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Seed ageing of four Western Australian species in relation to storage environment and seed antioxidant activity

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Abstract

The influence of the storage environment on seed viability and antioxidant potential was examined for four species native to Western Australia: *Acacia bivenosa* DC., *Anigozanthos manglesii* D. Don, *Banksia ashbyi* E.G. Baker, and *Mesomelaena tetragona* (R. Br.) Benth. Seeds were stored at four water contents (at *c.* 5%, 11–15%, 20–23% and 50% relative humidity) at each of five temperatures (–196, –18, 5, 23 and 50°C), and seed germination and seedling vigour monitored over an 18-month period. Deterioration was apparent in all species (except *A. bivenosa*) stored at 50°C, with 11% RH maximizing longevity for *B. ashbyi* and *M. tetragona* seeds, and 5% or 11% RH preventing deterioration for *A. manglesii* seeds. Seed viability generally remained high for all species stored at 23°C or less. Notably, however, germination and seedling vigour of *A. manglesii* and *M. tetragona* seeds gradually declined when stored at –18°C, suggesting that storage at this temperature was detrimental. The antioxidant activity of lipid extracts of seeds after 18 months storage at 5, 23 and 50°C was also examined to determine whether the seed viability decline was associated with a loss of antioxidants. Antioxidant activity varied between storage treatments and was not related to seed viability.

Keywords: *Acacia bivenosa*, *Anigozanthos manglesii*, antioxidants, *Banksia ashbyi*, cryostorage, *Mesomelaena tetragona*, seed storage, viability

Introduction

The long-term survival of many threatened Western Australian plant species is partly dependent upon the application of appropriate conservation programmes. Seed banking is viewed as an important component of *ex situ* conservation strategies for Australian species (Morse *et al*., 1993; Touchell *et al*., 1997). As one of the primary functions of *ex situ* conservation is to maintain germplasm suitable for re-establishing wild populations (Touchell *et al*., 1997), seed quality must be maintained over extended periods of time. During storage, seeds undergo ageing processes that lead to a deterioration in seed quality. Aged seeds show decreased vigour and may produce weak seedlings that are unable to survive once re-introduced into a habitat (Rokich *et al*., 2000). Genetic modifications also occur as seeds age (Rao *et al*., 1987). Such factors may decrease the biodiversity of re-introduced vegetation at rehabilitated sites and, thus, reduce the effectiveness of an *ex situ* collection. Therefore, it is important to determine the storage conditions that maintain initial seed quality for as long as possible.

Compared to domesticated species, there are few studies on seed storage behaviour of Australian species. However, studies suggest that seeds of most Mediterranean and dryland species have orthodox storage behaviour (Hong and Ellis, 1996). Orthodox seeds are able to withstand drying to low water contents and exposure to low temperatures, both of which increase longevity (Roberts and Ellis, 1989; Walters, 1998a). The water content that maximizes seed longevity at a given temperature is species specific (Ellis, 1998; Walters and Engels, 1998); however, it appears that this critical/optimum water content occurs at a constant relative humidity for all species (Ellis, 1998; Walters and Engels, 1998). Numerous studies of orthodox species contend that there is a critical water content (that in equilibrium with approximately 10% RH at 20°C) that maximizes longevity at all storage temperatures (Ellis *et al*., 1989, 1995, 1996), and that drying seeds below this water content has no effect on longevity. Other studies

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suggest that there is an optimum water content for storage in equilibrium with approximately 20% RH at the temperature at which the seeds are to be stored (and thus the optimum water content increases with decreasing temperature), and that drying seeds below this water content increases the rate of deterioration (Vertucci and Roos, 1990, 1993; Walters, 1998b). Further evidence for a temperature-dependent optimum water content is provided by recent studies investigating molecular mobility in the cytoplasm of seeds and pollen, demonstrating that the water content at which molecular mobility is minimized (and storage life is suggested to be greatest) increases with decreasing temperature (Buitink *et al*., 1998, 2000).

Many of the processes implicated in seed ageing during storage appear to be free-radical mediated, and lipid peroxidation is suggested to be a primary cause of deterioration in dry stored seeds (Wilson and McDonald, 1986; McDonald, 1999). Seeds are known to contain numerous antioxidant compounds, both enzymic and non-enzymic, that act to prevent oxidative damage by scavenging free radicals before they attack membranes or other seed components (Leprince *et al*., 1993; McDonald, 1999). In dry seeds, lipid-soluble, non-enzymic antioxidants (such as tocopherol) are a potential mechanism of defence when enzyme systems may be impaired at low seed water contents (Senaratna and McKersie, 1986). Some studies have measured a decrease in the activity of lipid-soluble antioxidants in aged seeds (Senaratna *et al*., 1988; Pukacka, 1991), while others have found no correlation between lipid-soluble antioxidant concentration and seed viability (Fielding and Goldsworthy, 1980; Priestley *et al*., 1980). Thus, while the degree and rate of free-radical-induced injury in seeds may be influenced by antioxidant status, the importance of endogenous antioxidants in limiting seed deterioration during storage remains uncertain.

Based on the limited knowledge of the seed storage characteristics of Australian indigenous species and the importance of seed storage in conservation programmes, this study investigated the effects of storage temperature and seed water content on seed germination and seedling vigour over an 18-month storage period, to test the hypotheses that: (1) seeds of each species are able to survive the combined effect of low water content and low temperature (sub-zero) storage; (2) at a given temperature, seed longevity is greatest at a common relative humidity for these diverse species; and (3) there is an optimum water content above and below which seed longevity is reduced. In addition, the lipid-soluble antioxidant activity of seeds stored in selected environments was measured to determine whether the assay may be used as an alternative measure of seed quality and aid in identifying optimum storage environments. The hypotheses tested were: (1) seed ageing is associated with a loss of antioxidants; and (2) seeds with greater longevity and/or tolerance to adverse storage conditions have a greater pre-storage antioxidant capacity.

