

Seed Storage Characteristics and Dormancy of Australian Indigenous Plant Species:

from the seed store to the field



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Summary

This chapter highlights some of the gaps in our knowledge of the seed biology of many Australian species. Firstly, it discusses the problematic dormancy often encountered in Australian species, such as after-ripening and cyclical dormancy, and points to a number of research areas that could be followed in this area. The chapter also presents data on the applicability of “typical” *ex situ* gene bank conditions for the long-term storage of four Australian species. The results indicate that -18°C is a suitable temperature for the storage of two species. However, -18°C may not be suitable for the storage of two other species, although -196°C may. This raises the possibility that a number of Australian species, despite being desiccation tolerant, may be sensitive to storage at some sub-zero temperatures. Thus, this chapter raises a number of important issues which are, currently, gaps in our knowledge of the seed biology of Australian species and therefore points to a number of possible future research areas.

Introduction

Seed science for highly endemic floras is often assumed from links with the abundance of studies associated with domesticated plants, or based on phylogenetic or taxonomic relatedness. The Australian flora, comprising in excess of 25,000 plants with almost 80% continental endemism, is an example of an extensive and relatively un-researched flora in terms of seed biology, seed storage requirements and seed dormancy release mechanisms. Recent issues associated with the crises involving land degradation, clearing for agriculture and ecological collapse, and the related decline in plant biodiversity, provide new foci for developing more effective and widely applicable standards in the use of seed as a conservation and rehabilitation tool. For example, 48% of the Australian continent has been disturbed by humans, 75% of the rainforests are gone (yet they occupied less than 1% of the continent) and of 2 million ha of lowland native grasslands, only 0.5% remain. Combined with the remarkable levels of local and regional endemism in plant species, the conservation needs of Australia are at a critical point. Seed banks and means for delivery of germinable seed to site present two key areas in developing effective plant conservation strategies.

What do we know of the value of seed for long term conservation of Australian plant species? This chapter reviews a recent case study of some of the storage characteristics of Australian seed, combined with a review of how to effectively release dormancy in seed from the *ex situ* seed bank. Seed dormancy release has received extensive attention in recent years as the demands for large scale, biologically diverse rehabilitation increase (Tieu *et al.*, 2001a, b, c; Adkins and

Bellairs, 2000; Koch and Dixon, 2000; Bell, 1999; Roche *et al.*, 1997a, b; 1998; Bell *et al.*, 1995) and this chapter will provide a review of known and new methods for dormancy release. Seed storage science for indigenous Australian species is, on the other hand, a relatively new area where most Australian seed banks have had to rely on the adoption of international standards to guide storage practices. Aspects of recent research will be presented in the light of new findings on the applicability of established standards for storage of Australian seed.

Seed Dormancy in Australian Species:

from the Ex Situ Seed Bank to the Field

Seed dormancy is a key issue in the development of Australian species for horticulture, conservation and restoration. Effective use of an *ex situ* seed bank as a source of propagules depends upon a reliable understanding of the factors contributing to dormancy. Much of the continental flora of Australia is of ancient origin (Hopper *et al.*, 1996), with the western half of the continent containing over 11,000 plant species and many Gondwanan links extending beyond 65m years. For example, the present day species of the genus *Banksia* are known to produce fruiting structures and seed types similar to fossil *Banksia* species dated at 50m years. Many other species share remarkable inter-continental linkages with South America and southern Africa, again suggesting many species are of ancient origin (Hopper *et al.*, 1996). Since the flora is of considerable antiquity, what is known of the seed dormancy mechanisms, and how do these mechanisms relate to known mechanisms for more established floras?

1. Dormancy Characteristics of Australian Seed

For many Australian species, their unique deep dormancy means that substantial difficulties are encountered in achieving germination. For example, a recent survey of 'germination recalcitrance' in Australia species (Baskin and Baskin, 2002) showed that the majority of species in some families, including the *Rutaceae* (particularly *Boronia* and *Eriostemon*), *Dilleniaceae* (*Hibbertia*), *Cyperaceae* (most genera) and *Restionaceae* (most genera), and notable genera such as *Persoonia*, failed to respond to well established dormancy release cues.

For many groups of Australian plants, the nature of the dormancy mechanism(s) has been postulated (Figure 42.1). Permeability barriers (physical dormancy) represent the most well known of dormancy mechanisms in Australian species, with an emphasis on legumes (Figure 42.1). Baskin and Baskin (2002), however, consider that there needs to be a reappraisal of the criteria used to establish the nature of dormancy in many Australian species outside of groups with well established patterns (such as physical dormancy).

