to self/cross-pollinate. As seeds reached maturity, flowers dried and dehisced. Therefore, the first seeds per plant were dispersed naturally and lost. The remaining and vast majority of seeds were harvested just before the point of natural dispersal; whole flower heads were cut from plants when they were dry and grey in colour, placed in labelled paper bags (per plant, per day), and placed in drying conditions (15 ± 2°C, 15–20% RH) for a total of 4 weeks. Within the first 2 weeks of drying, seeds were processed; plant material was gently pushed through a 2 mm and then a 500 μm sieve using a hand-held rubber stopper, and filled seeds were separated from chaff and immature seeds using an aspirator. At the end of 4 weeks, mature seeds, inside paper envelopes, were vacuum-sealed in aluminium envelopes and placed at −20 ± 1°C for 3 months. The processes of drying seeds to between 15 and 20% RH and storing at −20°C are together referred to as 'banking', methods commonly employed by seed banks for germplasm conservation (Linington, 2003).

Three months post-banking, all aluminium foil bags containing seeds were removed from −20°C storage and allowed to thaw for 24 h at room temperature (*c.* 20°C) before being opened. Two hundred seeds from each of ten individual randomly selected plants, per treatment, were weighed and then, per treatment, the total number of seeds harvested per plant and the mean seed mass were estimated.

Assessing seed viability and germinability

Tests were carried out to assess the effect of banking on seed viability and germinability. Pre-banking, 20 seeds per replicate, per treatment were harvested at the beginning, middle and end of the harvest period, and sown directly on to 1% water–agar medium, with and without 200 mg l^{-1} GA_3, in 9 cm diameter plastic Petri dishes. Dishes were sealed in transparent plastic bags and placed in incubators at alternating 25/15°C (12/12 h photoperiod). Final germination was counted after 4 weeks, and any remaining non-germinated seeds were examined for viability (see below). Post-banking, these tests were repeated using seeds from the same harvest times.

Seeds that did not receive GA_3 did not germinate and were tested for viability using the tetrazolium chloride (TZ) staining technique (ISTA, 2003). The outer gelatinous layer was removed from each seed, and the seed coat carefully punctured with a scalpel blade (away from the embryo and axis). Seeds were submerged in TZ solution and placed at 30°C for 24 h in darkness. Each seed was then dissected, and the embryo was examined for its staining pattern. Embryos that uniformly stained dark pink/red were considered viable, and those that were pale pink, unstained or irregularly stained were considered non-viable.

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Assessing seed dormancy status

Approximately 20% of all seeds collected per week, from within each of the four treatments, were bulked together within replicates, and the seed dormancy status investigated after various durations of dry after-ripening (DAR). Additional seeds from the cool–dry treatment were used to investigate the effect of DAR on response to GA_3, and seeds from the cool–dry treatment collected in the first and last weeks of the harvest period were used to investigate the effect of timing of seed development on seed dormancy status.

The DAR treatment of 34/20°C, 40% RH mimicked conditions seeds experience in the field, post-dispersal and before germination (Hoyle *et al.*, 2008b). The treatment was considered to be 'dry' since seeds were not imbibed. Seeds were equilibrated for 17 d inside an air-tight, thermo-stable, plastic box over a LiCl solution (450 g l^{-1}) that created an environment of *c.* 40% RH, in darkness, at 15°C. Seeds were then divided into replicates of 100, vacuum-sealed in small aluminium foil envelopes and placed at 34/20°C, 12/12 h photoperiod. Every 4 weeks for 36 weeks, three replicates of 100 seeds per treatment were removed from the DAR conditions, and three replicates of 50 seeds per treatment were tested for germination at both constant 15°C and alternating 25/15°C (12/12 h photoperiod). After each retrieval from DAR, additional seeds from the cool–dry treatment (three replicates of 50 seeds) were sown on agar containing 200 mg l^{-1} GA_3, and tested for germination at both 15°C and 25/15°C. Final germination was counted after 4 weeks, and any non-germinated seeds were cut open and examined for viability. Seeds with a firm, fresh endosperm and white embryo were considered viable, and empty or necrotic seeds were excluded when calculating percentage germination.