Materials and methods

Seed material

Seeds of four species native to Western Australia, *Acacia bivenosa* DC., *Anigozanthos manglesii* D. Don, *Banksia ashbyi* E.G. Baker, and *Mesomelaena tetragona* (R. Br.) Benth., were selected as representatives of key seed morphological types and germination characteristics. *A. bivenosa* was collected approximately 50 km east of Wittenoom in north-western Western Australia (WA)(22°30′S, 119°20′E). *A. manglesii* was collected from bushland in Kings Park and Botanic Garden, Perth, WA (31°57′S, 115°50′E). *B. ashbyi* was collected from the coastal regions of Shark Bay, central western WA (25°92′S, 114°30′E). *M. tetragona* was collected within a 10 km radius of Collie, south-western WA (33°21′S, 116°09′E). Seeds were collected in the season (1998) immediately prior to the commencement of experiments, and were stored in glass jars with screw-top lids at 5°C until required (4–12 weeks). The initial seed viability of each species was estimated on a random sample of 50 seeds, using a cut test, whereby the seed coat was removed and the presence or absence of healthy embryonic tissues scored.

Seed storage

Seeds were stored at four water contents (*c*. 5%, 11–15%, 20–23% and 50% relative humidity) at each of five temperatures, –196, –18, 5, 23 and 50°C. For storage at 5, 23 and 50°C, seeds were placed in nylon mesh bags and sealed in polyethylene boxes containing different saturated salt solutions to regulate relative humidity. The salts (and corresponding relative humidities) used at each temperature were: $ZnCl_2$ (5%), LiCl (11%), KF (20%) and NaBr (50%) at 50°C; $ZnCl_2$ (5%), LiCl (13%), CH_3COOK (23%) and $Ca(NO_3)_2$ (50%) at 23°C; and $ZnCl_2$ (5%), LiCl (15%), $CaBr_2$ (23%) and KNO_2 (50%) at 5°C [relative humidity data from Winston and Bates (1960) and Vertucci and Roos (1993)]. All boxes were stored in the dark, and the salt solutions were checked and stirred regularly over the storage period to ensure that the correct consistency was maintained. For storage at sub-zero temperatures, seeds were first dried at 23°C in each of the four relative humidities for 4 weeks. Seeds were then removed and sealed in aluminium-foil bags and placed in a freezer at –18°C,

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or placed in polyethylene cryovials (Nunc®) and plunged directly into liquid nitrogen (–196°C). Seed water content was determined gravimetrically (g H_2O g^{-1} DW) after drying three replicates of 10 seeds in an oven at 103°C for 17 h (ISTA, 1985), and was measured: (1) after the initial 4–12 weeks at 5°C, just prior to the beginning of the storage treatments; and (2) again following 18 months' storage in all environments.

Determination of seed germination and seedling vigour

Seed germination and seedling vigour tests were performed prior to storage (control) and following 6, 12 and 18 months' storage. Seeds of three species required dormancy-breaking treatments prior to incubation to elicit the maximum germination response. For *M. tetragona* seeds, the embryos were extracted under sterile conditions using a dissecting microscope and placed on half-strength Murashige and Skoog (MS) medium containing 60 mM sucrose, supplementary vitamins (2.5 µM pyridoxine-HCl, 3 µM thiamine-HCl and 4 µM niacin) and growth regulators (3 µM GA_3 and 1 µM zeatin) and solidified with 0.6% (w/v) agar, according to Meney and Dixon (1995). Seeds of *A. bivenosa* were placed in near-boiling water (90–95°C) for 10 min and allowed to cool (Cavanagh, 1987). Seeds of *A. manglesii* were given a heat-pulse treatment by placement in an oven at 100°C for 3 h (Tieu *et al*., 2001). Following pre-treatment, seeds of *A. bivenosa*, *A. manglesii* and *B. ashbyi* (which required no dormancy breaking treatment), were sterilized in a 2% calcium hypochlorite [$Ca(OCl)_2$] solution for 10–15 min, washed with sterile H_2O, and incubated on 0.7% (w/v) water agar plates. Seeds of all species were incubated in the dark at 18°C. All germination trials were replicated three times with 10 seeds per replicate. Seeds were considered germinated upon radicle emergence, and germination was scored every 3–4 days for 3 weeks. Total seedling length was measured after 3 weeks as a measure of seedling vigour. To aid in distinguishing between treatment effects and determining the optimum storage environment, seed quality was also assessed using a germination index, calculated as the maximum germination percentage multiplied by the total seedling length (after 3 weeks) of germinated seeds (Vertucci *et al*., 1994).

Determination of lipid-soluble antioxidant potential

For each species, seeds stored at three temperatures (5, 23 and 50°C) and two relative humidities (5% and 50% RH) were assayed prior to, and after 18 months' storage. To extract seed lipids, three replicate samples of 10–30 seeds (between 0.15 and 0.25 g, depending on seed size) were homogenized in 9 ml of chloroform:methanol (2:1 by volume) and the suspension centrifuged at 12,000 rpm for 15 min. The supernatant was removed and partitioned with 5 ml of 0.7% (w/v) NaCl and centrifuged at 10,000 rpm for 10 min to remove non-lipid material. The organic layer was removed and evaporated to dryness under N_2, and the total lipid content determined gravimetrically before the samples were redissolved in 1 ml of chloroform:methanol (Christie, 1973). The antioxidant activity was determined by the potential of the lipid extracts to inhibit linoleic acid oxidation, according to the methods of Senaratna *et al*. (1985, 1988). A test emulsion was formed by mixing 0.2 ml of 0.2 M linoleic acid with 0.1 ml of each sample extract in ethanol (800 µg of lipid), ethanol alone (control), or α-tocopherol (standard) and 3 ml of 10 mM KH_2PO_4 buffer at pH 6.8. The reaction was initiated at 37°C by the addition of 1.5 ml of 0.2 mM $FeSO_4$. A 0.1 ml aliquot was removed immediately and every 30 min for 3 h, and the reaction stopped by the addition of 2 ml of 0.01 M NaOH in 10% ethanol. Oxidation of linoleic acid was measured as the absorbance of an aliquot of the test emulsion at 232 nm. The absorbance was plotted over time, and per cent inhibition was calculated over the linear region of the reaction (between 30 and 150 min) as $[(\Delta A_{232}\ \text{control} - \Delta A_{232}\ \text{sample})/(A_{232}\ \text{control}) \times 100]$.