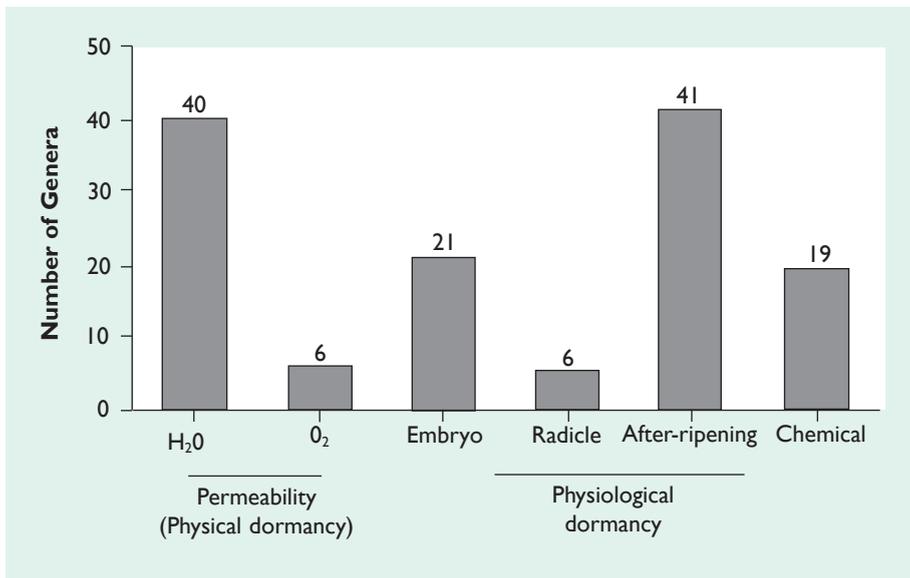


Figure 42.1 Number of genera of Australian species with known systems of dormancy.

After-ripening phenomena are not well understood in Australian species, and Figure 42.1 represents after-ripening as a consideration in defining dormancy. Many species previously thought to be deeply dormant were found to be germinable following after-ripening. For example, Wells and Dixon (2003) found that ‘deep’ dormancy in *Triodia* species (*Poaceae*) from the arid tropics of Australia was a result of an after-ripening requirement with seed being readily germinable after one year of storage at ambient conditions.

Cyclical dormancy is a newly found phenomenon which may account for erratic germination events in seed of some Australian species. Tieu *et al.* (2001a) found in the herbaceous *Conostylis neocymosa* Hopper (*Haemodoraceae*), an endemic genus from the south west of Australia, that seed entered a smoke-germinable phase in the first and second autumn period after collection from the mother plant, with seed being more dormant to the smoke cue in other seasons. The need to catalogue cyclical dormancy in seed is emerging as an important and necessary area of research, particularly for ensuring that seed being returned from the seed bank to the field is provided during the most germinable period.

Table 42.1 Dormancy release agents known to operate for geosporous species in the Australian flora

Agent	Number of Genera	Mode of Action
Scarification	27	Removal of embryo constraints
Leaching	2	Removal of possible inhibitory materials
Chemical applications	36	GA ₃ ; cytokinins
Nutrient applications	9	Nitrogen compounds
Light stimulation*	16	
Thermic pulse	8	60–120°C for up to 3h
Smoke	95	

* Light suppression of germination is known for 13 genera

2. Dormancy Release Agents for Australian Geosporous Species

Table 42.1 illustrates all known agents recognised as being important in the release of seed dormancy in Australian geosporous species. The most commonly encountered cue is smoke (95 genera). The fire-prone nature of much of the flora of Australia (Dixon and Barrett, 2003) underpins the probable reason for the high incidence of smoke-mediated germination in Australian plants.

Smoke is the most effective agent for releasing dormancy in species from a wide spectrum of geosporous and some brady-sporous species (Roche *et al.*, 1997a). The action of smoke in releasing dormancy is complex and varies between species – in some cases involving the permeation of lipoidal deposits in the sub-testa (Egerton-Warburton, 1998), and in others, the denaturing of inhibitory compounds in the seed endosperm and/or embryo (Tieu *et al.*, 1999). The active principle in smoke is water soluble, acts at extremely low concentrations (nanograms) and is deposited in the soil surface following fire (Dixon and Barrett, 2003). Rainfall solubilises the germination-active chemical species, transmitting the chemicals to the soil seed bank in the first 25–50 mm of precipitation. Smoke is therefore a reliable and precise measure of the action of fire in the landscape in terms of Australian geosporous species. More detailed information on the action of smoke is found in Dixon *et al.* (1995), Lloyd *et al.* (2000a, b), Roche *et al.* (1997a, b; 1998) and Tieu *et al.* (1999; 2001a, b, c).

