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A continental-scale study of seed lifespan in experimental storage examining seed, plant, and environmental traits associated with longevity

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Abstract Management of seed banks conserving the biodiversity of phylogenetically diverse species requires insight into seed longevity. This study determined the seed longevity of 172 species sourced from across the mega-diverse flora of the Australia continent. Seeds were aged via a controlled ageing experiment through storage at 45 °C and 60 %

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RH, or 60 °C and 60 % RH, and regularly tested for germination. Relative seed longevity between species was determined by comparing the time to 50 % viability loss (p_{50}), calculated via probit analysis of seed survival curves. Seed, plant, and environmental traits were examined for associations with longevity. The p_{50} values varied between species from 3.0 to 588.6 days. Serotinous species, and woody trees and shrubs, had significantly longer-lived seeds than geosporous species, and species of herbaceous habit. Seeds that possess physical dormancy, and seeds with large embryos with little endosperm, were also long-lived. There was a weak, but significant, positive correlation between seed mass and longevity. Seeds sourced from regions of higher mean annual temperature and rainfall were significantly longer-lived than seeds from cooler and drier regions, although both environmental factors were weakly associated with longevity. Compared with species from other regions of the world, prolonged longevity is a feature of many Australian species. Nevertheless, seed life-spans vary substantially between species and close consideration of seed traits along with biotic and abiotic components of the plants and their environment can assist to differentiate between potentially long- and short-lived seeds.

Keywords Biodiversity conservation · Climate · Gene bank · Germination · Seed bank · Serotiny

Introduction

The collection and storage of seeds of wild species in *ex situ* seed banks is a major strategy for plant biodiversity conservation (Smith et al. 2011; Godefroid et al. 2011). Conservation seed banks can establish and maintain genetically and geographically representative samples of wild plant germplasm able to be used years, decades, or even centuries into the future (Walters et al. 2005; Li and Pritchard 2009). Seed banks also have a major role to play in facilitating the restoration of degraded land (Merritt and Dixon 2011). The longevity of orthodox (desiccation tolerant) seeds increases as seed moisture content and storage temperature is reduced (Ellis and Roberts 1980). However, all seeds have a finite lifespan and even under ideal storage conditions the longevity of seeds varies greatly between species (Walters et al. 2005; Probert et al. 2009). For the effective management of seed banks, data on seed longevity must be established. For example, seed banks conserving wild species and providing seeds for use in restoration programs commonly manage large, seasonal seed collection programs that result in many collections being received at the same time. Identifying species with short-lived seeds assists in prioritising these for immediate post-harvest processing, provides a focus for the development of more specialised storage techniques such as cryostorage (Li and Pritchard 2009), and informs viability testing and regeneration cycles.

Direct measurements of seed longevity under gene banking conditions (storage at −20 °C) are difficult to obtain in real time as it might take decades, perhaps centuries, for germination to begin to decline (Walters et al. 2005). As an alternative, seed ageing can be accelerated by storing seeds in warm, moist conditions (Newton et al. 2009). Controlled ageing experiments have recently been employed to compare the seed longevity of a diverse range of species held in the Royal Botanic Gardens, Kew, Millennium Seed Bank, in relation to seed traits and environmental parameters (Probert et al. 2009). Similar

comparative studies have been undertaken on some Australian (Hay et al. 2010; Tuckett et al. 2010; Crawford et al. 2011) and European species (Mondoni et al. 2011).

Controlled ageing experiments allow the seed longevity of species to be ranked relative to each other. Species are ranked either by the time it takes for 50 % of the seeds within an accession to lose viability (p_{50}), or by the standard deviation of the frequency distribution of seed deaths in time (σ) (Newton et al. 2009). An assessment of the comparative longevity of seeds of 195 species sourced from many countries demonstrated that p_{50} values ranged from 0.1 to 771 days (Probert et al. 2009). The seeds of the Australian species tested were amongst the longest-lived. On average seeds of Australian species had a p_{50} value of 202.7 days, compared with seeds from Chile, England, and the USA, which had p_{50} values of 25.2, 28.8, and 38.6 days, respectively (Probert et al. 2009). However, the study assessed relatively few Australian species (22 species in eight families), and whilst another study of the seed longevity of 276 species from across the globe noted that the seven Australian species tested were long-lived (Walters et al. 2005), further research has shown that not all Australian species are long-lived. Seeds of some Australian semi-aquatic species were found to have p_{50} values between 5.8 and 44.3 days (Tuckett et al. 2010), and some Australian terrestrial orchid species have p_{50} values of between 0.5 and 36.3 days, even when aged at a cooler temperature of 40 °C (Hay et al. 2010).

In a broader context, seed ageing data can be used to investigate the relationship between seed longevity and seed morphological, physical, and physiological traits, as well as the environment in which the parent plants grow. Examining such correlates provides the potential for predicting the longevity of untested species and also contributes to understanding ecological and plant/seed physiological traits associated with seed longevity. Recent studies have shown that climate influences seed longevity, with seeds originating in hot, dry environments having greater longevity than those from cool, wet climates (Walters et al. 2005; Probert et al. 2009). Taxonomic relatedness also influences seed longevity, with species from the Apiaceae and Brassicaceae being short-lived, and species from the Malvaceae and Chenopodiaceae being long-lived (Walters et al. 2005). In terms of seed traits, endospermic seeds have been shown to be shorter-lived than non-endospermic seeds (Probert et al. 2009). Other potential correlates of seed longevity that have been postulated include seed mass, oil content, and carbohydrate composition (Walters et al. 2005), but recent studies have failed to corroborate any correlation between these factors and seed longevity (Walters et al. 2005; Probert et al. 2009).