Statistical analysis

Germination, seedling length and antioxidant activity data were analysed for statistical significance by analysis of variance. Percentage values for germination and antioxidant activity were arcsine-transformed prior to analysis (untransformed data appear in all tables and figures). Fisher's least significant difference ($P < 0.05$) was used to determine significant differences between treatments.

Results

Effects of the storage environment on seed water content

The water content of seeds prior to storage (air dry) ranged from approximately 5% for *A. bivenosa* seeds, to 8.5% for *A. manglesii* seeds (Table 1). Both temperature and relative humidity influenced the water content of stored seeds, with seed water content generally increasing with an increase in relative humidity, or a decrease in temperature. However, the range of water contents of *A. bivenosa* seeds stored at –18°C and –196°C was narrow compared to the other species.

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Table 1. Water content (mean ± SE) of seeds. Data presented are seed water contents prior to any drying or storage (air dry), and seed water contents following 18 months' storage at indicated temperatures and relative humidities

Species	Temperature (°C)	Relative humidity (%)	Water content (%)	Species	Temperature (°C)	Relative humidity (%)	Water content (%)
Acacia bivenosa		air dry	5.0 ± 0.4	*Anigozanthos manglesii*		air dry	8.5 ± 0.3
	50	5	0.8 ± 0.1		50	5	1.4 ± 0.1
		11	0.8 ± 0.2			11	2.5 ± 0.3
		20	2.7 ± 0.2			20	4.0 ± 0.7
		50	6.4 ± 0.7			50	7.3 ± 0.3
	23	5	1.9 ± 0.1		23	5	2.9 ± 0.4
		13	3.9 ± 0.0			13	5.4 ± 0.0
		23	4.8 ± 0.2			23	7.0 ± 0.3
		50	6.4 ± 0.4			50	9.7 ± 0.1
	5	5	2.9 ± 0.1		5	5	3.4 ± 0.2
		15	4.5 ± 0.4			15	6.0 ± 0.5
		23	4.0 ± 0.3			23	7.3 ± 0.4
		50	5.4 ± 0.4			50	9.5 ± 0.0
	–18[a]	5	3.7 ± 0.1		–18[a]	5	3.3 ± 0.5
		13	4.2 ± 0.4			13	6.4 ± 0.5
		23	5.2 ± 0.4			23	7.8 ± 0.4
		50	5.7 ± 0.3			50	10.5 ± 0.3
	–196[a]	5	2.9 ± 0.1		–196[a]	5	3.0 ± 0.6
		13	4.4 ± 0.2			13	5.8 ± 0.6
		23	4.5 ± 0.2			23	7.9 ± 0.3
		50	4.7 ± 0.3			50	10.4 ± 0.9
Banksia ashbyi		air dry	7.4 ± 0.2	*Mesomelaena tetragona*		air dry	6.8 ± 0.1
	50	5	1.3 ± 0.1		50	5	1.0 ± 0.1
		11	2.4 ± 0.1			11	2.2 ± 0.1
		20	3.3 ± 0.1			20	2.9 ± 0.1
		50	5.4 ± 0.0			50	5.0 ± 0.1
	23	5	2.9 ± 0.0		23	5	1.9 ± 0.1
		13	3.9 ± 0.2			13	3.0 ± 0.0
		23	5.2 ± 0.2			23	4.4 ± 0.0
		50	7.3 ± 0.2			50	6.8 ± 0.1
	5	5	3.3 ± 0.1		5	5	2.3 ± 0.0
		15	5.0 ± 0.1			15	3.5 ± 0.0
		23	5.9 ± 0.3			23	5.3 ± 0.1
		50	7.3 ± 0.2			50	6.9 ± 0.1
	–18[a]	5	3.1 ± 0.2		–18[a]	5	2.6 ± 0.2
		13	5.3 ± 0.4			13	3.9 ± 0.1
		23	6.7 ± 0.1			23	5.4 ± 0.1
		50	7.9 ± 0.2			50	7.3 ± 0.2
	–196[a]	5	3.5 ± 0.4		–196[a]	5	2.5 ± 0.1
		13	4.8 ± 0.2			13	3.4 ± 0.1
		23	5.9 ± 0.2			23	5.3 ± 0.1
		50	7.6 ± 0.1			50	7.5 ± 0.1

[a] Seeds stored at –18 and –196°C were equilibrated for 4 weeks at 23°C prior to freezing, data represent water content of seeds frozen for 18 months.