In Situ Seed Storage

Seed storage in the soil seed bank represents a transitory element for many taxa. Whereas long-lived legumes exhibit traditional physical dormancy based on water-impermeable testas, many dominant geosporous groups in the Australian flora exhibit a level of physical dormancy, yet have remarkably short-lived soil seed banks. Figure 42.2 shows the substantial decline after one year in the viability of the soil seed bank for 25 monocotyledon and 99 eudicotyledon species from the species-rich sandplain floras of south western Australia. The transient and vulnerable nature of the soil seed bank is only now being recognised in terms of the long-term conservation of species (Meney *et al.*, 1994). For example, Meney *et al.* (1994) and Roche *et al.* (1998), cite the frequency of fire as a determining factor in the recruitment biology for geosporous species in the biodiverse vegetation of south western Australia, with high frequency burning (some taxa requiring at least a 15 year fire-free interval) resulting in possible localised species extinction. The lack of longevity in the soil seed bank (other than for legumes and fire ephemeral species – see Figure 42.3) combined with a general lack of knowledge of the *in situ* biology of seed means that effective conservation of Australian species, particularly the 512 nationally threatened species, will rely on a combination of seed research and seed conservation science for *ex situ* seed banking.

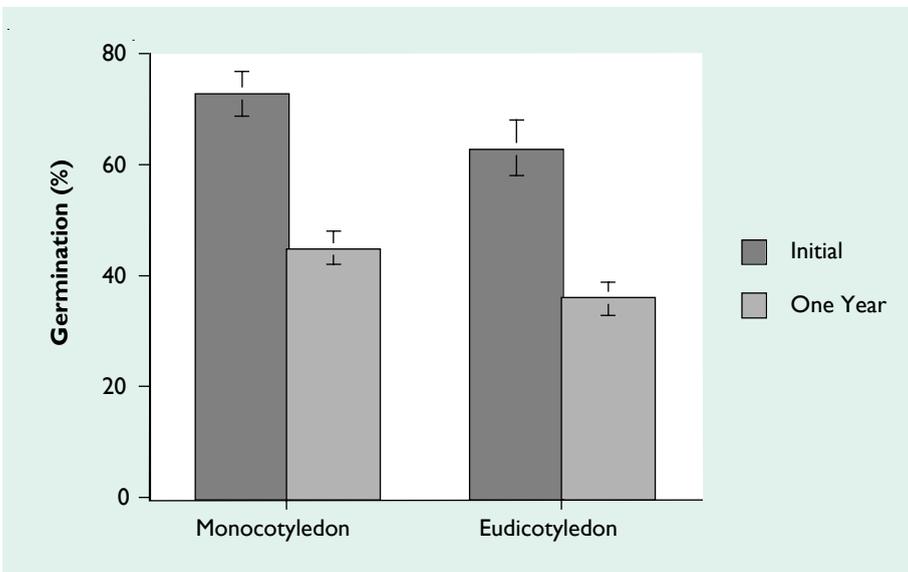


Figure 42.2 Viability of 25 monocotyledon and 99 eudicotyledon species prior to storage (initial) and after 1 year of soil storage. (Figure modified from Roche *et al.*, 1997a).

