Physiological dormancy in forbs native to south–west Queensland: Diagnosis and classification

G.L. Hoyle a,⁎, K.J. Steadman b, M.I. Daw c, S.W. Adkins a

a Integrated Seed Research Unit, School of Land, Crop and Food Sciences, The University of Queensland, St. Lucia, QLD, 4072 Australia
b School of Pharmacy, The University of Queensland, St. Lucia, QLD 4072, Australia
c Seed Conservation Department, Royal Botanic Gardens Kew, Wakehurst Place, Ardingly, West Sussex RH17 6TN, United Kingdom

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Abstract

Seed physiological dormancy (PD) is reportedly the primary reason why many Australian native plants are not currently used for revegetation of degraded land. However knowledge of germination and dormancy of forb species from semi-arid environments is lacking. Consequently, we investigated germination of 15 Australian forb species from four families, particularly Asteraceae, native to south–west Queensland (Qld). Seeds were tested for viability using tetrazolium chloride (TZ) and sown at 5 to 35 °C. Nine species, including seven Asteraceae, achieved germination exceeding or not significantly lower (P>0.05) than TZ test results. Despite spring dispersal, the majority of species had optimal germination at temperatures reminiscent of winter months. Only six species exhibited low germination across all temperatures investigated when compared to TZ results (P<0.05), i.e. low germination could not be attributed to low seed viability. Of these, Actinobole uliginosum (Asteraceae) had non-deep PD since seeds responded to gibberellic acid (GA3) and dry after-ripening. In contrast, Goodenia fascicularis appeared to exhibit deep PD since seeds did not respond to GA3 or dry after-ripening, and scarification led to germination of abnormal seedlings. It appears that, contrary to expectations, seeds of many forbs native to south–west Qld (9 of 15 in this study), possess negligible or no dormancy and may therefore be suitable for use in land rehabilitation. Other species e.g. G. fascicularis require further work to investigate dormancy mechanisms and develop reliable germination techniques before seeds can be used effectively.

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1. Introduction

Dormancy is a seed characteristic, the degree of which defines the conditions necessary for seed germination. This block or series of blocks(s) within a dormant seed prevents germination, despite adequate water availability, temperature and gaseous conditions for germination (Benech-Arnold et al., 2000). Therefore dormancy is not due to the absence of environmental factors required to initiate germination and must be considered separately from germination requirements (Vleeshouwers et al., 1995). For both conservation and restoration, it is crucial that dormant seeds are distinguished from non-viable seeds. Viability can be underestimated if, in the absence of germination, seed dormancy is not diagnosed. In addition, reliable methods for alleviating (or at least bypassing) dormancy are essential for effective use and conservation of seeds. Once dormancy is diagnosed, classification of the type and level of dormancy can aid investigation of ‘dormancy breaking’ treatments and germination requirements (Baskin and Baskin, 2004).

It is often possible to bypass the block(s) to germination and stimulate PD seeds to germinate by applying agents such as gibberellins, e.g. GA3 (Cohn et al., 1989; Foley, 1992). However, such ‘germination inducing factors’ do not affect dormancy status (Vleeshouwers et al., 1995) and can result in seedling abnormalities. In contrast, PD can be alleviated altogether by applying treatments that regulate temperature and seed moisture content (Benech-Arnold et al., 2000; Vleeshouwers and Bouwmeester, 2001). Such approaches are also likely to promote the development of normal, healthy seedlings.
The most recent attempt to classify the observed diversity of dormancy types and levels (Baskin and Baskin, 2004) builds upon Nikolaeva’s comprehensive system (Nikolaeva, 1969). Physiological dormancy (PD), the most common form of dormancy (Baskin and Baskin, 2004), is thought to be caused by a physiological state of the embryo and possibly a decreased gas permeability of seed covering structures (Baskin and Baskin, 2001).