Effects of the storage environment on seed viability

The initial seed viability (according to cut tests) and germination of all species was high prior to storage, ranging between 94 and 100% and 86 and 97%, respectively. *A. bivenosa* seeds remained of high quality in all storage environments. Germination remained similar to that prior to storage, and seedling vigour generally increased over time, resulting in the germination indices of stored seeds being greater than those prior to storage (Fig. 1). In contrast, significant ($P < 0.05$) ageing was evident in seeds of the other three species stored at 50°C, and longevity was dependent on storage relative humidity. At 50°C, seeds stored at 50% RH aged most rapidly, followed by seeds stored at 20% RH (Fig. 2). Seed longevity was greatest at 11% RH for *B. ashbyi* and *M. tetragona* seeds, whereas for *A. manglesii*, no loss of germination or vigour was evident in seeds stored at 5% or 11% RH (Figs 1, 2). The germination and seedling vigour of seeds stored at 23, 5 and –196°C generally remained similar to that of seeds prior to storage (Fig. 1). Only *A. manglesii* and *M. tetragona* seeds stored at 23°C and 50% RH showed signs of deterioration, with a significant reduction ($P < 0.05$) in seedling vigour noted after 12 and 18 months, resulting in a significant reduction in the germination index for these seeds (Fig. 1). Notably, however, deterioration was evident in *A. manglesii* and *M. tetragona* seeds stored at –18°C. A progressive loss in both germination and seedling vigour over time was noted. Germination of *A. manglesii* seeds decreased by 10–15% (depending on seed water content), and by 20–30% for *M. tetragona* seeds. Seedling vigour declined by 15–55% for *A. manglesii* seeds, and 5–40% for *M. tetragona* seeds. As a result, the germination indices were significantly reduced ($P < 0.05$) (except for *M. tetragona* seeds stored at 5% RH) (Fig. 1). For both species, deterioration was greater at higher water contents.

Lipid-soluble antioxidant potential

The antioxidant activity of seeds prior to storage varied between species. Seeds of *A. bivenosa* and *B.*

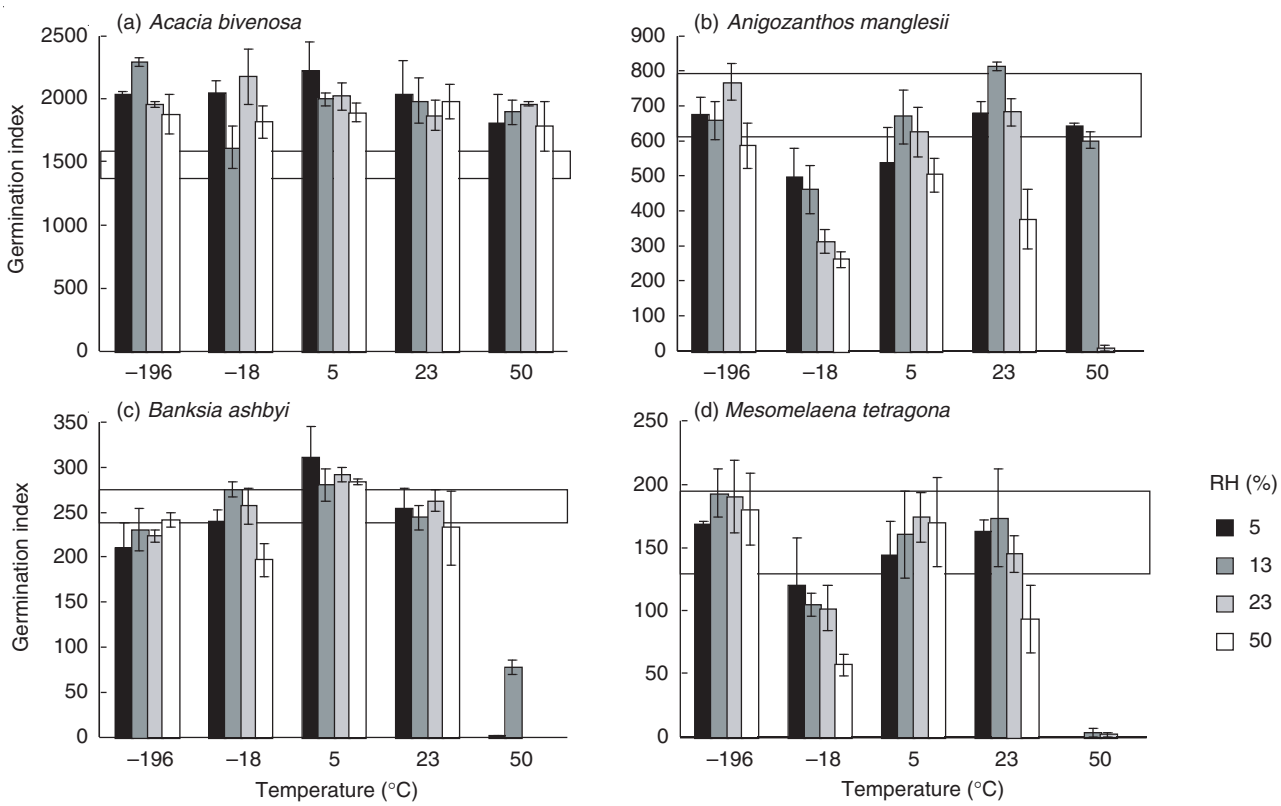

Figure 1. Mean germination index (germination × seedling length) of (a) *Acacia bivenosa*; (b) *Anigozanthos manglesii*; (c) *Banksia ashbyi*; and (d) *Mesomelaena tetragona* seeds stored for 18 months at the indicated temperature and relative humidity. The horizontal bar represents the germination index (± SE) of seeds prior to storage. (Note that the relative humidity varied slightly between temperatures.)