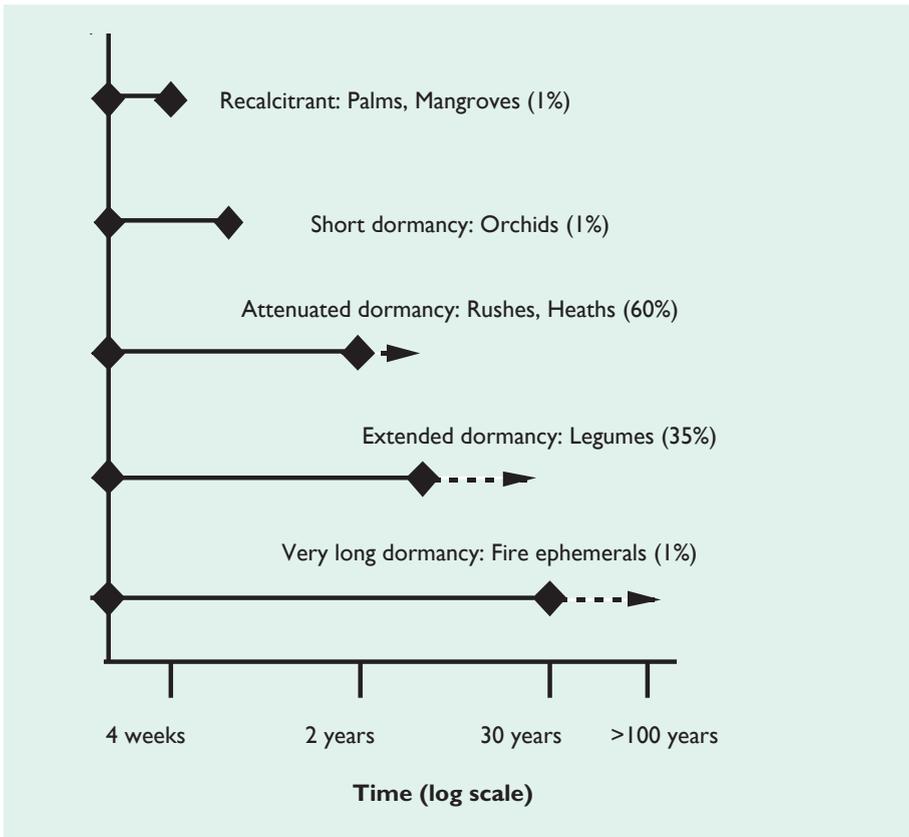


Figure 42.3 Longevity in the soil seed bank of Australian geosporous species with different modes of dormancy. Values in parentheses indicate estimated frequency in the flora. Dashed line represents a period of residual dormancy in a proportion of the seed bank following a germination event.

Serotiny (or bradyspory) occurs in between 20–34% of Western Australian species reviewed by Bell (2001). Serotinous species demonstrate a remarkable level of *in situ* longevity, with taxa including *Hakea platysperma* Hook. (the cricket ball Hakea), *Xylomelum* spp. and many *Myrtaceae* (particularly *Callistemon* and *Melaleuca* spp.) retaining viable seeds in protective woody fruits for periods of 20 years or longer. Not surprisingly, once released from their protective fruit walls, seeds of serotinous species lose viability within weeks of wetting if they don't germinate (Figure 42.4). Crosti (unpublished data) has

