Ecophysiology of Seed Dormancy in the Australian Endemic Species
Acanthocarpus preissii (Dasypogonaceae)

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• **Background and Aims** Seedlings of Acanthocarpus preissii are needed for coastal sand dune restoration in Western Australia. However, seeds of this Western Australian endemic have proven to be very difficult to germinate. The aims of this study were to define a dormancy-breaking protocol, identify time of suitable conditions for dormancy-break in the field and classify the type of seed dormancy in this species.

• **Methods** Viability, water-uptake (imbibition) and seed and embryo characteristics were assessed for seeds collected in 2003 and in 2004 from two locations. The effects of GA3, smoke-water, GA3 + smoke-water and warm stratification were tested on seed dormancy-break. In a field study, soil temperature and the moisture content of soil and buried seeds were monitored for 1 year.

• **Key Results** Viability of fresh seeds was >90%, and they had a fully developed, curved-linear embryo. Fresh seeds imbibed water readily, with mass increasing approx. 52% in 4 d. Non-treated fresh seeds and those exposed to 1000 ppm GA3, 1 : 10 (v/v) smoke-water/water or 1000 ppm GA3 + 1 : 10 (v/v) smoke-water/water germinated <8%. Fresh seeds germinated to >80% when warm-stratified for at least 7 weeks at 18/33 °C and then moved to 7/18 °C, whereas seeds incubated continuously at 7/18 °C germinated to <20%.

• **Conclusions** Seeds of *A. preissii* have non-deep physiological dormancy that is released by a period of warm stratification. Autumn (March/April) is the most likely time for warm stratification of seeds of this species in the field. This is the first report of the requirement for warm stratification for dormancy release in seeds of an Australian species.

**Key words:** Acanthocarpus preissii, Dasypogonaceae, physiological dormancy, seed dormancy, seed germination, warm stratification.

**INTRODUCTION**

Although cold and/or warm stratification can break dormancy in seeds of many species (Baskin and Baskin, 1998, 2004a), their effect on dormancy-break has not been thoroughly documented in Australian species. Periods of (moist) cold stratification (4–5°C) for up to 90 d can increase germination percentage and rate in the woody species Allocasuarina verticillata (Moncur et al., 1997), Eucalyptus pauciflora (Beardsell and Mullett, 1984) and in one provenance of Banksia sasicola (Middleton et al., 1996), but no studies have tested the effect of warm stratification on seed dormancy-break in Australian species. Other studies have found that seeds of many Australian species are responsive to smoke and smoke products (Dixon et al., 1995; Flematti et al., 2004; Merritt et al., 2006), thermic pulsing (Tieu et al., 2001a), after-ripening (Schatz et al., 2002) and light (Plummer and Bell, 1995). Nevertheless, there remain a number of common Australian species that do not respond to these treatments and are difficult to germinate (Dixon et al., 1995; Tieu et al., 2001b; Merritt and Dixon, 2003). *Acanthocarpus preissii*, the subject of the present study, is a prime example of a species in this latter category.

The importance of incubation temperature on germination of seeds of Australian species has been demonstrated previously, particularly for those of south-west Western Australia (Bell et al., 1995). However, as yet there are no published studies highlighting the importance of sequential incubation temperatures (see Baskin and Baskin, 2004b) for release of intractable seed dormancy of Australian species.

*Acanthocarpus preissii* is a rhizomatous, tufted perennial herb 0·2–0·7 m tall that grows along the west coast of Western Australia from Exmouth to Pemberton, and is particularly common in sandstone/limestone sites and on coastal dunes that extend almost to the shoreline (Marchant et al., 1987; Paczkowska and Chapman, 2000; Wheeler et al., 2002; Barrett and Tay, 2005). Due to its rhizomatous habit, tough leathery leaves and ability to grow in nutrient-deficient soils that are subject to strong seasonal droughting, *A. preissii* is a highly desirable plant for coastal restoration and sand dune stabilization, particularly in exposed, high wind-energy environments where few other plants will grow and thrive. For these reasons, efficient propagation methods are needed for *A. preissii*. However, vegetative and seed-based propagation has proven to be difficult for this species.