Three levels of PD are recognized: non-deep, intermediate and deep (see Baskin and Baskin, 2004). Physical dormancy (PY) is due to a water impermeable seed or fruit coat, morphological dormancy (MD) requires that an underdeveloped embryo grows before germination can commence and combinations of PD and MD also exist (MPD) (Baskin and Baskin, 2001).

In recent years, partly due to increasing demands for biologically diverse land rehabilitation, seed dormancy of Australian native seeds has received considerable attention. It is frequently reported that the majority of Australian plant species possess seed dormancy mechanisms of some sort (Bell, 1999; Koch and Dixon, 2000; Tieu et al., 2002; Merritt et al., 2007), and that species in diverse families, including Euphorbiaceae, Asteraceae and Goodeniaceae (i.e. herbaceous, non-grassy species), have unknown dormancy alleviation and germination requirements. As a result, dormancy is cited as the greatest obstacle to the effective use of native seed in land revegetation (Merritt et al., 2007). Furthermore, the majority of previous studies have focused on native grasses, hard-coated PY seeds, and species native to temperate and Mediterranean-type regions within Australia. Consequently, this study aimed to identify seed dormancy in a range of previously unstudied Australian forb species spanning four families native to the semi-arid tropical region of south-west Qld.

### 2. Materials and methods

#### 2.1. Seed collecting and processing

Seeds of 15 species across four families of native forbs were collected in south-west Qld between October and December 2004 (see Table 1). Where possible, at least 10,000 seeds/species were collected from >50 individual plants. Only mature seeds close to the point of natural dispersal were collected. Following collection, seeds were treated as if they were to be used in land revegetation by being stored in paper envelopes at 15 °C and 15 to 20% relative humidity (RH) for between 10 and 16 weeks before being used in germination experiments. Non-seed material was removed by hand, aspirator or by rubbing seed capsules/flowers through a sieve using a rubber bung.

#### 2.2. Seed viability and germination testing

Viability of 3 replicates of 20 seeds/species was assessed using the tetrazolium chloride (TZ) staining technique (ISTA, 2003). Seeds were initially hydrated on plain agar for 24 h at room temperature before being scarified (away from the embryo axis) and placed in TZ solution at 30 °C and darkness for 24 h. Seeds were then cut in half and examined. Only uniformly stained red/dark pink embryos were considered ‘viable’.

Germination tests used 3 replicates of 15 seeds each sown into 9 cm diameter plastic Petri dishes containing 1% agar-water. Petri dishes were sealed inside plastic bags to avoid agar desiccation. Seeds of all species were placed in germination incubators at 15, 20, 25 and 30 °C, with constant light provided by fluorescent tubes (ca. 50 μmol m⁻² s⁻¹). Five of the species (A. uliginosum, Brachyscome melanocarpa, Camptacra barbata, Plantago cunninghamii and Goodenia fascicularis) were also sown at 5, 10 and 35 °C. Germination, defined as radicle emergence by at least 1 mm, was scored every 7 d, germination tests ran for 60 d and any non-germinated seeds were assessed using a cut test. Empty or necrotic seeds were excluded when calculating percentage germination.

#### 2.3. Treatment conditions

Based on high viability (results of TZ tests) and low germination percentages, two species (A. uliginosum and G. fascicularis) underwent further investigation. Gibberellic acid (GA₃) was applied to seeds via incorporation into the agar germination medium; seeds received either constant application of 250, 125 or 62.5 mg/L throughout the germination test, or a pulsed application of 250 mg/L for 24 h before seeds were moved to plain agar for germination. Mechanical scarification...
was applied to seeds using a scalpel blade; *G. fascicularis* seeds were completely de-coated, or a small piece of seed coat removed from the vicinity of the radicle or cotyledons, before seeds were moved to germination test conditions. Seeds receiving a dry after-ripening (DAR) treatment were stored in paper envelopes inside a drying oven at 40 °C, 15–20% RH and darkness for four weeks before being moved to germination test conditions. Imbibition curves for scarified and non-scarified seeds were obtained by periodically weighing seeds as they imbibed on agar at room temperature. Germination tests were carried out at constant 20 °C (*G. fascicularis*) or 15 °C (*A. uliginosum*), 12/12 h light/dark.