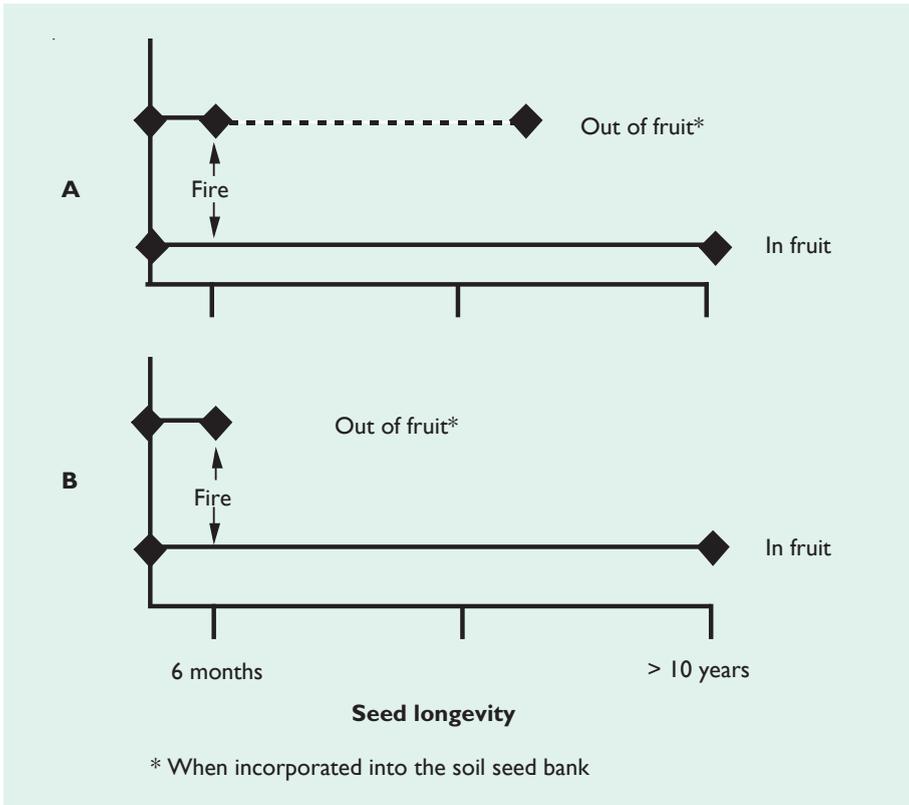


Figure 42.4 *In situ* longevity of Australian bradydysporous species. (A) represents bradydysporous species with seed dormancy (3 genera), and (B) represents bradydysporous species with no seed dormancy (most genera).

shown for *Banksia menziesii* R.Br. from the south west of Australia, that loss of viability occurs within one week of seeds being exposed to moisture under natural conditions. The ecological benefits of serotiny in Australian species are possibly many – protection from fire and predation, and synchronised seed release and seedling recruitment after fire (Lamont *et al.*, 1991). Regardless of the benefits ascribed to the serotinous habit, what is clear is that serotinous structures provide a unique environment for promoting seed longevity. Seed longevity in bradydysporous species is so remarkable that *ex situ* seed banks could benefit from a deeper understanding of the physiological and physical environments within the fruit structures which protect the seed.

Ensuring Quality Standards:

Seed Entering the Ex Situ Seed Bank

Establishing the quality of seed batches is becoming an increasingly important aspect in the storage and use of seed of Australian species. For example, Wells *et al.* (2000) showed that seed quality levels in spinifex species were in the order of less than 5% viable seed as a result of premature seed collection. Protocols have been established for the determination of seed quality prior to storage or use (Touchell *et al.*, 1997). These involve three key steps after a seed batch is provided to the seed bank: purity analysis (degree to which a seed batch is free from non-seed materials such as foreign and inert plant material); viability testing (estimating the degree to which a seed represents a potential germination event); germination testing (degree to which the viable seed unit is capable of being released from dormancy – can also involve after-ripening steps).

Each step in determining the quality of the seed batch represents a decrease in the quantum of seed which is ultimately available for species restoration. In some instances such as in some members of the Australian rush (*Cyperaceae*) and sedge (*Restionaceae*) families, seed can be highly viable as evidenced by turgid, white endosperms and intact embryos capable of growth when excised on *in vitro* medium (Meney and Dixon, 1995), yet intractably dormant (Roche *et al.*, 1997a, b).

Seed Storage for Australian Species

A recent national survey of seed use in Australia found that 70%–80% of all native seed collected in Australia is used for land rehabilitation, and is obtained from commercial suppliers (Mortlock, 1998). Compared to these uses, only limited quantities of seed are presently consigned to conservation collections. In addition, most seed banks fail to adequately address many of the unique issues associated with dormancy release in native Australian species, and a recent review of Australian seed testing standards by Baskin and Baskin (2002) highlights the need to understand the interactions between seed dormancy state and the *ex situ* storage environment. A 1993 report into Australian seed banks found that while appropriate facilities for successful *ex situ* seed conservation existed in all states of Australia (Morse *et al.*, 1993), seed handling, monitoring, storage regimes and equipment varied widely, and in

only a few cases were they adequate for viable long-term seed storage. For example, out of 30 respondents who indicated some consideration of seed water content during storage, only ten reported routinely controlling seed water content, and fewer than half of those actually measured the water content prior to storage. While seed banking is increasingly recognised as an important component for *ex situ* conservation of Australian flora (Morse *et al.*, 1993; Touchell *et al.*, 1997), there remains a dearth of information on the storage requirements of native species, and the application of effective storage methods for long-term conservation.

To address some of these issues, recent studies at Kings Park and Botanic Garden have focussed on investigating the storage characteristics of Australian native species, as well as evaluating the applicability of IPGRI (International Plant Genetic Resources Institute) storage guidelines for Australian species (Merritt *et al.*, 2000a, b; 2003a, b). This research was undertaken on four indicative native species, *Acacia bivenosa* DC. (*Leguminosae* subfam. *Mimosoideae*), *Anigozanthos manglesii* D. Don (*Haemodoraceae*), *Banksia ashbyi* Baker (*Proteaceae*) and *Mesomelaena tetragona* (R. Br.) Benth (*Cyperaceae*), selected as representative of key plant families and significant seed morphological/dormancy types (i.e., monocots/eudicots, hard seeded, deeply-dormant to non-dormant, variation in seed size).