Statistical analysis

All statistical analysis was carried out in Minitab 15 (Minitab Inc., State College, Pennsylvania, USA, 2007). Since controlled-temperature glasshouses were not replicated, the effect of soil moisture and replicate were analysed nested within temperature environment. A nested ANOVA [General Linear Model (GLM)] analysed seed numbers per plant (square root transformed), mean seed weight and pre- and post-banking seed viability and germination data (arcsine transformed). Sigmoidal Gompertz 3-parameter curves were generated with SigmaPlot (Version 7, SPSS Inc., Chicago, Illinois, USA) and fitted to non-transformed germination data (one curve per replicate). A nested ANOVA (GLM) was performed to compare curve parameters a (maximum percentage germination), b (slope) and $x0$ (time to reach 50% germination, in weeks or t_{G50}). A one-way ANOVA compared curve parameters between the two germination temperature regimes. A split-plot ANOVA (GLM) analysed the effect of treatment duration on seed dormancy status.

Results

Parental seed germination

Application of GA_3 stimulated germination of the majority of parental *A. uliginosum* seeds at 25/15°C, 12/12 h photoperiod following storage at 15°C and 15–20% RH for 18 months (data not shown). Immediate transferral to fresh agar medium avoided seedling abnormalities often observed following the use of GA_3.

Plant growth and seed production

The time between the start of flowering and the beginning of mature seed dispersal in the warm environment (mean 26°C) was 43 d. Warm–wet plants produced *c.* 18,000 seeds and warm–dry plants *c.* 14,000 seeds over the course of the next 32 d, before

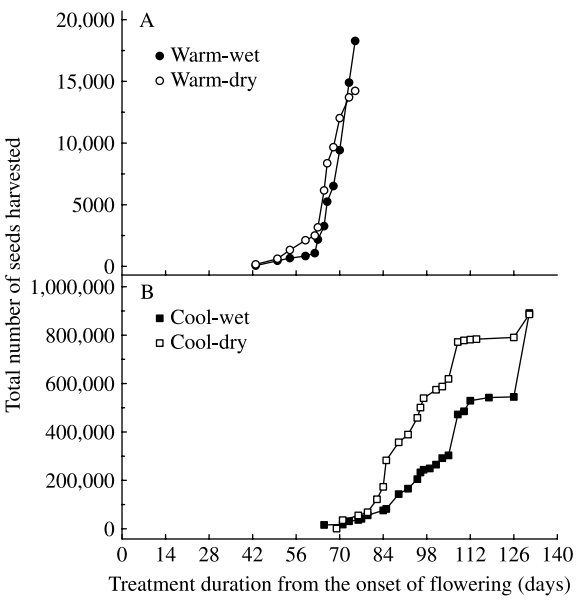

Figure 1. Cumulative total number of seeds harvested from *Actinobole uliginosum* plants grown in a (A) warm or (B) cool environments, with adequate (wet) or limited (dry) soil water during the reproductive phase. Day zero of treatment duration refers to the onset of flowering, and the experiment was terminated after 131 d of treatment. Each treatment contained three replicates of 12 plants.

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plants died after a total of 75 d in warm treatment conditions (Fig. 1A). In the cool environment (mean 17°C), times between flowering and the onset of seed dispersal were 65 and 69 d in the cool–wet and cool–dry treatments, respectively. Approximately 800,000 seeds were produced by both cool–wet and cool–dry plants, over the course of the next 66 or 62 d, respectively (Fig. 1B). Seeds in the cool–wet treatment matured more slowly than those in under cool–dry conditions, and after a total of 131 d in cool treatment conditions, all remaining seeds were harvested regardless of maturity, and the experiment was terminated (Fig. 1B).