Australia is a continent rich in plant biodiversity, containing one of the world's 34 biodiversity hotspots and an ancient flora with a high level of endemism on a global scale (Myers et al. 2000; Hopper and Gioia 2004). Pressing conservation issues include a high rate of plant species extinction and habitat loss (Mackey et al. 2008; Lindenmayer et al. 2010) and a flora predicted to be significantly impacted by climate change. Such issues are motivating increasingly urgent protection measures (Fitzpatrick et al. 2008; Lindenmayer et al. 2010). Collaborative seed conservation initiatives in Australia, particular those formed with the Millennium Seed Bank Partnership (Royal Botanic Gardens, Kew, http://www.kew.org/msbp/index.htm), have seen seeds of more than 8,000 species, representing one third of the Australian flora, collected and banked from 2001 to 2010. To be effectively managed, these large-scale seed conservation programs must be underpinned by data on seed storage behaviour and longevity.

This study aimed to inform conservation seed banking programs and to test recent hypotheses proposing that seed traits (e.g. endosperm) and climatic traits (e.g. rainfall and temperature) are predictors of long- or short-lived seeds (Probert et al. 2009; Mondoni et al. 2011). Study species were sourced from across the Australian mainland continent,

selected to encompass a range of climatic zones and as representative of nine of the top ten families (by number of genera) in Australia. The study species also encompass variations in seed traits and plant life-forms. The experiments addressed the specific aims of: (1) determining seed longevity under controlled ageing conditions; (2) examining seed longevity in the context of phylogenetic relatedness, and; (3) investigating whether certain seed characteristics including seed size, presence/absence of endosperm, embryo type, post-maturation dispersal syndrome (serotiny vs. geospory), climate (mean annual rainfall and mean annual temperature), and plant life-form are associated with seed longevity.

Materials and methods

Study species

Seeds of 172 species from 44 plant families were selected for study. Species from across Australia (in the states of Western Australia (WA), Queensland (QLD), New South Wales (NSW), and South Australia (SA) were selected from seed collections held in the seed banks of: Kings Park and Botanic Garden, WA; the Australian Botanic Garden, Mount Annan, NSW; Botanic Gardens of Adelaide, SA. All experiments were undertaken in the laboratories of each of the three Botanic Gardens (with seeds sourced from WA and QLD tested at Kings Park and Botanic Garden). Prior to experimentation most seeds were stored according to international gene bank standards at −20 °C after drying at 15 °C, 15 % RH (FAO/IPGRI 1994), although some were used immediately following collection, cleaning, and drying. Seed viability, based on germination testing, exceeded 90 % for all species prior to the ageing experiments. Species were selected based on a number of criteria including: seed accessions available of known germination requirements and high initial seed viability; representative of dominant Australian plant families; variations in seed morphological types and plant life-forms; species that originate from a range of climatic zones including Mediterranean, temperate, arid, and tropical regions (Appendix).

Controlled seed ageing

Controlled seed ageing experiments were conducted via a modification of the comparative seed longevity protocol (Newton et al. 2009; Probert et al. 2009). To first hydrate the seeds, for each species a minimum of 10 samples of 50 seeds were placed in open 7.5 mL glass vials and the vials placed for 14 days in air-tight polycarbonate electrical enclosure boxes (28 × 28 × 14 cm; NHP Fibox, Australia) above a non-saturated solution of LiCl (370 g L^{-1}; anhydrous, Sigma®, Australia), creating a relative humidity (RH) of 47 % at 20 °C (Hay et al. 2008). For seed ageing, vials containing seeds were then transferred to polycarbonate boxes above a non-saturated solution of LiCl (either 300 or 280 g L^{-1}, to create a RH of 60 % at 45 or 60 °C, respectively). Seeds were aged at either 45 or 60 °C depending on their expected longevity. Preliminary experiments on a range of seed types indicated that ageing at 45 °C was too slow for the practical determination of longevity. Therefore, seeds of 44 species predicted to be long-lived were aged at 60 °C to speed the ageing process (ageing temperature for each species is noted in the Appendix). The RH within the polycarbonate boxes was monitored via a hygrometer HygroPalm AW1 (Pryde Measurement Pty Ltd, Ashburton, Australia) throughout the ageing experiment. One sample of 50 seeds was removed from the ageing conditions at each predetermined sample time for germination testing. To ensure the experimental procedures were comparable

amongst laboratories, seeds of *Brassica napus* L. originating from the same seed lot served as a control (aged at 45 °C and 60 % RH). The variation in p_{50} values for *B. napus* seeds tested in the three laboratories was small (mean $p_{50} = 54.7 \pm 4.81$ days) and no manipulation of the raw germination data collected in each laboratory was undertaken prior to analyses.

Germination testing

Pre-treatments were applied to seeds of some species to alleviate dormancy (Appendix). Some seeds were pre-treated prior to being placed into polycarbonate boxes for hydration. Species known to possess physical dormancy (water impermeable seed coats) were scarified by nicking the testa with a scalpel, or via dipping in hot water (HW) at 95 °C for between 30 s and 2 min. For three species, dry seeds (15 % RH at 15 °C) were heat treated in an oven at 40 °C for 4 weeks (*Thysanotus patersonii*), or at 100 °C for 3 h (*Anigozanthos manglesii* and *Austrostipa elegantissima*).

Following removal from the ageing environment, seeds of all species were sown in 90 mm Petri dishes containing 25 mL solidified water agar (0.7 % w/v), or water agar containing either gibberellic acid (GA$_3$ at 0.29, 1.44, or 2.89 mM; Sigma®, Australia) or karrikinolide (KAR$_1$ at 0.67 µM; synthesised as described in Flematti et al. (2005) as required to alleviate dormancy (Appendix). Petri dishes were incubated at either a constant temperature regime, or a daily alternating (12/12 h) temperature regime (Appendix). All seeds were incubated under a daily 12 h photoperiod of 30 µmol m^{-2} s^{-1}, 400–700 nm, cool-white fluorescent light, with the light provided during the warm temperature phase for seeds under the alternating temperature regime. Seeds of species known to be susceptible to contamination were sterilised prior to incubation in Petri dishes by immersion in a 2 % (w/v) Ca(OCl)$_2$ solution for 10 min, and then rinsed three times in sterile de-ionised water. Germination was assessed based on the emergence of a radicle to >2 mm, and subsequent normal seedling development.