1. Seed Water Content in Relation to the Storage Environment

To characterise the relationship between the storage environment and seed water content, water sorption isotherms were determined by equilibrating seeds of the four study species over 12 weeks in a range of storage environments (Merritt *et al.*, 2003a). Isotherms for *A. manglesii*, *B. ashbyi* and *M. tetragona* seeds exhibited a sigmoidal relationship (Figure 42.5b, c, d) common to many crop species with orthodox storage behaviour (Walters, 1998). However, for intact seeds of the legume *A. bivenosa*, seed water content remained relatively constant at 5°C and 23°C, regardless of relative humidity (Figure 42.5e). As isotherms of physically scarified seeds show a similar sigmoidal relationship to the other species (Figure 42.5a), it may be concluded that the intact seed coat exhibits a degree of impermeability to water which impedes equilibration with the storage environment. This suggests extended equilibration times may be required for hard-seeded species to achieve water contents appropriate for optimal storage – a comparison of the isotherms for intact versus scarified *A. bivenosa* seeds indicates that intact seeds do not equilibrate within a 12 week period at an appropriate drying temperature (e.g., 23°C).

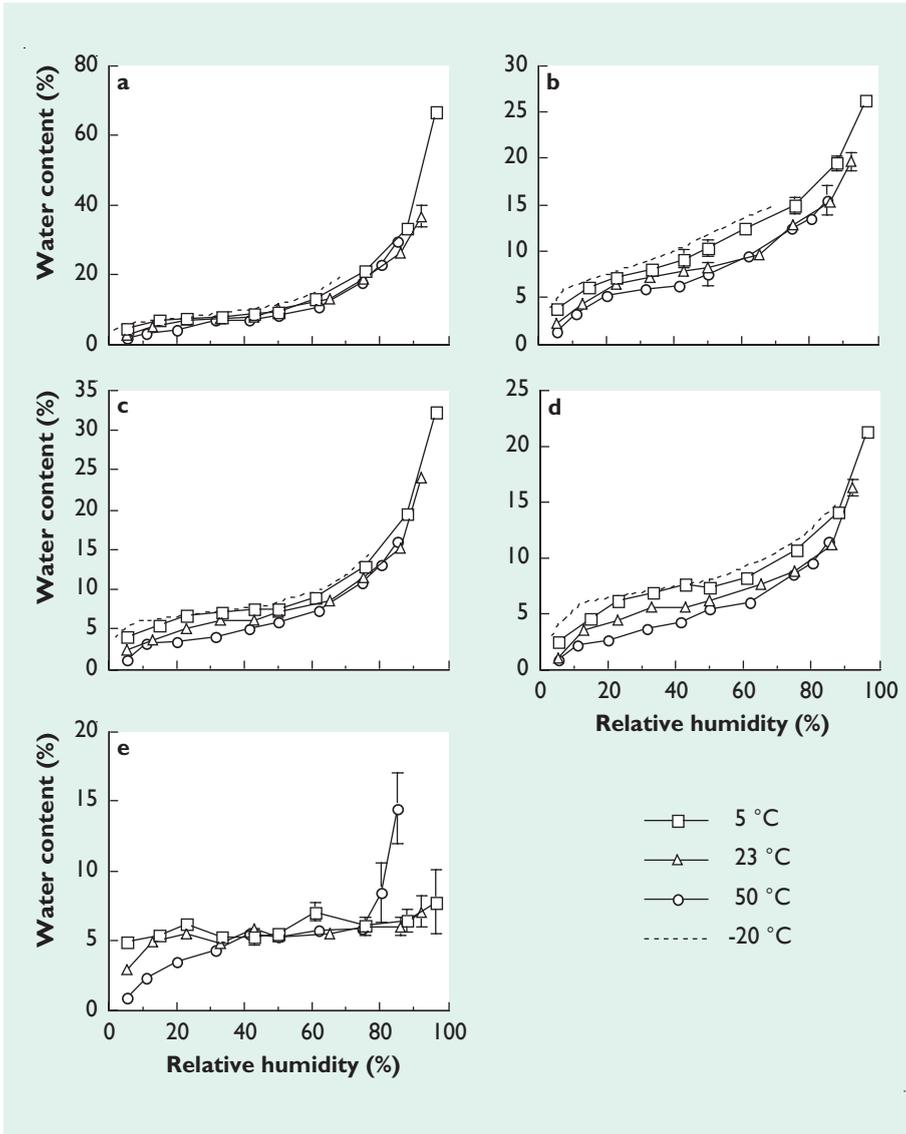


Figure 42.5 Isotherms for scarified seeds of *Acacia bivenosa* (a) and whole seeds of *Anigozanthos manglesii* (b); *Banksia ashbyi* (c); *Mesomelaena tetragona* (d); and *Acacia bivenosa* (e). Data points represent seed water content (mean \pm S.E.) at indicated temperature. The dashed lines are predicted water contents for seeds stored at -20°C calculated from van't Hoff analyses. Reproduced from the Australian Journal of Botany vol. 51, issue 1 (D.J. Merritt, D.H. Touchell, T. Senaratna, K.W. Dixon, K. Sivasithamparam, 2003) with permission of CSIRO Publishing.

2. Impact of the Storage Environment on Seed Germination and Seedling Vigour

To investigate the influence of the storage environment on seed longevity, seeds were stored at a range of temperatures (-196°C, -18°C, 5°C, 23°C and 50°C) and seed water contents (c. 5%, 11–13%, 20–23% and 50% relative humidity) (Merritt *et al.* 2003b) for 18 months. Seeds stored at sub-zero temperatures were dried for 4 weeks at 23°C prior to freezing, while seeds stored at 5°C, 23°C and 50°C were stored at the required humidity level for the duration of the experiment.