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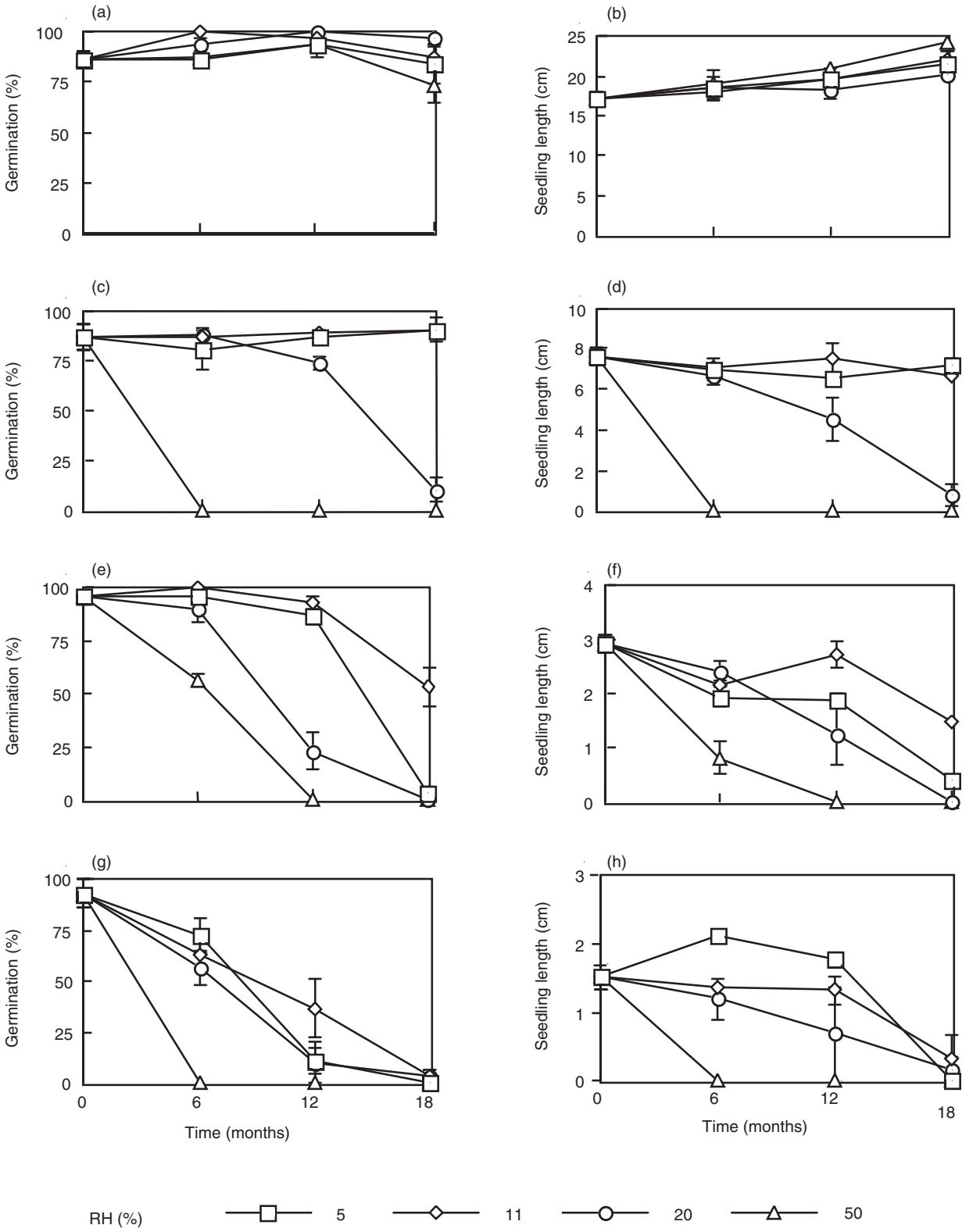

Figure 2. Germination and seedling length (mean ± SE) of: (a, b) *Acacia bivenosa*; (c, d) *Anigozanthos manglesii*; (e, f) *Banksia ashbyi*; and (g, h) *Mesomelaena tetragona* seeds stored at 50°C and indicated relative humidity.

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ashbyi had greater activity (*c*. 46% and 47% inhibition, respectively) than *A. manglesii* and *M. tetragona* seeds (*c*. 32% and 39% inhibition, respectively) (Table 2). Following 18 months' storage, no relationships were evident between antioxidant activity and the storage treatment or seed viability. For *A. bivenosa* seeds, although the antioxidant activity of stored seeds was generally lower than that prior to storage, the decrease was evident across all storage treatments, and was not accompanied by a decrease in germination. For *A. manglesii*, a similar antioxidant activity was measured in all seeds, with no significant differences observed between control seeds and stored seeds, or between different storage treatments. The antioxidant activity of *B. ashbyi* and *M. tetragona* seeds varied significantly ($P < 0.05$) between several treatments. However, for both species, the variation was disparate amongst treatments, and there was no correlation with seed viability.

Discussion

Numerous studies have demonstrated the importance of temperature and seed water content during *ex situ* storage, with a reduction in either (or both) generally increasing the longevity of orthodox seeds (Roberts and Ellis, 1989; Walters, 1998a). In this study, the benefits to longevity of reducing seed water content were highlighted for seeds of *A. manglesii*, *B. ashbyi* and *M. tetragona* stored at 50°C. However, progressive drying did not continually increase longevity; as for *B. ashbyi* and *M. tetragona* seeds, damaging effects of over-drying below an optimum water content (at 11% RH) were apparent, with seeds stored at 5% RH ageing at a rate comparable to those stored at 20% RH. A longer storage period is necessary to determine whether this trend also exists for *A. manglesii* seeds, as there was no ageing detected in seeds stored at either 5% or 11% RH after 18 months. Some studies have found reduced germination of seeds stored at very low water contents to be a result of imbibitional damage (Hong and Ellis, 1996). However, the reduced germination of seeds stored at 5% RH in this study does not appear to be a result of imbibitional damage. The germination of *B. ashbyi* seeds remained similar to that of control seeds for 12 months before a rapid decline was observed, and the progressive decline in germination of *M. tetragona* seeds over time suggested an ageing effect. Vertucci and Roos (1990) and Vertucci *et al*. (1994) also reported increased rates of seed deterioration in many orthodox species stored above or below the optimum water content at which maximum longevity occurred. In addition, increased ageing rates have been noted in very dry (<4% water content) wheat, rice, millet and peanut seeds stored at a range of temperatures between 0 and 45°C (Hu *et al*., 1998), and in several vegetable species stored at 20 or 40°C (Kong and Zhang, 1998). Similar to the findings in these studies, the detrimental effects of over-drying *B. ashbyi* and *M. tetragona* seeds only became apparent after a period of time in storage (12–18 months). These results contrast to those of Ellis *et al*. (1989, 1990a, 1992), which suggest that drying seeds below the critical water content, at which maximum longevity is achieved, does not increase the rate of seed deterioration. The increased deterioration of very dry seeds observed in several studies may be a result of membrane damage due to the removal of structural water (Walters, 1998a). Alternatively, oxidative processes may be increased at low water contents. Above approximately 6% water content, lipid peroxidation is thought to be reduced as the water acts as a buffer against free-radical attack on membranes (McDonald, 1999). Thus, over-drying seeds may expose macromolecular surfaces to free-radical attack and reduce seed longevity (Walters, 1998a; McDonald, 1999).