Plants from the warm environment yielded far fewer mature seeds per plant (GLM: $F = 1164.5$, df $= 1$, $P \leq 0.001$; Fig. 2A) than those from the cool environment, but seeds were greater in mass (GLM: $F = 26.1$, df $= 1$, $P \leq 0.001$; Fig. 2B). Across all treatments, pre-banking seed viability was high (mean 80.4 ± 2.3%), and although there was an apparent increase in viability post-banking for seeds from the warm-dry treatment, viability was not significantly affected by the seed-banking process overall (GLM: $F = 3.31$, df $= 4$, $P = 0.017$; Fig. 2C).

Seed dormancy status

There was no germination of F_1 seeds, pre- or post-banking, in the absence of GA_3 at 25/15°C (data not shown). When germination was assessed at 15°C, *c.* 15% of seeds harvested from the warm environment were able to germinate (Fig. 3A). Seed PD was gradually alleviated by increasing durations of DAR at 20/34°C and 40% RH in darkness, evident by the increasing percentage germination of seeds when retrieved and incubated in germination test conditions (Fig. 3). Beyond *c.* 20 weeks of DAR, dormancy alleviation of all seeds no longer increased with further DAR, so that *c.* 20% of seeds were unable to

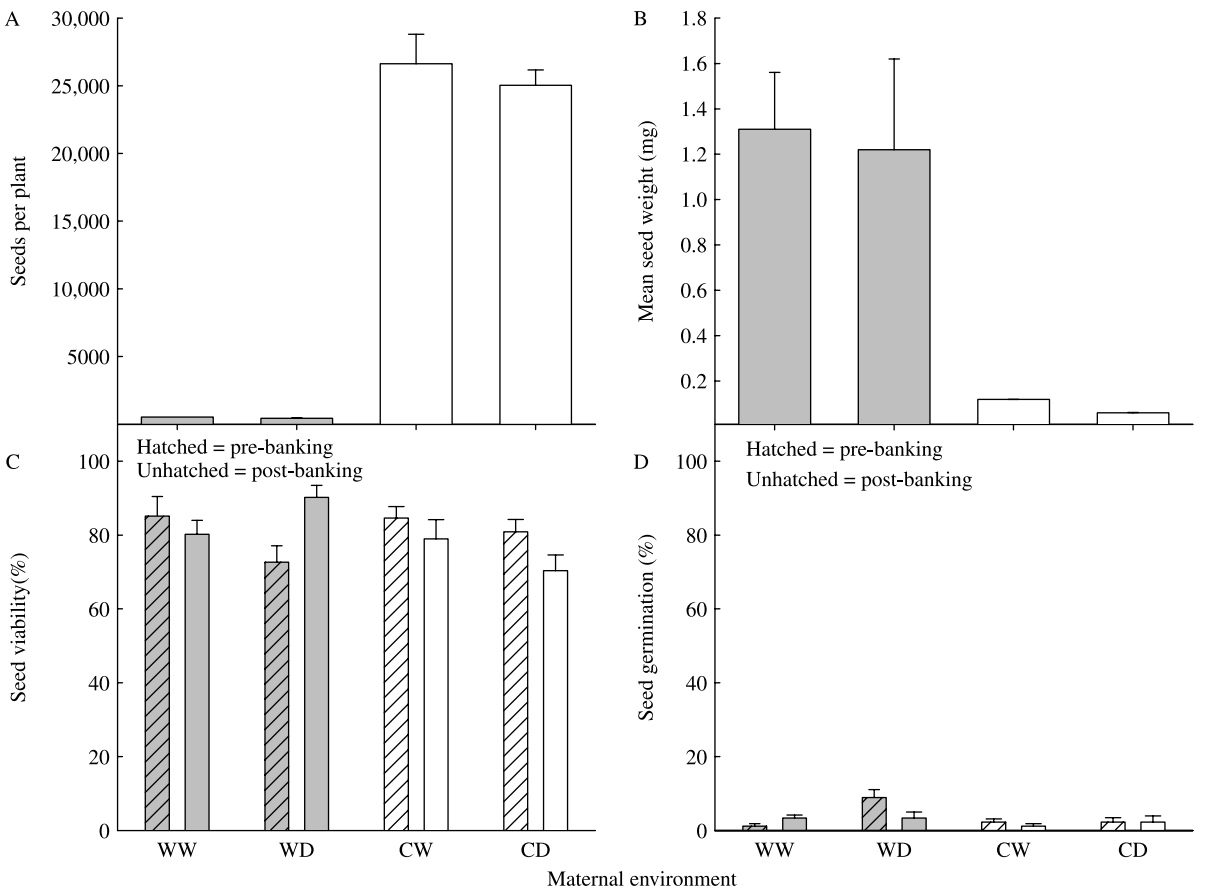

Figure 2. The effect of a warm–wet (WW), warm–dry (WD), cool–wet (CW) and cool–dry (CD) environment during the reproductive phase of *Actinobole uliginosum* on (A) the number of seeds harvested per plant, (B) mean seed weight, (C) seed percentage viability as determined by tetrazolium staining pre- and post-banking, and (D) seed germination at 25/15°C, 12/12 h photoperiod after 4 weeks application of GA_3 pre- and post-banking (mean ± SE).