Seed, plant, and climatic characteristics

To determine seed size, mean seed dry weights (of seeds dried to 15 % RH at 15 °C) were obtained by weighing three replicates of 100 seeds (WA and SA), or three replicates of 50 seeds (NSW) using a four decimal-point balance. Seed morphological traits were determined using five replicate seeds of each species, whereby seeds were sectioned under a dissecting microscope and the presence/absence of endosperm was recorded, and seed embryo type classified, according to Martin (1946).

The seed dispersal syndrome of each species was classified as geosporous (seeds are released annually into the soil seed bank) or serotinous (the release of mature seeds is delayed and seeds are instead retained on the parent plant to form a canopy seed bank) (Lamont et al. 1991). Plant life-form data (forb, grass, herb, herb/shrub, rush, sedge, shrub, climber, shrub/tree, or tree) for each species were collated from seed accession collection notes and via PlantNET—The Plant Information Network System of The Royal Botanic Gardens and Domain Trust, Sydney, Australia (Version 2.0) (http://plantnet.rbgsyd.nsw.gov.au) for species from NSW; via eFloraSA—The Electronic Flora of South Australia (http://www.flora.sa.gov.au/) for species from SA, and; via Western Australian Herbarium (1998)—FloraBase—the Western Australian Flora, Department of Parks and Wildlife (http://florabase.dpaw.wa.gov.au/) accessed in January 2012. Mean annual temperature and mean annual rainfall for all recorded years for the collection location of each species was

determined from statistics available from the weather station nearest to each species' collection location, with data gathered from the Australian Bureau of Meteorology (http://www.bom.gov.au accessed in January 2012).

Data analysis

The time taken for seed viability to fall to 50 % (p_{50}) was calculated through probit analysis of the seed ageing data using Genstat version 10.0 (VSN International Ltd., UK) fitting the seed viability equation (Ellis and Roberts 1980):

$$v = K_i - p/\sigma$$

where v is the viability in normal equivalent deviates (NED) at time p (days); K_i is the initial viability (NED) and σ is the standard deviation of the normal distribution of seed deaths in time (Ellis and Roberts 1980).

To enable comparison of the p_{50} values of all species across the entire dataset seeds of 21 species were aged at both 45 °C, 60 % RH and 60 °C, 60 % RH. Species with a representative range of seed types and source locations were selected for this comparison (Appendix). There was an 8.44 ($\pm$1.154 SE) fold difference between p_{50} values of these seeds aged at 45 and 60 °C. Therefore, for those seeds aged at 60 °C, the p_{50} at 45 °C was estimated by multiplying the p_{50} values at 60 °C by 8.44 following the methods of Probert et al. (2009). The transformed p_{50} values for those seeds aged at 60 °C are presented in the Appendix and were used for all subsequent analyses and comparisons.

Direct effects of phylogeny on seed longevity were estimated via graphical analysis. Variation of p_{50} values for families and orders of species were plotted via boxplots using SigmaPlot 10.0 (Systat Software Inc., USA). Only families and orders are presented if data had been obtained for three or more species in each test group.

The relationship between p_{50} and seed mass, seed embryo type, plant life-form, seed dispersal syndrome (serotinous or geosporous), endosperm presence or absence, and local climatic conditions (mean annual rainfall and mean annual temperature) was investigated through generalized least-squares regression analysis in R (R: Development core team). Seed mass and p_{50} data were $\log_{10}$ transformed to ensure normality of data (Probert et al. 2009). The effect of phylogenetic relationships (based on APGIII 2009) was removed by defining a covariance matrix based on phylogenetic branch lengths between species. Initial branch lengths were assumed to be one between species within the same genus, two between species in different genera but the same family, and three between species in different families but the same order. The R package caper (Orme 2012) allows the creation of a covariance matrix based on those branch lengths. The model assumes a Brownian model of evolution: how related two species are determines how similar their traits are. However, this is not necessarily an appropriate evolutionary model. Thus, caper offers several methods of transforming the covariance matrix to improve the fit to the data. The simplest of the three transformation parameters was chosen: the internal branch lengths were multiplied by a constant λ. By multiplying branch lengths by a constant, the strength of similarity between species was altered, based on their relatedness. The optimal value of λ was found for each individual model using maximum likelihood fitting. Additionally, in using equal branch lengths (even under transformation), a punctuated model of trait evolution—that a trait only changes at a branching event—was assumed.

Initial analysis showed a high correlation between the seed dispersal syndrome and the presence of endosperm. Both parameters were tested individually in a model with all other variables. The adjusted r^2 values were compared between the two models and showed that seed dispersal syndrome explained significantly more variation than the presence of

endosperm. Thus, the presence of endosperm was dropped and only seed dispersal syndrome was used. The maximal model therefore included all of the explanatory variables mentioned above (except endosperm presence) and all second order interactions excluding any interactions with embryo type and plant life-form. The phylogenetic generalised linear model function that was used in caper requires that all categorical interactions exist. Embryo type and plant life-form led to interaction terms that did not exist within the sample, and thus those interactions were excluded from the maximal model. A stepwise deletion was performed to minimize the model for the simplest final result (Crawley 2007; Zuur et al. 2009). Because the phylogenetic branch length was optimized within each individual model, it was not possible to compare models using ANOVA tests. Rather, a comparison of adjusted r^2 values was made. If the adjusted r^2 value decreased after a term was dropped, the term was retained. An additional objective was to minimize the number of categories being tested. Plant life-form and embryo type were both factors with a relatively large number of categories. Once interaction terms were minimized, non-significantly different categories were combined together, checking at each combination step that the adjusted r^2 value was improved.