The Dasypogonaceae is a predominantly Australian (Gondwanan) family with most species restricted to the

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south-west corner of Western Australia. Species belonging to this family are typically highly adapted and specialized to xerophytic perennials. Very little is known about the germination characteristics of species in this family. Plummer et al. (1995) reported for Lomandra sonderi and L. drummondii (Dasypogonaceae) seeds, that possess a white/yellow, adhering inner pericarp at the time of shedding, that dormancy could be overcome by pericarp removal and subsequent treatment with 50 mg L\(^{-1}\) gibberellic acid (GA\(_3\)) to promote embryo growth at the optimum temperature of 15–20°C.

The purpose of this study was to better understand the seed dormancy and germination characteristics of *A. preissii* by: (a) describing seed and embryo morphology and estimating the percentage of viable seeds; (b) determining via measurements of imbibition whether seeds are water-permeable or water-impermeable; (c) testing the effects of GA\(_3\), smoke-water and GA\(_3\) + smoke-water on germination of fresh seeds; (d) evaluating the effect of a warm stratification pre-treatment on germination responses of nearly fresh seeds; and (e) weekly monitoring of soil temperature and moisture and buried-seed moisture content in the field to identify the most likely time of dormancy-break in nature.

**MATERIALS AND METHODS**

**Seed sources**

Dry fruits of *Acanthocarpus preissii* Lehm. (Dasypogonaceae), consisting of a one, two or three-locular capsules (Fig. 1), were collected from coastal dunes in City Beach, Western Australia (approx. 10 km west of Perth) in December 2003 and in early November 2004 and from coastal dunes at Shark Bay (approx. 830 km north of Perth) in October 2004. Fruits were dried and dehiscence occurred over about a 2-week period. Seeds were cleaned and stored at ambient room conditions (approx. 22°C, approx. 50% RH) prior to experimentation. Experiments commenced in January 2004 (2003 City Beach accession) and in December 2004 (2004 City Beach and Shark Bay accessions).

**Seed characteristics and viability**

Seed viability for all accessions was estimated using a cut test on a random sample of three replicates of 20 non-imbibed seeds, rated on presence or absence of white endosperm and embryo. Length and mass of three replicates of 20 non-imbibed (dry) seeds were measured for each accession. Several additional non-imbibed seeds also were bisected longitudinally to determine embryo morphology based on Martin (1946). Seed lengths were measured using a binocular microscope equipped with an ocular micrometer.

**Imbibition of seeds**

An imbibition test was conducted at room temperature (22–24°C) on the 4-week-old 2003 City Beach accession using three replicates of 20 seeds. Seeds were weighed, moistened for 5 min in 90-mm Petri dishes lined with water-moistened seed germination papers, removed from the dishes, blotted dry and reweighed (time 0) and returned to the dishes. Then, seeds were removed from Petri dishes after 1, 2, 4, 8, 24, 48 and 96 h, blotted and reweighed. Percentage increase in seed mass was determined as described by Turner et al. (2006) and calculated as follows: 

\[
\text{Percentage increase in mass} = \frac{W_1 - W_d}{W_d} \times 100, \text{where } W_1 \text{ and } W_d = \text{mass of imbibed and dry seeds, respectively.}
\]

**Germination**

**Effects of GA\(_3\) and/or smoke-water.** Three replicates of 25 seeds from the 4-week-old 2003 City Beach accession were soaked for 24 h in deionized water (control) and solutions of 1000 ppm GA\(_3\), 1 : 10 (v/v) smoke-water/water or 1000 ppm GA\(_3\) + 1 : 10 (v/v) smoke-water/water. After soaking they were blotted dry and sown at a depth of 5 mm in punnets containing four parts composted jarrah (*Eucalyptus marginata*) sawdust, two parts nursery sand and one part river sand. Punnets were incubated at 17 ± 2°C with 12 h of dark and 12 h of light, which was supplied by standard white fluorescent tubes. Punnets were monitored and watered regularly, and emergence (i.e. appearance of a seedling above the soil surface) was scored fortnightly for up to 24 weeks.