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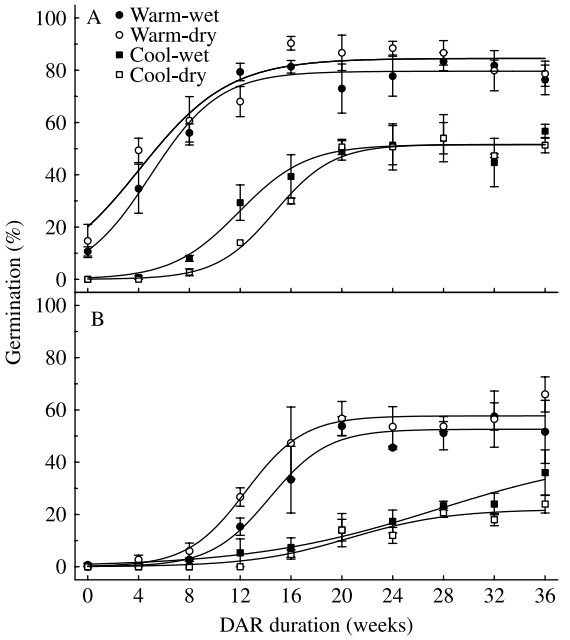

Figure 3. The effect of a warm–wet, warm–dry, cool–wet and cool–dry environment during the reproductive phase on final percentage germination (mean ± SE) of *Actinobole uliginosum* at (A) constant 15°C and (B) alternating 25/15°C, 12/12 h photoperiod, after increasing durations of dry after-ripening (DAR) at 34/20°C. A sigmoidal three parameter model was fitted to the data.

germinate in any circumstances. Apparent dormancy alleviation was dependent on germination test temperature. At constant 15°C (Fig. 3A), maximum germination was greater (one-way ANOVA: $F = 7.57$, df = 2, $P = 0.003$), and 50% germination was achieved after less DAR (one-way ANOVA: $F = 8.29$, df = 1, $P = 0.009$), when compared to alternating 25/15°C (Fig. 3B). Between maternal treatment temperatures, a greater proportion of the seeds from the warm environment lost PD during the DAR treatment before germinating at 15°C (one-way ANOVA: $F = 93.9$, df = 1, $P = 0.001$) or 25/15°C (one-way ANOVA: $F = 2.82$, df = 1, $P = 0.001$). Similarly, seeds from the warm environment required less DAR before germination to alleviate PD of 50% of seeds at 15°C when compared to seeds from the cool environment (one-way ANOVA: $F = 145.1$, df = 1, $P \leq 0.001$; Fig. 3A).