Results

Variation of p_{50} between species

The viability of seeds aged at either 45 or 60 °C declined with time, following a negative sigmoidal curve (Fig. 1), and seeds aged more rapidly at 60 °C than at 45 °C. Across all species, the calculated p_{50} values of seeds varied by three orders of magnitude (Appendix). The variation in p_{50} for the 172 species ranged from 3.0 days in *Callitris pyramidalis* to 588.6 days in *Acacia colei* var. *colei*. When seed longevity was compared across orders (Fig. 2, Appendix) species from the Fagales (mean $p_{50} = 223.2 \pm 59.12$ days), Sapindales (mean $p_{50} = 192.8 \pm 46.68$ days), Fabales (mean $p_{50} = 178.6 \pm 21.76$ days), Myrtales (mean $p_{50} = 169.3 \pm 25.49$ days), Rosales (mean $p_{50} = 162.2 \pm 54.68$ days), and Proteales (mean $p_{50} = 123.9 \pm 25.85$ days) were amongst those with the longest-lived seeds. Species from the Asparagales (mean $p_{50} = 32.5 \pm 7.38$ days), Asterales (mean $p_{50} = 32.1 \pm 4.31$ days), Caryophyllales (mean $p_{50} = 28.5 \pm 9.26$ days), Liliales (mean $p_{50} = 29.0 \pm 9.72$ days), Apiales (mean $p_{50} = 24.8 \pm 7.97$ days), and Lamiales (mean $p_{50} = 19.4 \pm 7.98$ days) were amongst those with the shortest-lived seeds. However, the variation of p_{50} values within orders was large in many cases. For example, for species from the Poales (18 species, 5 genera tested), p_{50} values ranged from 11.8 days (*Poa hiemata*) to 183.1 days (*Cyperus ixiocarpus*). At the family level, species of the Casuarinaceae (mean $p_{50} = 223.2 \pm 59.12$ days), Rhamnaceae (mean $p_{50} = 208.9 \pm 66.09$ days), Sapindaceae (mean $p_{50} = 192.8 \pm 46.68$ days), Fabaceae (mean $p_{50} = 178.6 \pm 21.76$ days), and Myrtaceae (mean $p_{50} = 176.0 \pm 25.93$ days) comprised species with the longest-lived seeds. Species of the Asteraceae (mean $p_{50} = 32.4 \pm 5.03$ days), and Poaceae (mean $p_{50} = 30.6 \pm 7.04$ days) tended to have short-lived seeds, along with many other families for which only a single, or a few, representative species were tested (Fig. 2, Appendix). In some families there was a large variation in p_{50} values. For example, within the Proteaceae ($n = 12$), five species (*Banksia attenuata*, *Banksia ericifolia*, *Banksia menziesii*, *Banksia prionotes*, and *Hakea petiolaris*) were amongst the longest-lived ($p_{50} > 150$ days), whereas three species (*Grevillea robusta*, *Lomatia myricoides*, and *Telopea speciosissima*) were amongst the shortest-lived ($p_{50} < 20$ days). Similarly, for the Fabaceae, p_{50} values ranged from 39.0 days (*Daviesia cordata*) to 588.6 days (*Acacia colei* var. *colei*).
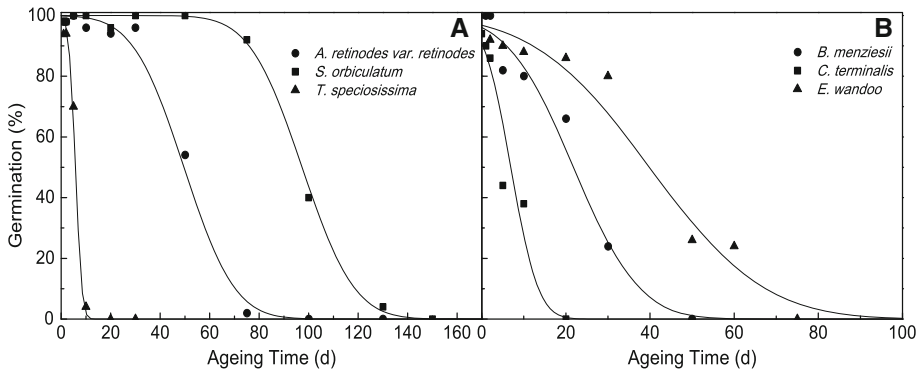
Fig. 1 Seed survival curves fitted by probit analysis for seeds of **a** *Acacia retinodes*, *Solanum orbiculatum* and *Telopea speciosissima* aged at 45 °C and 60 % RH, and seeds of **b** *Banksia menziesii*, *Corymbia terminalis*, and *Eucalyptus wandoo* aged at 60 °C and 60 % RH
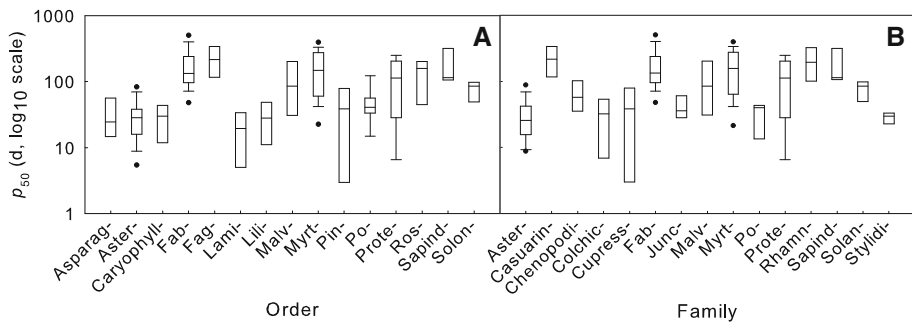