**Warm stratification.** Six- to 8-week-old seeds (collected in November 2004) from City Beach and from Shark Bay (collected in October 2004) were soaked in 1 : 10 (v/v) smoke-water/water or in deionized water for 24 h. Seeds were surfaced sterilized in 1% (w/v) solution of calcium hypochlorite [Ca(OCl)\(_2\)] for 30 min and then rinsed three times in sterile deionized water. For all treatment combinations, 20 seeds were placed in 90-mm-diameter Petri dishes on sterilized white sand moistened with deionized water. For each treatment, five replicates were warm-stratified (pretreated) at 13/26°C or at 18/33°C for 2.5, 5, 7, 10 or 15 weeks, after which they were moved to 7/18°C for an additional 12 weeks. Seeds incubated continuously at 7/18°C for the duration of the experiment (27 weeks) served as the control. All seeds were incubated in darkness for the duration of the experiment (by wrapping Petri dishes with aluminum foil), except when the dishes were unwrapped every few weeks and seeds were exposed to room light for a few minutes to monitor seed germination. Seeds were scored for germination on the day they were moved from 13/26°C to 7/18°C or from 18/33°C to 7/18°C and thereafter every 2 weeks, with final germination scored after 12 weeks at 7/18°C (except control Petri dishes left for 27 weeks at 7/18°C). Petri dishes were moistened with deionized water as required.

**Embryo growth.** The purpose of this part of the study was to determine the size of *A. preissii* embryos and whether they grow within seeds before radicle emergence, in which case they would have morphological or morphophysiological dormancy (Baskin and Baskin, 2004a). For the 2004 City Beach accession, seed and embryo lengths (mm ± s.e.) were determined for three replicates each of ten 2-d hydrated seeds (time 0 control),
of seeds that had been warm stratified for 7 weeks at 18/33 °C and of seeds that had been warm stratified for 7 weeks at 18/33 °C followed by 3 weeks of incubation at 7/18 °C, just prior to radicle emergence.

Seed burial experiment. Soil temperature and soil and seed moisture content were monitored in the field to determine if seeds were hydrated during a time when temperatures were appropriate for warm stratification. Approximately 50 seeds from the 2003 City Beach provenance were placed in each of 52 bags consisting of 75-μm nylon mesh that allowed water penetration but excluded soil and other particles. Bags were placed 1 cm apart at a depth of 10–20 mm at Kings Park, Perth, Western Australia. After the seeds had been buried, plastic mesh was laid over the top of the soil to prevent disturbance by animals. Bags containing seeds were buried in December 2004 and retrieved weekly for 52 weeks.

After seeds were retrieved and removed from bags, moisture content was determined on three replicates of 20 seeds at 7/18 °C. Imbibition (increase in seed mass), germination percentage, seed length and mass, embryo length and embryo:seed ratio (E:S) data were analysed for statistical significance by analysis of variance (ANOVA). Germination data were arcsine-transformed prior to ANOVA (non-transformed data appear in all figures). Where ANOVA detected significant differences, Fisher’s least significant difference (LSD) test was used to determine if treatments were significant (P < 0.05).

RESULTS

Seed characteristics and viability

Seeds from the 2003 City Beach collection were 4.3 ± 0.1 mm (mean ± s.e.) in length and weighed 42.8 ± 1.0 mg. Length and mass of 2004-collected seeds were 4.4 ± 0.1 mm and 46.5 ± 2.0 mg, respectively, for those from City Beach, and 3.9 ± 0.1 mm and 35.6 ± 0.6 mg, respectively, for those from Shark Bay. Seed mass for the Shark Bay provenance was significantly less than it was for those from both City Beach collections (P < 0.05). More than 95 % of seeds from all accessions were viable. Embryos were curved-linear in shape (Fig. 1).