The cool–dry treatment was expected to mimic most closely conditions that *A. uliginosum* would experience during the reproductive phase, *in situ*. Therefore, seeds produced during this treatment were used for further investigation. Seeds produced in the cool–dry treatment at the beginning of the harvest period responded better to DAR than seeds produced at the end, when germinated at both 15°C (GLM: $F = 14.2$, df = 1, $P = 0.020$) and 25/15°C (GLM: $F = 10.9$, df = 1, $P = 0.030$; Fig. 4). As PD was alleviated, germination was again higher at 15°C (Fig. 4A) compared to 25/15°C (Fig. 4B).

Seed germination in the presence of GA_3 at 25/15°C was low across all four treatments (mean 3 ± 1% germination), and was unaffected by the seed-banking process (GLM: $F = 2.14$, df = 4, $P = 0.088$; Fig. 2D). Increasing durations of DAR caused seeds from the cool–dry treatment to become increasingly responsive to GA_3 (Fig. 5). Germination reached 97% at 15°C following just 4 weeks of DAR, and between 86 and 95% of seeds were stimulated to germinate after 36 weeks of DAR, regardless of germination temperature, which was far greater germination than when seeds were tested without GA_3 (Fig. 5).

Discussion

There was a gradual increase in germination of *A. uliginosum* following dry after-ripening (DAR) at 20/34°C, 40% RH, in darkness, confirming that mimicking summer field conditions alleviates PD of *A. uliginosum*. Many plant families worldwide respond

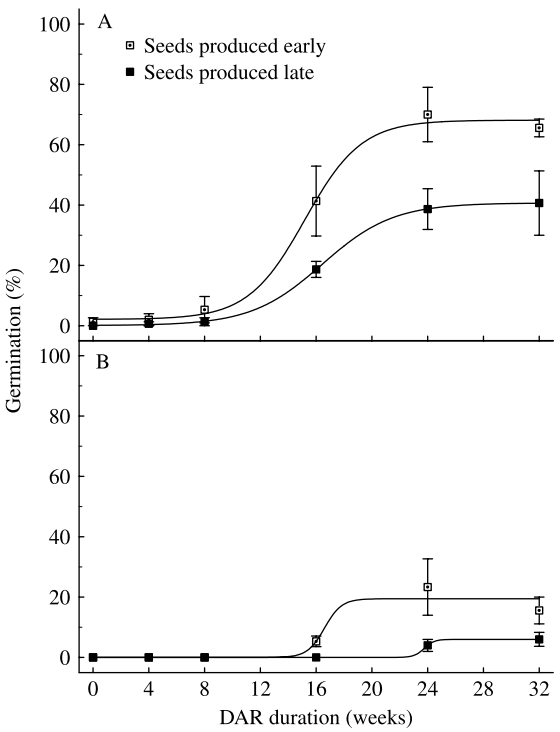

Figure 4. The effect of seed production time on final percentage germination (mean ± SE) at (A) 15°C and (B) 25/15°C, 12/12 h photoperiod, of *Actinobole uliginosum* seeds from a cool–dry environment after increasing durations of dry after-ripening (DAR) at 34/20°C. Sigmoidal three parameter curves were fitted to the data.

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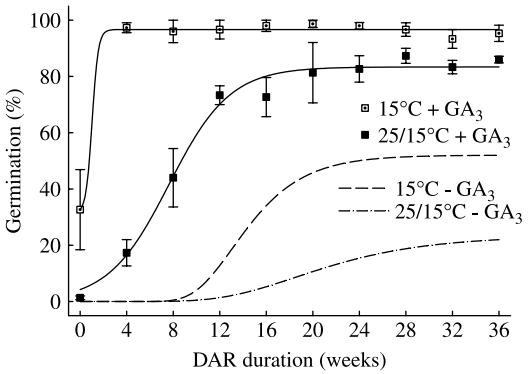