Fig. 2 *Box plots* of p_{50} values of plant **a** orders and **b** families. *Boxplots* show the 25–75th percentiles, *whiskers* span the 10 and 90th percentiles, and *circles* span the 5 and 95th percentiles. Groups that comprise less than three representative species are not shown

Seed, plant, and climatic correlates with seed longevity

Seed dispersal syndrome was found to be highly significantly correlated with seed longevity (Table 1). Serotinous species had seeds that were longer-lived (Fig. 3), with a mean p_{50} of 173.0 ± 19.18 days, compared with seeds from geosporous species, with a mean p_{50} of 91.1 ± 8.88 days. Seeds from species that were shrubs or trees (woody species) were significantly longer-lived than seeds from other life-forms that would form understorey and herbaceous components of vegetation communities (Fig. 4; Table 1). Certain seed characteristics were also found to be significant correlates of longevity. Seeds with folded embryos were found to be significantly longer-lived (mean $p_{50} = 196.7 ± 28.95$ days) than seeds with other embryo types (mean $p_{50} = 92.0 ± 8.12$ days) (Fig. 5; Table 1). Although seeds with investing (mean $p_{50} = 155.7 ± 18.74$ days) and bent (mean $p_{50} = 107.6 ± 12.31$ days) embryos were also relatively long-lived, they were not found to significantly differ from the remaining embryo types during the model minimisation process. Seed mass varied between 0.008 and 121.8 mg (for a single seed) and there was a weak, but significant, positive correlation between seed mass and p_{50}, with heavier seeds having greater p_{50} values (Fig. 6; Table 1).

Table 1 Results for the minimized model for the effects of seed, plant, and environmental traits on seed longevity

Variable	Estimate	SE	t value	Pr (>|t|)
(Intercept)[a]	−1.201	1.202	−0.999	0.319
Serotiny	3.574	0.705	5.069	<0.001
Seed mass (log)	0.375	0.173	2.169	0.032
Mean temperature	0.149	0.041	3.612	<0.001
Mean rainfall	0.003	0.001	2.750	0.007
(Life-form) herbaceous[b]	1.568	0.871	1.801	0.074
(Life-form) woody[c]	2.153	0.873	2.466	0.015
Embryo: folded	0.599	0.265	2.261	0.025
Serotiny × temperature	−0.153	0.037	−4.158	<0.001
Seed mass × rainfall	−0.0004	0.0002	−2.0	0.047
Temperature × rainfall	−0.0001	0.0001	−2.411	0.017
Multiple R-squared: 0.3241				
Adjusted R-squared: 0.2819				

The variables listed are those retained within the final model with estimate, standard error of the estimate, t-value, and estimated p value

[a] Seed dispersal syndrome = geosporous; life-form = climber; embryo type = all except folded

[b] Life-form herbaceous = forb, grass, herb, herb/shrub, rush, sedge

[c] Life-form woody = shrub, shrub/tree, tree

Climatic factors were found to correlate with seed longevity (Fig. 7; Table 1), though with relatively weak effect estimates. Species from warmer environments with higher rainfall averages were found to have longer-lived seeds than species from cooler, drier regions. Additionally, environmental factors were found to have significant interactions with some plant traits: serotiny and temperature interacted significantly, as well as seed mass and rainfall average. However, these interactions were also weak.

Discussion

Seeds of many species proved to be long-lived under the experimental storage conditions and it is clear that species with long-lived seeds are relatively abundant in the Australia flora. Of the 172 species studied, 28 had seeds with p_{50} values exceeding 200 days. To date, out of more than 460 species originating from across the globe, only species of Australian origin have been found to have p_{50} values > 200 days (Hay et al. 2006; Kochanek et al. 2009; Probert et al. 2009; Hay et al. 2010; Tuckett et al. 2010; Crawford et al. 2011; Mondoni et al. 2011). The longest-lived species yet identified is the Australian species *Calothamnus rupestris*, with a p_{50} of 771 days (Probert et al. 2009). This exceeds the longest-lived species in the present study (*Acacia colei* var. *colei* with a p_{50} of 589 days), but is consistent with the correlations identified between seed traits and longevity; *C. rupestris* being a serotinous, woody shrub/tree species of Myrtaceae with non-endospermic seeds.

The finding that endospermic seeds were shorter-lived than non-endospermic seeds is consistent with a previous study of the longevity of 195 species (Probert et al. 2009). However, as compared with presence/absence of endosperm, in this study seed dispersal syndrome (i.e. serotiny vs. geospory) had the stronger explanatory power, with seeds of
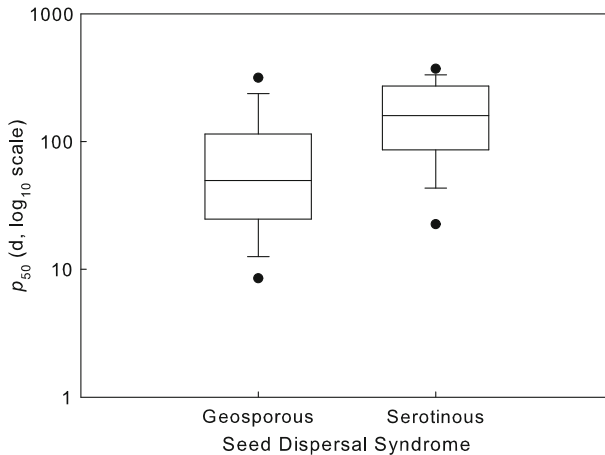
Fig. 3 *Box plots* of p_{50} values of geosporous and serotinous species. *Boxplots* show the 25–75th percentiles, *whiskers* span the 10 and 90th percentiles, and *circles* span the 5 and 95th percentiles