Imbibition of seeds

Seeds of A. preissii imbibed water readily and followed a typical pattern of initial rapid water uptake with seed mass increasing by 30.1 ± 0.9 % after 24 h, and 51.5 ± 0.9 % after 96 h with seed mass stabilizing beyond this point (data not shown) (P < 0.05).

Effect of GA3 and smoke-water

Neither GA3 (4.0 ± 2.3 % germination), smoke-water (5.3 ± 2.7 %), nor GA3 + smoke-water (6.7 ± 1.3 %) promoted germination compared with control seeds (4.0 ± 2.3 %) (P > 0.05) (data not shown).

Warm stratification

Germination responses were similar for fresh seeds from City Beach and Shark Bay that were warm stratified at 13/26 °C or at 18/33 °C (Fig. 2). In both cases, control seeds continuously incubated at 7/18 °C for 27 weeks germinated to <20 % (Fig. 2). Germination for all stratification treatments scored when Petri dishes were moved from 13/26 °C or 18/33 °C to 7/18 °C was 0–15 % (data not shown). Following movement of seeds to 7/18 °C, germination percentage increased significantly (P < 0.05), with germination commencing within 2–3 weeks for both Shark Bay and City Beach seeds warm stratified (pre-treated) either at 13/26 °C or 18/33 °C. Pre-treatment for ≥5 weeks at 18/33 °C resulted in ≥82 % germination for
Germination at 7/18°C of seeds from both provenances, pretreated at 13/26°C, was slower than that of seeds pretreated at 18/33°C. The highest germination percentage following stratification at 13/26°C for both provenances was for seeds stratified for 10 weeks and then moved to 7/18°C, with 92.0 ± 2.6% of the City Beach provenance and 74.0 ± 6.2% of the Shark Bay provenance germinated. No seeds germinated while they were at 13/26°C for 10 weeks (data not shown). Germination after stratification for 15 weeks at 13/26°C or 18/33°C and immediately prior to transfer to 7/18°C was 13–15% (13/26°C) and 4–14% (18/33°C) for City beach seeds and 4–9% (13/26°C) and 0% (18/33°C) for Shark Bay seeds. Seeds stratified for shorter periods (2.5, 5 and 7 weeks) germinated to lower percentages after they were moved to 7/18°C than either those warm-stratified for 10 weeks at 13/26°C or for 2.5, 5 and 7 weeks at 18/33°C. Overall, after they were moved to 7/18°C Shark Bay seeds responded more positively to pretreatment at 13/26°C (highest germination 89.0 ± 2.9%) than they did to pretreatment at 13/26°C (highest germination 74.0 ± 6.2%). The City Beach seeds pretreated at both regimes (13/26°C or 18/33°C) germinated to similar percentages. However, for the City Beach seeds, the duration of the warm-stratification pre-treatment required to achieve >80% germination after movement to 7/18°C was different, i.e. 10 weeks at 13/26°C (90.0 ± 5.7%) versus 5 weeks at 18/33°C (84.0 ± 1.0%). The only significant difference between germination of smoke-water-treated and water-control seeds was for seeds stratified at 13/26°C for 5 weeks prior to movement to 7/18°C (P < 0.05). In this case, 87.0 ± 3.4% of the smoke-water-treated seeds germinated compared with 52.0 ± 11.3% of those that were water-treated (Fig. 2). Seeds in the other treatments did not respond to smoke-water in terms of provenance, stratification temperature or duration of stratification.
Embryo growth

Length of 2-d hydrated 2004 City Beach seeds was 5.29 ± 0.02 mm and embryo length 3.71 ± 0.02 mm. Thus, mean E:S was 0.70, and it did not significantly change (P > 0.05) during stratification for 7 weeks at 18/33 °C, or even after incubation of seeds for an additional 3 weeks at 7/18 °C, just prior to radicle emergence (Table 1).