The storage environment was found to have little impact on *A. bivenosa* seeds over an 18 month period, with germination percentage and seedling vigour under all storage conditions actually increasing slightly over the storage period, resulting in germination indices somewhat greater than that observed prior to storage (Figure 42.6a). The extraordinary tolerance of *A. bivenosa* seeds to adverse storage conditions (50°C) may be partly a result of the hard seed coat impeding water movement during storage. The degree of hard-seededness and tolerance to storage at 50°C in this species has a strong basis in the extreme ecological conditions (soil temperatures of 65°C) of the arid environments in which this species grows.

For the other three study species, significant ageing was noted in seeds stored at 50°C, with longevity dependent on seed water content. Seeds of *A. manglesii*, *B. ashbyi* and *M. tetragona* stored at 50% RH lost viability within 6–12 months (Merritt *et al.*, 2003b), whilst seeds stored at 20% RH lost viability after 12–18 months. For *B. ashbyi* and *M. tetragona* seeds, longevity was greatest at 11% RH, with seeds stored at 5% RH deteriorating at a similar rate to those stored at 20% RH. Therefore, maximum longevity (at 50°C) of these two diverse species (*B. ashbyi* being a bradysporous eudicot and *M. tetragona* a geosporous monocot) occurred at the same relative humidity, similar to the findings of other studies on domesticated species (reviewed in Walters, 1998). For *A. manglesii* seeds, germination remained similar at both 5% and 11% RH after 18 months, and a longer storage period is required to establish whether 11% RH provides maximum longevity.

For seeds stored at 23°C or less, seed viability generally remained high, although some evidence of ageing (loss of seedling vigour) was noted in *A. manglesii* and *M. tetragona* seeds stored at 23°C and 50% RH (Figure 42.6b,d). Notably, however, storage of *A. manglesii* and *M. tetragona* seeds at -18°C appeared to be detrimental, with a progressive decrease in germination (10–30%, depending on seed water content) and seedling vigour (5–40%) over the storage period (Figure 42.6b,d and Figure 42.7). Therefore, although both of these species have other physiological characteristics suggestive of orthodox storage behaviour (Merritt *et al.*, 2003a,b), the results demonstrate that the ability of seeds to survive initial desiccation and exposure to -18°C does not necessarily translate to long-term survival under these conditions. Further,