serotinous species being significantly longer-lived than seeds of geosporous species. Serotinous species have been similarly recognised as producing long-lived seeds in a study on three *Banksia* species (Crawford et al. 2011). Many *Banksia* species are strongly serotinous, retaining viable seeds within protective, woody cones for >10–15 years (Lamont et al. 1991), and species of *Allocasuarina* and *Hakea* are also strongly serotinous (Clarke et al. 2011). Thus, a plausible hypothesis is that seed traits conferring longevity in the canopy seed bank contribute to the longevity of serotinous seeds in *ex situ* storage. However, it is notable that some of the longest-lived serotinous seeds in the results reported here, such as those of *Corymbia* and *Eucalyptus* spp., are only weakly serotinous (House 1997). Also, Crawford et al. (2011) found no relationship between the degree of serotiny and seed longevity amongst the three species of *Banksia* studied. So a direct translation of longevity in the canopy seed bank to longevity in *ex situ* storage is not forthcoming. Nevertheless, serotiny *per se* is a good indicator that seeds will be long-lived in storage.

Seed morphological traits that are shared amongst serotinous species and that may contribute to their longevity include large embryos (relative to the size of the seed) and little (e.g. *Callitris*, *Corymbia*, *Eucalyptus*) to no (e.g. *Allocasuarina*, *Banksia*, *Hakea*) endosperm. Underdeveloped (i.e. small) embryos are plesiomorphic amongst angiosperms (Forbis et al. 2002), and Probert et al. (2009) hypothesised that the moist environment in which early angiosperms evolved accounts for the poor longevity of endospermic seeds with small embryos. That is, seed longevity is proposed to have evolved with a drying climate where the ability to survive for longer periods in the dry state would be advantageous (Probert et al. 2009). In hot or seasonally dry climates, greater relative embryo size is also viewed as advantageous through conferring the competitive advantage of rapid germination (Parsons 2012; Vandelook et al. 2012). Thus, larger relative embryo size, along with an increased ability to survive in the dry state (i.e. resistance to ageing) may have evolved in seeds as adaptive traits to increasingly seasonality and aridity. In this study, seeds with folded embryos (embryo:seed ratio of 0.8–1.0) were significantly longer-lived than all other embryo types. Many of the serotinous plants had seeds with folded embryos (*Corymbia*, *Eucalyptus* spp.). Other seeds from serotinous plants possessed large spatulate (*Allocasuarina* spp.) (E:S = 1.0), or investing (E:S = 0.7–1.0) (*Banksia, Hakea* spp.) embryos and these seeds also tended to be long-lived, albeit not to a significant degree from other embryo types.
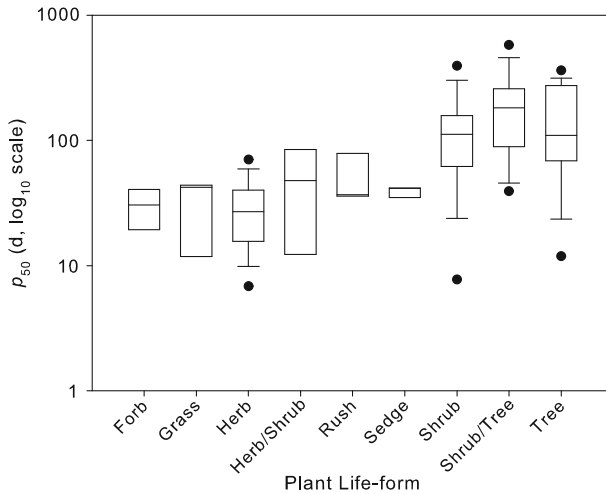
Fig. 4 *Box plots* of p_{50} values of seeds from varying plant life-forms. *Boxplots* show the 25–75th percentiles, *whiskers* span the 10 and 90th percentiles, and *circles* span the 5 and 95th percentiles
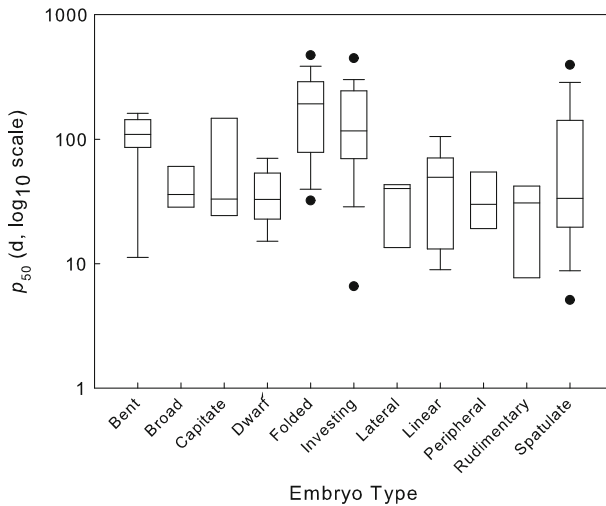
Fig. 5 *Box plots* of p_{50} values of seeds of differing embryo types. *Boxplots* show the 25–75th percentiles, *whiskers* span the 10 and 90th percentiles, and *circles* span the 5 and 95th percentiles. Groups that comprise less than three representative species are not shown

Large folded, investing, and spatulate embryos were also common in seeds with physical dormancy (found in Fabaceae, Malvaceae, Rhamnaceae, and Sapindaceae) and these seeds were dominant amongst the longest-lived. In fact, half of the 30 longest-lived species tested had seeds with physical dormancy, including the four longest-lived (*Acacia colei* var. *colei*, *Mirbelia dilatata*, *Acacia holosericea*, *Trymalium ledifolium*). Notably, in these seeds it was not the presence of an impermeable seed coat (and therefore an ability to maintain a low seed water content during ageing) that was a contributing factor to longevity, as the seeds were