Seed burial experiment

The moisture content of A. preissii seeds buried under field conditions increased by 31 March 2005. From this time on, seeds remained constantly moist until 4 November 2005, after which moisture content began to decrease (Fig. 3) in concert with the onset of seasonal soil drying (see following data). Seed moisture content was highly variable, ranging from 5–9 % prior to hydration (January to March) to 70–99 % during the cooler wet season. Soil moisture was <0.2 % during the dry season (January to March) and 0.4 –11 % (average approx. 5 %), during the wet season (late March to early November) (Fig. 3). Germination of buried seeds was first observed on 19 May 2005, 7 weeks after they had begun imbibing water under field conditions, and germinating seeds were observed weekly from this point onwards, although most seedlings were dead by 30 September, by the time the seed bags were retrieved. Seeds were partially hydrated (approx. 23 % moisture content) on 10 March 2005, which was 2 weeks before the onset of constant wet conditions. Soil temperatures during the time seeds were constantly moist (31 March until the first germination event) were 13.8 to 25.2 °C. During the period of intermittent seed moisture (10–31 March), soil temperatures reached a maximum of 35.7 °C. Field-stored seeds were exposed to approx. 73 h of moist conditions at 20–25 °C prior to the first germination event (Table 2) and to 11 h at 25–30 °C while partially hydrated (during March; Table 2). Fully hydrated seeds were also exposed to warm moist conditions in spring (September to November), when temperatures were >20 °C for 241 h. In comparison, seeds stratified at 13/26 °C or at 18/33 °C for 2.5 or 7 weeks received 210, 420 and 588 h, respectively, at 26 °C or at 33 °C. Seeds incubated for 2 weeks under standard germination conditions (i.e. in darkness at 7/18 °C on moistened sand in Petri dishes) had a moisture content of 79.2 ± 1.1 %.

DISCUSSION

Seeds of Acanthocarpus preissii can germinate to high percentages after exposure to several weeks of warm, moist conditions followed by incubation in cool, moist conditions, a temperature–soil moisture sequence that simulates that of the autumn to winter transition in the southwest of Western Australia. This appears to be the first report of an Australian species for which a period of warm stratification promotes alleviation of seed dormancy. Prior to this study, the types of dormancy [morphological, physiological, morphophysiological, physical or combinational (physical + physiological); Baskin and Baskin, 1998, 2004a] and methods to release dormancy in A. preissii seeds was not known. Seeds of A. preissii imbibed water readily, increasing in mass by 52 % in 4 d under ex situ conditions, thus ruling out a water impermeable seed (or fruit) coat (i.e. physical dormancy and combinational dormancy). Morphological and morphophysiological dormancy were excluded as possible reasons for the delay in germination, since the embryo is not underdeveloped, i.e. it does not grow within seeds prior to germination (radicle emergence). Thus, seeds of A. preissii have physiological dormancy, i.e. they are water permeable, have a fully-developed embryo and require warm stratification to come out of dormancy (Baskin and Baskin, 1998, 2004a). Further, A. preissii seeds have non-deep physiological dormancy, since only a few weeks of warm stratification overcame dormancy (Fig. 2). Non-deep physiological dormancy is the most common kind of dormancy found in seeds (Baskin and Baskin, 1998, 2003), and therefore is most likely to be commonly encountered in the Australian angiosperm flora (Baskin and Baskin, 1998).

Fresh seeds of A. preissii did not respond to GA3, smoke-water or GA3 + smoke-water. GA3 has been shown to release seed dormancy in many species with physiological dormancy (Hagon, 1976; Bunker, 1994; Plummer et al., 1995). However, 50 mg L−1 GA3 was only slightly effective in promoting germination of seeds of Lomandra sonderi (Dasypogonaceae), and fails to promote germination of all physiologically dormant seeds (e.g. Boscaglì and Sette, 2001). On the other hand, whereas smoke-water failed to stimulate seeds of A. preissii to germinate, two species, Lomandra preissii and L. purpurea, from taxa closely related to A. preissii are responsive to both smoke and warm-stratification (S. R. Turner and D. J. Merritt unpublished results).