2.4. Statistical analysis

A 2-sample *t*-test was carried out/species to assess whether there was a difference between observed maximum percentage germination and seed viability. To assess differences between combinations/concentrations within treatments upon *A. uliginosum* and *G. fascicularis*, one-way ANOVA was carried out on
arcsine transformed data (Zar, 1984). ANOVA pair-wise comparisons were made using Fisher’s 1 SD test at 5% significance level. Statistical analysis was carried out in Minitab 15 (Minitab Inc., 2007).

3. Results

3.1. Seed viability and germination

Seed viability of all species ranged from 65 to 100% according to TZ staining (Fig. 1). Seeds of all 15 species responded to at least one of the constant temperatures, to achieve between 10% (A. uliginosum at 15 °C) and 100% (C. barbata at 15 °C) final germination (Fig. 1). The majority of species reached maximum germination at 15 °C (9 species in total). However B. melanocarpa responded better to 10 °C and G. fascicularis responded better to 20 °C (Fig. 1). Of the five species sown at the full range of germination temperatures (5 to 35 °C) four germinated at 5 °C; A. uliginosum (5%), B. melanocarpa (38%), C. barbata (100%) and P. cunninghamii (100%), and there was no germination of any species above 25 °C. In relation to seed viability, a total of 9 species achieved germination that exceeded, equaled or was not significantly lower (2-sample t-test: \( P \leq 0.05 \)) than results from TZ testing: B. melanocarpa (Fig. 1b), Calotis cuneifolia (Fig. 1c), C. barbata (Fig. 1d), Leiocarpa brevicompta (Fig. 1f), Leptorhynchos baileyi (Fig. 1g), Pycnosorus chrysanthus (Fig. 1i), Wahlenbergia timidiiflora (Fig. 1o). The remaining six species achieved significantly lower maximum germination (2-sample t-test: \( P \leq 0.001 \)) compared with viability, at the temperatures investigated; A. uliginosum (Fig. 1a), Gnephosis arachnoidea (Fig. 1e), Rhodanthe floribunda (Fig. 1j), Rhodanthe moschata (Fig. 1k), Vittadinia pterochaeta (Fig. 1l) and G. fascicularis (Fig. 1n). A. uliginosum and G. fascicularis exhibited the highest viability coupled with the lowest germination and were therefore chosen for further investigation.

3.2. G. fascicularis

Intact and scarified G. fascicularis seeds imbibed at the same rate and to the same extent (Fig. 2a) and it was observed that seeds contained large spatulate embryos and relatively little endosperm. There was little response to constant application of any concentration of GA3 at 20 °C for 60 days (one-way ANOVA: \( F = 2.39, \) \( \text{d.f.} = 3, \) \( P = 0.144 \)); maximum germination was only 18% with 125 mg/L GA3 (Fig. 2b). Similarly, there was little response to the DAR either with or without GA3 in the germination media (one-way ANOVA: \( F = 1.62, \) \( \text{d.f.} = 3, \) \( P = 0.260 \)); DAR followed by constant application of 125 mg/L GA3 achieved only 22% germination (Fig. 2c). Seeds responded to mechanical scarification and de-coating prior to germination testing (one-way ANOVA: \( F = 6.94, \) \( \text{d.f.} = 3, \) \( P = 0.013 \)), for example scarification above the radicle stimulated 71% germination after 60 days at 20 °C (Fig. 2d). However de-coating seeds caused seedlings to
Many Australian plant species synchronise seed germination with the reliable winter–rainfall period in their native habitat (Bellairs and Bell, 1990; Curtis, 1996). While semi-arid tropical Qld does not experience a ‘drought’ season in the strict sense (rainfall is distributed throughout the year), summer temperatures are high (mean min. and max. temperatures of 22 and 35 °C respectively). Consequently, lower mean min. and max. autumn (14 to 28 °C) and winter (7 to 20 °C) temperatures (BoM, 2000), which will result in reduced evapo-transpiration, may signal optimal germination conditions and increased chances of successful seedling establishment. In support of this we found that maximum germination of many of the forbs investigated was dependent upon cooler temperatures (ca. 15 °C) that are not necessarily present at the time of seed dispersal. Thus, rather than possessing seed dormancy, germination in these species may simply be temperature dependent (Baskin and Baskin, 2001), i.e. seeds can only germinate in autumn/winter despite being dispersed in summer.