Fig. 6 Relationship between p_{50} values and seed mass. The *line* is a simple linear *trend line* to highlight the relationship between the two variables
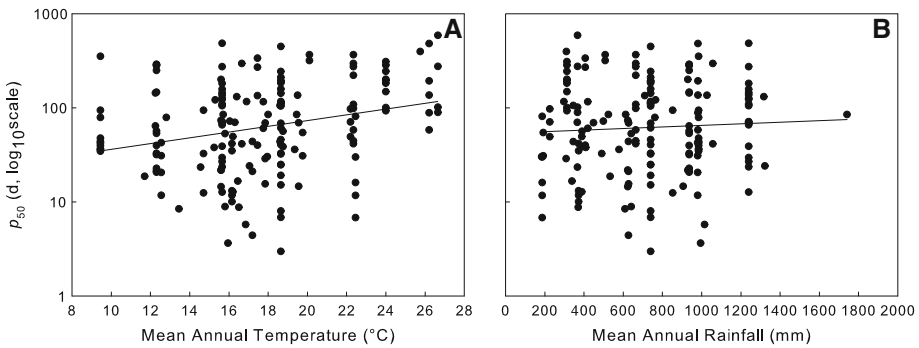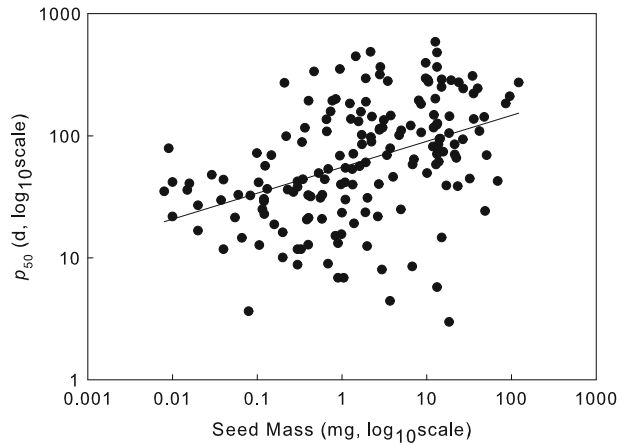
Fig. 7 Relationship between p_{50} values and **a** mean annual temperature, and **b** mean annual rainfall at the collection location of each species. The *lines* are simple *linear trend lines* to highlight the relationships between the p_{50} values and the environmental variables

rendered permeable to water prior to ageing. Other attributes of physically dormant seeds, perhaps associated with large relative embryo size, contribute to their longevity in storage.

Whilst long-lived seeds were a feature of the dataset, longevity still varied widely and a range of species could also be considered relatively short-lived. For example, 42 species had p_{50} values of ≤30 days. Seeds of the northern hemisphere species *Ranunculus sceleratus* L. have a p_{50} of 13.7 days (Probert et al. 2009), and this species has been suggested as a useful marker species for short-lived seeds (Newton et al. 2009). Based on this criterion, 18 species (across 14 plant families) could be considered short-lived in the present study. In their study of the seed longevity of species sourced from across the globe, Probert et al. (2009) found longevity to vary across species by four orders of magnitude. This compares with a variation in longevity of three orders of magnitude in the results presented here. However, Hay et al. (2010) found seeds of Australian Orchidaceae to have p_{50} values as low as 0.5 days when aged at 40 °C and 60 % RH. Therefore, the p_{50} values of Australian seeds also extend over at least four orders of magnitude.

This significant variation in seed longevity of wild species requires careful consideration by curators of conservation seed banks (Hay and Probert 2013). Ideally, seed viability testing should be conducted at intervals coinciding to one-third of the time expected for viability to decline by 15 % (FAO 2013). The comparative longevity approach taken in this study does

not provide for a direct prediction of seed longevity for seeds stored under gene banking conditions (-20 °C). However, the data does provide guidance as to those species with short-lived seeds that should be prioritised for viability testing after 5 years of storage (FAO 2013). The ability to predict the longevity of seeds stored under a range of conditions can be achieved through resolution of the viability equation of Ellis and Roberts (1980) that has been developed for many crop species. But this method requires extensive experimental testing for each species, and consumes large numbers of seeds and significant resources. New approaches being explored to address the difficulty of predicting seed deterioration under gene banking conditions include identifying potential biochemical markers of longevity based on the oxidative damage known to accumulate in seeds during ageing (Kranner et al. 2010), and the measurement of volatile compounds during storage (Colville et al. 2012).

The positive correlation between seed mass and longevity is the first instance of a correlation between these seed traits. Other studies specifically addressing whether seed mass and longevity are correlated have found no relationship (Walters et al. 2005; Probert et al. 2009). This positive correlation, whilst significant, is weak, and may be interpreted with some caution given the absence of corroboration from two previous studies. It is not clear why the data in the present study differs from previous studies, but there are some notable differences in the data sets. The study of Probert et al. (2009) did not comprise many large seeds (only 14 of 195 species were ≥ 10 mg, as compared to 49 species in the present study) and, whilst not significant, the relationship between seed mass and longevity was positive so it could be speculated that a greater sample of species with larger seeds might have strengthened the relationship. The study by Walters et al. (2005) encompassed many species with seeds >10 mg in mass, but their seed mass data was derived from published literature, and their approach to data analysis was different; in particular no account of phylogenetic relatedness was considered. Nonetheless the disparities between studies suggest seed mass is perhaps not a reliable predictor of longevity, and it is difficult to ascribe an ecological or physiological reason for an association between seed size and longevity in *ex situ* storage. Seed size is recognised as an important ecological trait influencing plant fecundity, seed dispersal, and seedling emergence, and bigger seeds are strongly associated with larger adult plants (Moles 2007). In the present study, woody species (i.e. larger trees and shrubs) produced longer-lived seeds compared with other plant life-forms, and seeds of these woody species were indeed larger (mean seed mass of 13.35 ± 1.940 mg, vs. 2.18 ± 1.014 mg, respectively). But an association between woody habit, large seed size, and longevity was not always present as some woody species had small, but very long-lived seeds, particularly those of *Eucalyptus* with a mean seed mass of 0.57 ± 0.185 mg, compared with a mean seed mass of 8.84 ± 1.298 mg for all species.