Field data on soil temperature and soil and seed moisture, combined with those from laboratory experiments, can be used to identify the likely time(s) of year when seeds of A. preissii are warm-stratified in the soil seed bank and the optimal temperatures and times required to promote seed germination. Results for 2005 (when the rainfall in Perth, Western Australia, was slightly above the long-term average) indicate that seeds were intermittently hydrated from 7 to 30 March, after which they were continuously fully hydrated. Maximum temperatures

| Treatment                                | Seed length (mm ± s.e.) | Embryo length (mm ± s.e.) | E:S ratio (± s.e.) |
|------------------------------------------|-------------------------|---------------------------|-------------------|
| Hydrated seeds (2 d at 18 °C)            | 5.29 ± 0.02             | 3.71 ± 0.02               | 0.70 ± 0.01       |
| Warm stratified seeds (18/33 °C for 7 weeks) | 5.71 ± 0.11             | 3.85 ± 0.15               | 0.67 ± 0.02       |
| Warm stratified seeds (18/33 °C for 7 weeks) + 3 weeks incubation (7/18 °C) | 5.68 ± 0.03             | 3.89 ± 0.047              | 0.69 ± 0.01       |

Three replicates of ten seeds/embryos were measured for each experimental condition.
during this period of intermittent moisture were 25-2-35-7°C. Thus, under natural conditions, seeds of *A. preissii* and other species in this area of Western Australia are exposed to several weeks of intermittent warm, moist conditions in early to mid-autumn. In comparison, under laboratory conditions a significant increase in germination percentage was obtained with as few as 2.5 weeks of stratification at 13/26°C, and >60% of the seeds subsequently germinated at 7/18°C following 5 weeks stratification at 13/26°C, which simulates field conditions in late March to early May. Interestingly, more hours of warm (>20°C) moist conditions occurred in spring than in autumn, suggesting that seeds are also warm-stratified in spring prior to the onset of the summer drought.

Further, there are preliminary data that suggest that seeds of *A. preissii* can after-ripen during dry storage in ambient laboratory conditions. Thus, it seems probable that events occurring in seeds of this species when soil and seed moisture contents are low also contributed to dormancy loss in the field. However, further studies are needed on dry after-ripening of *A. preissii* seeds under field conditions before conclusions can be made about its role in the ecology of dormancy-break in this species.

While responses of seeds collected at City Beach and at Shark Bay were similar, there were some subtle differences. For example, no control seeds (7/18°C) germinated from the Shark Bay accession, but about 15% of seeds in the City Beach accession did so.

**Table 2.** Number of hours seeds of *Acanthocarpus preissii* were exposed to particular temperature intervals (5–70°C) over the course of 1 year (31 December 2004 to 30 December 2005) while dry (<20% moisture content), partially hydrated (20–60% moisture content) and fully hydrated (>60% moisture content)