Seeds of *A. bivenosa* showed greater longevity at 50°C than those of the other species, with no reductions in germination, and an increase in seedling vigour over time. These seeds, like those of many legumes, have a hard seed coat that requires scarification before imbibition and germination can proceed. The narrow range of water contents for seeds dried for 4 weeks and stored at –18°C and –196°C (Table 1) suggests that the seed coat also impedes equilibration with the storage environment. Indeed, subsequent studies on water sorption characteristics of the species in this study demonstrated that a 4-week drying time was not sufficient to obtain equilibrium water contents for *A. bivenosa* seeds (Merritt *et al*., 2003). Thus, it is possible that, by acting as a barrier to water movement during storage, the hard seed coat may be a contributing factor to greater longevity at high temperatures. The increase in seedling vigour (particularly after 18 months' storage) suggests that the dormancy-breaking treatment employed (boiling water) became more effective with time, perhaps as the seed coat naturally weakened with age, or as a result of freezing and thawing in the case of seeds stored at –18°C and –196°C.

In general, seed viability remained high over the storage period for all species stored at 23°C or below, and it was not possible to determine the optimum water content. However, detrimental effects of storage at –18°C were evident for *A. manglesii* and *M. tetragona* seeds. Sensitivity to storage at sub-zero temperatures is not characteristic of orthodox seeds, and seeds of some species, such as coffee and citrus, have been classified as intermediate, as longevity at low water contents is reduced by exposure to sub-zero temperatures (Ellis *et al*., 1990b; Hong and Ellis,

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Table 2. Antioxidant activity (expressed as % inhibition of linoleic acid oxidation) of lipid extracts of seeds stored for 18 months at indicated temperature and relative humidity (RH) in relation to germination (mean ± SE)

Species	Temp (°C)	RH (%)	Germination (%)	Antioxidant activity (% inhibition)	Species	Temp (°C)	RH (%)	Germination (%)	Antioxidant activity (% inhibition)
Acacia bivenosa	Control		87 ± 7^{a}	46 ± 8^{a}	*Banksia ashbyi*	Control		97 ± 3^{a}	47 ± 6^{ae}
	50	50	73 ± 9^{a}	29 ± 3^{bd}		50	50	0 ± 0^{b}	57 ± 6^{e}
		5	83 ± 9^{a}	22 ± 3^{bcd}			5	3 ± 3^{b}	35 ± 4^{ade}
	23	50	97 ± 3^{a}	25 ± 6^{acd}		23	50	93 ± 4^{a}	43 ± 9^{ae}
		5	90 ± 10^{a}	14 ± 3^{c}			5	97 ± 3^{a}	29 ± 8^{bcd}
	5	50	93 ± 3^{a}	28 ± 5^{acd}		5	50	93 ± 3^{a}	19 ± 2^{bcd}
		5	97 ± 3^{a}	35 ± 3^{ad}			5	97 ± 7^{a}	30 ± 2^{ac}
Anigozanthos manglesii	Control		86 ± 4^{a}	32 ± 8^{a}	*Mesomelaena tetragona*	Control		93 ± 7^{a}	39 ± 2^{a}
	50	50	0 ± 0^{b}	32 ± 2^{a}		50	50	0 ± 0^{b}	27 ± 2^{ac}
		5	90 ± 6^{a}	33 ± 2^{a}			5	0 ± 0^{b}	17 ± 4^{bc}
	23	50	90 ± 6^{a}	22 ± 9^{a}		23	50	57 ± 3^{b}	30 ± 3^{ac}
		5	93 ± 3^{a}	35 ± 2^{a}			5	93 ± 3^{a}	12 ± 2^{b}
	5	50	90 ± 6^{a}	33 ± 10^{a}		5	50	73 ± 14^{a}	31 ± 7^{ac}
		5	83 ± 12^{a}	37 ± 3^{a}			5	83 ± 3^{a}	35 ± 9^{a}

Values in a column for each species followed by the same letter are not significantly different ($P < 0.05$).

1995; Eira *et al*., 1999). Seeds categorized as intermediate also typically suffer desiccation damage when dried to water contents of less than 10% (Ellis *et al*., 1990b; Eira *et al*., 1999). Seeds of *A. manglesii* and *M. tetragona* do not fit the intermediate classification, as they survived desiccation to water contents of approximately 1%, and germination after storage at –18°C was not dramatically reduced, instead declining slowly over time. Similar results have been noted in other species that otherwise exhibit orthodox characteristics. For example, Kraak and Vos (1987) found that lettuce seeds stored at –20°C deteriorated faster than those stored at –5 or –10°C. In addition, Zewdie and Ellis (1991) found damage to *Eragrostis tef* and *Guizotia abyssinica* seeds stored at –18°C to increase with increasing water content (between 15 and 26%), and Pritchard *et al*. (1999) reported a decrease in the germination of seeds of various orchid species at temperatures ranging between –50 and –30°C. Similar to the results obtained for *A. manglesii* and *M. tetragona* seeds in the present study, Pritchard *et al*. (1999) found orchid seeds to be amenable to cryostorage. Therefore, there is some evidence to suggest that seeds of certain species are injured at specific sub-zero temperatures, and the results of the present study suggest that the ability of seeds to survive initial desiccation and exposure to sub-zero temperatures may not guarantee their long-term survival when stored under such conditions.