Figure 5. The effect of application of GA_3 ($200\,mg\,l^{-1}$) on final percentage germination (mean ± SE) at 15°C and 25/15°C, 12/12 h photoperiod, of *Actinobole uliginosum* seeds from a cool–dry environment after increasing durations of dry after-ripening (DAR) at 34/20°C. Sigmoidal three parameter curves were fitted to the data. Dotted lines taken from Fig. 4A and B for comparison.

to DAR as a means of alleviating PD (Baskin and Baskin, 2001), including other Australian *Asteraceae* such as *Schoenia filifolia* subsp. *subulifolia* (Peishi *et al.*, 1999), *Millotia myosotidifolia*, *Podotheca gnaphalioides*, *P. chrysantha* and *Ursinia anthemoides* (Schutz *et al.*, 2002). Durations of DAR longer than 20 weeks failed to increase dormancy alleviation of *A. uliginosum*, with *c.* 20% of seeds unable to germinate at 15°C or 25/15°C following 20–36 weeks of DAR treatment. This limited response may have been predetermined by the maternal environment during seed development, as previously seen for *Lolium rigidum* (Steadman *et al.*, 2004). Conversely, the DAR treatment may not have optimized PD alleviation, nor the germination test conditions maximized germination. Dormancy release of *L. rigidum* was slower during *ex situ* DAR than under field conditions (Steadman *et al.*, 2003), and *A. uliginosum* seeds dispersed in spring in south-western Queensland are unlikely to experience warm, dry periods of longer than 6 weeks *in situ* (Bureau of Meteorology, 2007). Indeed, parental *A. uliginosum* seeds responded better to DAR interspersed with short durations of warm stratification, mimicking the sporadic summer rain events in Queensland (Hoyle *et al.*, 2008b). In the present study, germination was significantly greater at a constant 15°C when compared to alternating 25/15°C, suggesting that germination of *A. uliginosum* is stimulated by the onset of cool autumn temperatures, to ensure that some seeds postpone germination until the hot summer months have passed.

Manipulating air temperature during the reproductive phase of *A. uliginosum* influenced dormancy status of the developing seeds. Seeds that developed at warmer temperatures responded better to DAR when compared to seeds from the cooler environment. A similar, although less dramatic, response to maternal temperature was reported for another forb native to south-western Queensland, *Goodenia fascicularis* (*Goodeniaceae*) (Hoyle *et al.*, 2008c). In addition, our results are similar to those observed for PD seeds of crop and exotic species. For example, *L. rigidum* seeds (Steadman *et al.*, 2004) and *Onopordum acanthium* (*Asteraceae*) cypselas (Qaderi *et al.*, 2006) that matured at *c.* 27°C exhibited less dormancy than those from plants grown at *c.* 18°C. Similarly, post-anthesis heat shocks of >30°C for >12 d reduced dormancy of five dormant *Triticum aestivum* (*Poaceae*) genotypes (Biddulph *et al.*, 2007).

In the warm environment, reproduction of *A. uliginosum* was accelerated so that within *c.* 11 weeks of treatment, plants had dispersed all seeds and died. In comparison, plants in the cool environment continued to produce seeds for a further 8 weeks. Accelerated physiological and biochemical processes in the warm environment may have acted to circumvent stages in the development of dormancy mechanisms, resulting in seeds requiring less DAR before germinating. Alternatively, having spent longer on the parent plant, seeds from the cool environment may have incurred greater PD through the accumulation of germination inhibitors, enzymes or plant growth regulators. *Avena fatua* (*Poaceae*) (O'Donnell and Adkins, 2001) and *O. acanthium* (Qaderi *et al.*, 2006) plants were also found to complete their development faster at warmer temperatures and disperse seed of lower dormancy status than plants grown at cooler temperatures. Increased dormancy with time spent on the parent plant may also explain why *A. uliginosum* seeds produced early in the reproductive phase, in the cool–dry treatment, responded better to DAR than those produced later. Similar results were reported for *O. acanthium* cypselas produced at high temperatures; those produced later were more dormant than those produced early, possibly due to increased amounts of phenolic compounds and seed coat wax content (Qaderi *et al.*, 2006). However, *G. fascicularis* seeds produced in four different maternal environments, early and late in the reproductive phase, exhibited no difference in PD status (Hoyle *et al.*, 2008c). Towards the end of the experiment, due to the difference in duration of seed development between temperature treatments, *A. uliginosum* plants in the cool environment will have experienced a slight increase in day lengths, which may also have acted to reduce seed germinability; a decreasing photoperiod during maturation of *Lactuca serriola* (*Asteraceae*) achenes in the field was associated with increasing germinability (Gutterman, 1992).