| Temperature interval (°C) | Month | 0–5 | 5–10 | 10–15 | 15–20 | 20–25 | 25–30 | 30–35 | 35–40 | 40–45 | 45–50 | 50–55 | 55–60 | 60–65 | 65–70 |
|---------------------------|-------|-----|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Seeds with <20% moisture content | January | – | – | – | – | 72 | 226 | 132 | 84 | 74 | 98 | 47 | – | – | – |
|                           | February | – | – | – | 26 | 130 | 216 | 119 | 93 | 72 | 36 | 6 | – | – | – |
|                           | March | – | – | – | – | 21 | 309 | 216 | 125 | 23 | 4 | – | – | – | – |
|                           | April | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
|                           | May | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
|                           | June | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
|                           | July | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
|                           | August | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
|                           | September | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
|                           | October | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
|                           | November | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
|                           | December | – | 6 | 203 | 158 | 72 | 57 | 35 | 44 | 28 | 29 | 25 | 22 | 4 | – |
| Total (H) | – | 0.0 | 0.0 | 5.8 | 365.2 | 771.2 | 782.8 | 457.3 | 257.8 | 217.0 | 183.2 | 113.2 | 46.7 | 36.2 | 4.7 |
| Seeds with 20–60% moisture content | January | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
|                           | February | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
|                           | March | – | – | – | 14 | 11 | 14 | 35 | 1 | – | – | – | – | – | – |
|                           | April | – | 22 | 135 | 35 | – | – | – | – | – | – | – | – | – | – |
|                           | May | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
|                           | June | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
|                           | July | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
|                           | August | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
|                           | September | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
|                           | October | – | 11 | 5 | 7 | 5 | 2 | – | – | – | – | – | – | – | – |
|                           | November | – | 47 | 98 | 35 | 25 | 21 | 23 | 12 | 4 | 1 | – | – | – | – |
| Total (H) | – | 0.0 | 0.0 | 79.3 | 240.3 | 88.7 | 37.3 | 21.0 | 23.3 | 11.7 | 3.5 | 1.2 | 0.0 | 0.0 | 0.0 |
| Seeds with >60% moisture content | January | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
|                           | February | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
|                           | March | – | – | – | 20 | 5 | – | – | – | – | – | – | – | – | – |
|                           | April | – | – | – | 20 | 5 | – | – | – | – | – | – | – | – | – |
|                           | May | – | 147 | 533 | 63 | – | – | – | – | – | – | – | – | – | – |
|                           | June | – | 104 | 504 | 113 | – | – | – | – | – | – | – | – | – | – |
|                           | July | – | 239 | 446 | 58 | – | – | – | – | – | – | – | – | – | – |
|                           | August | – | 119 | 515 | 111 | – | – | – | – | – | – | – | – | – | – |
|                           | September | – | 41 | 418 | 226 | 35 | – | – | – | – | – | – | – | – | – |
|                           | October | – | 5 | 218 | 256 | 116 | 62 | 40 | 40 | 7 | – | – | – | – | – |
|                           | November | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Total (H) | – | 0.0 | 507.5 | 2247.0 | 1337.0 | 222.8 | 61.8 | 39.7 | 16.3 | 7.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

Temperatures were recorded automatically every 70 min using a Tinytag Plus 2 data logger with external probe buried at a depth of 10 mm.
<20% of Shark Bay seeds stratified at 18/33°C for 2-5 weeks subsequently germinated at 7/18°C compared with 25–30% of those from City Beach. Thus, it appears that Shark Bay seeds may be more dormant than those from City Beach. This difference could be due to genetic, environmental (maternal) or genetic × environment effects (Baskin and Baskin, 1998). The climate at Shark Bay is arid with an average annual rainfall of 225.7 mm per year. The three wettest months are May (38.9 mm), June (54.8 mm) and July (41 mm). In comparison, City Beach receives >800 mm of rainfall per year, about 60% falling in June (182 mm), July (173 mm) and August (135 mm) (winter). Winter temperatures also are warmer in Shark Bay than at City Beach. The average minimum and maximum temperatures at Shark Bay for May to August are 12.8–16.4°C (min) and 21.7–25.6°C (max), respectively, while those at Perth are 9.0–11.7°C (min) and 17.4–20.9°C (max), respectively. Therefore, the climatic differences between collection sites may be influencing dormancy states in the two seed accessions in the same way that clinal studies of dormancy state-changes have been found for other Western Australian species (Tieu et al., 2001c).

Results of this study have important implications for dune restoration programmes where A. preissii is considered a primary species for stabilization of dunes. On a broader scale, the insight gained into the importance of a warm stratification pretreatment for overcoming dormancy in A. preissii seeds suggests that this approach may be useful in germinating seeds of other native Australian species that previously have proven to be difficult or impossible to germinate.

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