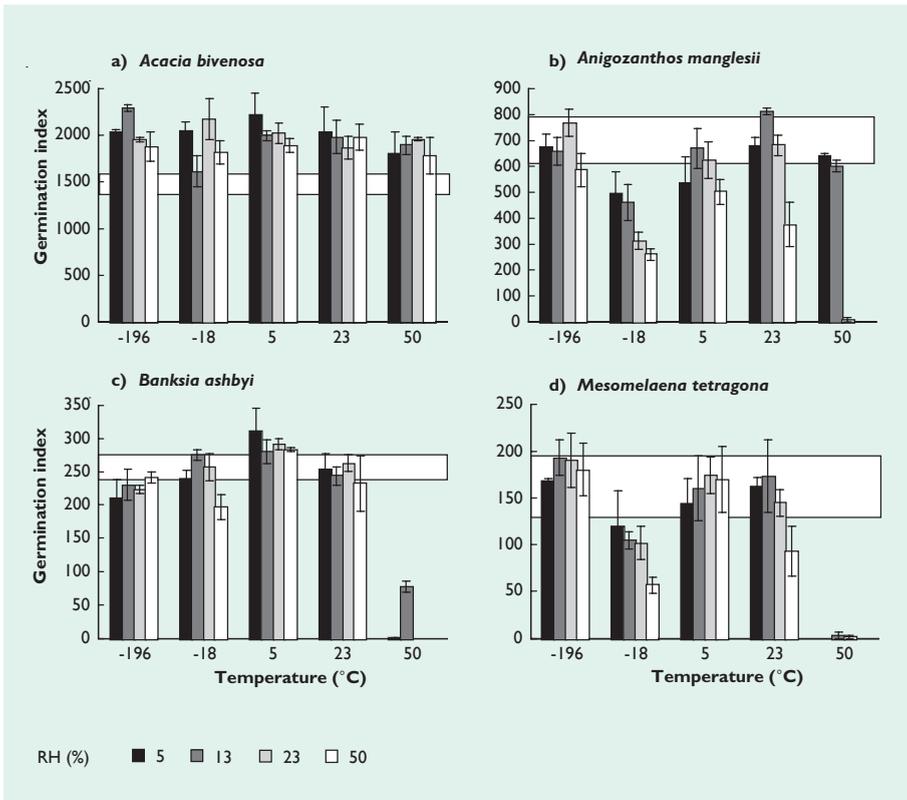


Figure 42.6 Mean germination index (germination * seedling length) after 3 weeks incubation of *A. bivenosa* (a); *A. manglesii* (b); *B. ashbyi* (c); and *M. tetragona* (d) seeds stored for 18 months at indicated temperature and relative humidity. Horizontal bar represents germination index (\pm S.E.) of seeds prior to storage. (Figure from Merritt *et al.*, 2003b).

storage conditions based on IPGRI recommendations (-18°C and $5 \pm 2\%$ water content) may not be appropriate for seeds of all Australian species. Importantly, however, cryostorage was found to be applicable to all four species tested, with no loss in germination or vigour at any of the water contents tested. Thus cryostorage appears to be a suitable alternative for the secure maintenance of seeds unable to be satisfactorily stored using conventional practices.

In conclusion, preliminary research now shows that the storage requirements of some Australian native species may be similar to those of other species studied and that internationally accepted guidelines are appropriate and applicable to maintain long-term, viable collections of seed germplasm.

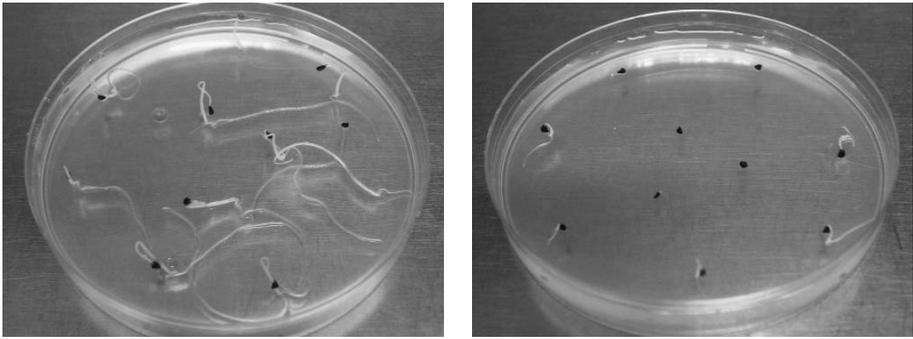


Figure 42.7 Germination of *Anigozanthos manglesii* seeds stored at 23°C (left) and -18°C (right), showing marked differences in germination percentage and seedling vigour after 3 weeks incubation.

However, equally, the results suggest that for species for which no storage physiology is known, and where there is a reasonable level of endemism at the family or genus level, that there is a need to adopt a testing regime which investigates the impact of a range of storage conditions on seed viability. Regular monitoring must also include assessment of germinant vigour – a feature not always assessed in seed banks. Such testing will prevent unnecessary loss of germplasm through inappropriate storage conditions.

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