In addition to examining seed viability during storage in different environments, the antioxidant activity of seed lipid extracts was measured to determine the influence of these antioxidants on seed longevity, and whether the assay may be used to identify optimum storage environments. The results gathered do not support the hypotheses that longer-lived seeds contain more antioxidants, and that seed deterioration is accompanied by a decline in lipid-soluble antioxidant activity. In contrast to these findings, Pukacka (1991) found that lipid-soluble antioxidant activity decreased in *Acer platanoides* seeds as seed age increased, and Senaratna *et al*. (1988) measured a 50% decrease in the lipid-soluble antioxidant activity of aged soybean seed axes. These studies found further evidence of free-radical-induced injury in seeds, with increased solute leakage, an increase in free fatty acids and a decrease in total phospholipids detected in both species, and a decrease in the proportion of unsaturated fatty acids measured in *A. platanoides*. However, confounding the reliable detection of oxidative injury to seeds, free-radical formation and antioxidant status in seeds is highly complex, and may be affected by storage temperature and seed water content, as well as the seed tissues employed for study and the distribution of the antioxidants within these tissues (Benson, 1990; Smith and Berjak, 1995). In this study antioxidant activity was not related to storage temperature or seed water content; thus, these factors do not account for the variability in activity between seeds of similar viability. The results suggest the assay employed is unlikely to be reliable for estimating seed longevity or quality during storage for Australian species.

Conclusion

Seeds of each of the species investigated in this study displayed a number of characteristics consistent with orthodox storage behaviour: seed water content increased with an increase in relative humidity or a decrease in temperature; all seeds survived initial desiccation to approximately 1% water content and exposure to freezing temperatures; and longevity at 50°C generally increased with decreasing water content. However, after 18 months' storage at –18°C, seeds of *A. manglesii* and *M. tetragona* had reduced germination and/or seedling vigour. Such a characteristic is not typical of orthodox seeds and warrants further investigation. Furthermore, this result suggests that storage conditions based on International Plant Genetic Resources Institute (IPGRI) recommendations (–18°C and 5 ± 2% water content) may not be appropriate for seeds of certain Western Australian species, and that adopting storage principles derived for predominantly agricultural species without experimentation may result in unnecessary loss of part or all of a seed accession. This study highlights the importance of investigating a range of storage environments for species of unknown storage characteristics and the need for regular viability testing to assess the suitability of the chosen environments.

References

Benson, E.E. (1990) *Free radical damage in stored plant germplasm.* Rome, International Board for Plant Genetic Resources.

Buitink, J., Claessens, M.M.A.E., Hemminga, M.A. and Hoekstra, F.A. (1998) Influence of water content and temperature on molecular mobility and intracellular glasses in seeds and pollen. *Plant Physiology* **118**, 513–541.

Buitink, J., Leprince, O., Hemminga, M.A. and Hoekstra, F.A. (2000) The effects of moisture and temperature on the ageing kinetics of pollen: interpretation based on cytoplasmic mobility. *Plant Cell and Environment* **23**, 967–974.

Cavanagh, T. (1987) Germination of hard-seeded species (Order Fabales). pp. 58–70 *in* Langkamp, P.J. (Ed.) *Germination of Australian native plant seed*. Melbourne, Inkata Press.

https://doi.org/10.1079/SSR2003133 Published online by Cambridge University Press

Christie, W.W. (1973) *Lipid analysis: Isolation, separation, identification, and structural analysis of lipids*. Oxford, Pergamon Press.

Eira, M.T.S., Walters, C., Caldas, L.S., Fazuoli, L.C., Sampaio, J.B. and Dias, M.C.L.L. (1999) Tolerance of *Coffea* spp. seeds to desiccation and low temperature. *Revista Brasileira de Fisiologia Vegetal* **11**, 97–105.

Ellis, R.H. (1998) Longevity of seeds stored hermetically at low moisture contents. *Seed Science Research* **8** (suppl. 1), 9–10.

Ellis, R.H., Hong, T.D. and Roberts, E.H. (1989) A comparison of the low-moisture-content limit to the logarithmic relation between seed moisture and longevity in twelve species. *Annals of Botany* **63**, 601–611.

Ellis, R.H., Hong, T.D., Roberts, E.H. and Tao, K.L. (1990a) Low moisture content limits to relations between seed longevity and moisture. *Annals of Botany* **65**, 493–504.

Ellis, R.H., Hong, T.D. and Roberts, E.H. (1990b) An intermediate category of seed storage behaviour? 1. Coffee. *Journal of Experimental Botany* **41**, 1167–1174.

Ellis, R.H., Hong, T.D. and Roberts, E.H. (1992) The low-moisture-content limit to the negative logarithmic relation between seed longevity and moisture content in three subspecies of rice. *Annals of Botany* **69**, 53–58.

Ellis, R.H., Hong, T.D. and Roberts, E.H. (1995) Survival and vigour of lettuce (*Lactuca sativa* L.) and sunflower (*Helianthus annuus* L.) seeds stored at low and very-low moisture contents. *Annals of Botany* **76**, 521–534.

Ellis, R.H., Hong, T.D., Astley, D., Pinnegar, A.E. and Kraak, H.L. (1996) Survival of dry and ultra-dry seeds of carrot, groundnut, lettuce, oilseed rape, and onion during five years' hermetic storage at two temperatures. *Seed Science and Technology* **24**, 347–358.

Fielding, J.L. and Goldsworthy, A. (1980) Tocopherol levels and ageing in wheat grains. *Annals of Botany* **46**, 453–456.

Hong, T.D. and Ellis, R.H. (1995) Interspecific variation in seed storage behaviour within two genera – *Coffea* and *Citrus*. *Seed Science and Technology* **23**, 165–181.

Hong, T.D. and Ellis, R.H. (1996) *A protocol to determine seed storage behaviour*. Rome, International Plant Genetic Resources Institute.