The effect of manipulating water availability during the reproductive phase had little effect upon PD status of *A. uliginosum*. This is contrary to results found for members of the *Poaceae*, including *Avena fatua* (Peters, 1982), *Sorghum halepense* (Benech Arnold *et al.*, 1992) and *Bromus tectorum* (Meyer and

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Allen, 1999), and others such as *Sinapis arvensis* (*Brassicaceae*) (Luzuriaga *et al.*, 2005). However, the effect of maternal water availability on dormancy status is commonly minimal (Steadman *et al.*, 2004; Swain *et al.*, 2006), particularly for species native to semi-arid regions (Llorens *et al.*, 2003; Gonzalez-Rodriguez *et al.*, 2005; Aragon *et al.*, 2008).

Germination response to GA_3 is routinely used to classify the level of PD (Baskin and Baskin, 2004). Parental *A. uliginosum* seeds were classified as having non-deep PD following dry storage for 16 weeks at 15°C, due to a significant germination response to the application of GA_3 (Hoyle *et al.*, 2008a). Eighteen months later, GA_3 was successfully used to germinate these same seeds for the present study. However, freshly harvested F_1 seeds would be classified as exhibiting deep PD due to their very limited response to GA_3. In addition, the present study showed that DAR at 34/20°C caused seeds to become increasingly responsive to GA_3 to achieve far greater percentage germination than when tested without GA_3. For many species, changes in dormancy status equate not only to changes in the conditions necessary for germination, but also seed sensitivity to the effect of germination triggers such as light, nitrate (e.g. Derkx and Karssen, 1993) and fluctuating temperatures (e.g. Benech-Arnold *et al.*, 1990). Results suggest that post-harvest handling and storage must be considered if response to GA_3 is to be used in the classification of PD level.

The level of stress experienced by a plant is relative to its natural environment, and higher stress results in a decline in overall plant performance (Lambers *et al.*, 1998). In the cool environment in our study, plants yielded *c.* 55 times more seeds than plants in the warm environment. Examination of plants from the warm environment failed to uncover any pathological reason for their seemingly premature death. Results suggest that the cooler environment, which closely matched temperatures common in south-western Queensland when these plants are reproducing *in situ* (Bureau of Meteorology, 2007), caused plants less overall stress. Larger seeds resulting from a warm environment may store more nutrients and, therefore, have a higher tolerance to nutrient deprivation upon dispersal, advantageous in a stressful environment (Krannitz *et al.*, 1991). Smaller, more dormant seeds from cooler environments may contribute to a more persistent seed bank (Thompson and Grime, 1979).

We can conclude that *A. uliginosum*, although equally adapted to and tolerant of variable rainfall events, is more sensitive to maternal air temperature than other Australian forbs such as *G. fascicularis* (Hoyle *et al.*, 2008c). Seeds are likely to exhibit deep PD at dispersal, which can be partially alleviated by *ex situ* DAR, regardless of maternal environment, before GA_3 will stimulate seed germination. Since *A. uliginosum* flowers during winter and early spring, cooler temperatures during seed development probably help to ensure that seeds remain discerning with regards to when and where germination occurs. However, the predicted climate change for Queensland (increased temperatures, fewer/less predictable rain events) may lead to the earlier production of less dormant seeds and consequently more inappropriate, 'risky' germination.

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