Low germination when compared to TZ results suggests that seeds of six of the species investigated possess dormancy mechanisms of some sort, although with the exception of G. fascicularis and A. uliginosum for which there was further investigation, we are unable to confirm dormancy type. Imbibition curves for intact and scarified seeds of G. fascicularis revealed that low germination was not a result of seed impermeability or PY (Fig. 2a), results that are supported by previous studies that have found no evidence of PY in this family (Baskin et al., 2006). After observing the large embryo size relative to seed size, MD and MPD were also ruled out. Germination of G. fascicularis was slow and gradual and continued to increase beyond 4 weeks (data not shown). Water permeability, low embryo length: seed length ratio and slow germination are common characteristics of seeds exhibiting PD (Baskin and Baskin, 2001). The fact that these seeds also failed to respond to GA3, dry after-ripening and exhibited seedling abnormalities post-scarification suggested deep PD (see Baskin and Baskin, 2004). Similarly, PD has been diagnosed for a number of species belonging to this family (Sugimoto and Lidbetter, 2002; Cochrane and Probert, 2006) with studies suggesting that a combination or series of factors may be required to achieve maximum germination of other Goodeniaceae species.

Germination of A. uliginosum remained low at all temperatures (Fig. 1a). Constant application of 250 mg/L GA3 bypassed PD to achieve 100% germination (Fig. 3a). In addition, the DAR treatment alleviated PD to achieve >60% germination (Fig. 3b). These results suggest that A. uliginosum possesses non-deep PD, a conclusion that is supported by reports of germination requirements for other Australian Asteraceae genera (Bunker, 1994; Plummer and Bell, 1995; Peishi et al., 1999).

The role of dormancy is to prevent germination during favorable conditions, when the resulting plant is not likely to survive and reproduce (Vleeshouwers et al., 1995). We conclude that the species investigated in this study exhibit strategies for avoiding germination during superficially suitable spring and early summer conditions. Some species exhibit dormancy mechanisms that are likely to exist to postpone germination until after the summer. However other species simply have a germination requirement for cool temperatures that are more indicative of autumn/winter. Since the majority of species studied germinated readily without

**3.3. A. uliginosum**

Constant application of 250 mg/L GA3 at 15 °C achieved 100% germination of A. uliginosum, and a 24 h pulse achieved 38% germination (Fig. 3a). Seeds were also responsive to the 4 week DAR treatment (one-way ANOVA: $F=23.6$, $d.f.=2$, $P=0.001$); germination increased gradually to 63% at 15 °C (Fig. 3b).

4. Discussion

Physiological dormancy (PD) is reported to be common in seeds of Australian native plants including species in the Asteraceae and other forb genera (Merritt et al., 2007). Despite this, 9 species out of 15, including 7 Asteraceae species, achieved germination that matched viability, without application of germination stimulants or dormancy alleviating treatments (Fig. 1). If dormancy alleviation occurred in the seed storage conditions, it was likely to have been very slow due to low seed moisture content and low temperature (Steadman et al., 2003). Therefore we concluded that seeds of these species possessed negligible or no dormancy upon dispersal and did not have a requirement for alternating temperatures.
‘dormancy breaking’ treatments, these native forbs are likely to be suitable for incorporation into land rehabilitation.

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References