Hu, C., Zhang, Y., Tao, M., Hu, X. and Jiang, C. (1998) The effect of low water content on seed longevity. *Seed Science Research* **8** (suppl. 1), 35–39.

International Seed Testing Association (ISTA) (1985) International rules for seed testing. *Seed Science and Technology* **13**, 299–355.

Kong, X.H. and Zhang, H.Y. (1998) The effect of ultra-dry methods and storage on vegetable seeds. *Seed Science Research* **8** (suppl. 1), 41–45.

Kraak, H.L. and Vos, J. (1987) Seed viability constants for lettuce. *Annals of Botany* **59**, 343–349.

Leprince, O., Hendry, G.A.F. and McKersie, B.D. (1993) The mechanisms of desiccation tolerance in developing seeds. *Seed Science Research* **3**, 231–246.

McDonald, M.B. (1999) Seed deterioration: physiology, repair and assessment. *Seed Science and Technology* **27**, 177–237.

Meney, K.A. and Dixon, K.W. (1995) *In vitro* propagation of Western Australian rushes (Restionaceae and related families) by embryo culture. Part 1. In vitro embryo growth. *Plant Cell, Tissue and Organ Culture* **41**, 107–113.

Merritt, D.J., Touchell, D.H., Senaratna, T., Dixon, K.W. and Sivasithamparam, K. (2003) Water sorption characteristics of seeds of four Western Australian species. *Australian Journal of Botany* **51**, 85–92.

Morse, J., Whitfeld, S. and Gunn, B. (1993) *Australian seed banks and cryogenic storage of rare and threatened flora*. Final Consultancy Report to the Endangered Species Unit, Australian Nature Conservation Agency. Canberra, Australian Tree Seed Centre, CSIRO Division of Forestry.

Priestley, D.A., McBride, M.B. and Leopold, C. (1980) Tocopherol and organic free radical levels in soybean seeds during natural and accelerated aging. *Plant Physiology* **66**, 715–719.

Pritchard, H.W., Poynter, A.L.C. and Seaton, P.T. (1999) Interspecific variation in orchid seed longevity in relation to ultra-dry storage and cryopreservation. *Lindleyana* **14**, 92–101.

Pukacka, S. (1991) Changes in membrane lipid components and antioxidant levels during natural ageing of seeds of *Acer platanoides*. *Physiologia Plantarum* **82**, 306–310.

Rao, N.K., Roberts, E.H. and Ellis, R.H. (1987) Loss of viability in lettuce seeds and the accumulation of chromosome damage under different storage conditions. *Annals of Botany* **60**, 85–96.

Roberts, E.H. and Ellis, R.H. (1989) Water and seed survival. *Annals of Botany* **63**, 39–52.

Rokich, D.P., Dixon, K.W., Sivasithamparam, K. and Meney, K.A. (2000) Topsoil handling and storage effects on woodland restoration in Western Australia. *Restoration Ecology* **8**, 196–208.

Senaratna, T. and McKersie, B.D. (1986) Loss of desiccation tolerance during seed germination: A free radical mechanism of injury. pp. 85–102 *in* Leopold, A.C. (Ed.) *Membranes, metabolism, and dry organisms*. Ithaca, Comstock Publishing.

Senaratna, T., McKersie, B.D. and Stinson, R.H. (1985) Antioxidant levels in germinating soybean seed axes in relation to free radical and dehydration tolerance. *Plant Physiology* **78**, 168–171.

Senaratna, T., Gusse, J.F. and McKersie, B.D. (1988) Age-induced changes in cellular membranes of imbibed soybean seed axes. *Physiologia Plantarum* **73**, 85–91.

Smith, M.T. and Berjak, P. (1995) Deteriorative changes associated with the loss of viability of stored desiccation-tolerant and desiccation-sensitive seeds. pp. 701–746 *in* Kigel, J.; Galili, G. (Eds) *Seed development and germination*. New York, Marcel Dekker.

Tieu, A., Dixon, K.W., Meney, K.A. and Sivasithamparam, K. (2001) The interaction of heat and smoke in the release of seed dormancy in seven species from southwestern Western Australia. *Annals of Botany* **88**, 259–265.

Touchell, D.H., Richardson, M. and Dixon, K.W. (1997) *Germplasm conservation guidelines for Australia*. Canberra, Australian Network for Plant Conservation.

Vertucci, C.W. and Roos, E.E. (1990) Theoretical basis of protocols for seed storage. *Plant Physiology* **94**, 1019–1023.

Vertucci, C.W. and Roos, E.E. (1993) Theoretical basis of protocols for seed storage II. The influence of temperature on optimal moisture levels. *Seed Science Research* **3**, 201–213.

Vertucci, C.W., Roos, E.E. and Crane, J. (1994) Theoretical basis of protocols for seed storage III. Optimum moisture contents for pea seeds stored at different temperatures. *Annals of Botany* **74**, 531–540.

Walters, C. (1998a) Understanding the mechanisms and kinetics of seed aging. *Seed Science Research* **8**, 223–244.

Walters, C. (1998b) Ultra-dry technology: Perspective from the National Seed Storage Laboratory, USA. *Seed Science Research* **8** (suppl. 1), 11–14.

Walters, C. and Engels, J. (1998) The effects of storing seeds under extremely dry conditions. *Seed Science Research* **8** (suppl. 1), 3–8.

Wilson, D.O. and McDonald, M.B. (1986) The lipid peroxidation model of seed aging. *Seed Science and Technology* **14**, 269–300.

Winston, P.W. and Bates, D.H. (1960) Saturated solutions for the control of humidity in biological research. *Ecology* **41**, 232–237.

Zewdie, M. and Ellis, R.H. (1991) Survival of tef and niger seeds following exposure to sub-zero temperatures at various moisture contents. *Seed Science and Technology* **19**, 309–317.

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