Significant relationships between seed longevity and the mean annual temperature and mean annual rainfall at the point of origin of the seeds were established. However, these relationships only accounted for a small proportion of the variation in p_{50} values. With respect to temperature, a similar positive correlation to longevity has been noted in other studies (Probert et al. 2009; Mondoni et al. 2011), and it seems that species in warmer regions commonly produce seeds with greater longevity, compared with those species in cooler regions. With respect to rainfall, the results show seeds sourced from higher rainfall zones proved longer-lived, in contrast to previous findings demonstrating that total annual rainfall is negatively correlated to seed longevity (Probert et al. 2009; Mondoni et al. 2011). One reason for the disparity between data sets may be that the present study comprises species from warm temperate, Mediterranean and arid environments, and there are few species from very wet environments in comparison with the other studies of Probert et al. (2009) and Mondoni et al. (2011). For example, only 26 species were from sites receiving between 1,000 and 1,300 mm of rainfall annually, whereas the previous

studies included many species from environments with >2,000 mm rainfall (Probert et al. 2009), and up to *c.* 7,500–9,500 mm (Mondoni et al. 2011). Notably, the study by Mondoni et al. (2011) also found a slightly stronger correlation between p_{50} values and rainfall during the period between flowering and seed set, as opposed to total annual rainfall. Nevertheless for each of these three data sets, rainfall at the point of origin has only been weakly associated with seed longevity and it is evident that further examination of the suitability of rainfall as a predictor of relative seed longevity amongst species is warranted.

Notwithstanding consideration of the potential for some intra-specific variation in seed longevity (Kochanek et al. 2009), plant orders comprising species with longer- (e.g. Fabales, Fagales, Myrtales) or shorter- (e.g. Liliales) lived seeds are consistent with findings of other studies (Walters et al. 2005; Probert et al. 2009). Also, the relationships between seed longevity and certain seed or plant habitat traits provide insight and guidance into the planning and management of conservation seed banks. In particular, certain seed traits appear useful predictors of longevity where genera within a family differ in seed dispersal syndrome. For example, within the Proteaceae a wide variation in longevity was noted (p_{50} values of five species exceeded 150 days, and those of three species were less than 20 days). Of these Proteaceae, the shortest-lived species (*Grevillea robusta*, *Lomatia myricoides*, and *Telopea speciosissima*) are all geosporous, whereas the longest-lived species (*Banksia* spp. or *Hakea* spp.) are all serotinous, and all from Mediterranean or semi-arid regions. Such relationships can be used to predict more easily species or habitats that produce shorter-lived seeds and these seeds assigned priority for post-harvest handling and storage, and the use of alternative storage methods, such as cryostorage, can be considered where appropriate (Li and Pritchard 2009; Hay et al. 2010).

Conclusions

This study provides comparative data for seed longevity across the dominant plant families of the Australian flora. The data demonstrate that seeds of Australian species from Mediterranean, temperate, and arid climates are amongst the longest-lived yet found world-wide. Whilst this is promising for the long-term conservation of biodiversity, it is important to recognise that seed longevity still varies amongst species by four orders of magnitude, and prolonged seed longevity should not simply be presumed. Seed dispersal syndrome is an important predictor of longevity in Australia species, and is likely so in other fire-prone environments where serotiny is common. Seeds with large embryos are also long-lived, relative to seeds with small embryos and copious endosperm. Although it seems clear that no single seed, plant, habitat, or environmental trait will explain a significant proportion of the variation in seed longevity between species, this study has identified traits that, taken together, allow for potentially long- and short-lived seeds to be identified for the management of seed banks storing phylogenetically diverse wild species where there is often no prior data on seed longevity.

Appendix

See Table 2.

Table 2 Characteristics of the species used in this study

Species	Order (ales)	Family (-aceae)	p_{50} (days)	Rank	Seed Pre-treatment	Germ. temp. (°C)	Embryo type	Endo-sperm	Seed disp.	Plant Life-form	Climatic zone
Abutilon otocarpum	Malv-	Malv-	30.9	129	Scarification	10/22	Folded	E	G	Forb	Desert
Acacia alata	Fab-	Fab-	126.3	48	HW, 2 min	15	Investing	N	G	Shrub	Temperate
Acacia ancistrocarpa	Fab-	Fab-	244.0*	23	HW, 2 min	25	Investing	N	G	Shrub/tree	Desert
Acacia blakei subsp. diphylla	Fab-	Fab-	121.4	50	Scarification	20	Investing	N	G	Tree	Temperate
Acacia colei	Fab-	Fab-	588.6*	1	HW, 2 min	25	Investing	N	G	Shrub/tree	Desert
Acacia cowleana	Fab-	Fab-	295.0*	13	HW, 2 min	20	Investing	N	G	Shrub/tree	Subtropical
Acacia decurrens	Fab-	Fab-	94.4	66	Scarification	20	Investing	N	G	tree	Temperate
Acacia extensa	Fab-	Fab-	122.9	49	HW, 2 min	15	Investing	N	G	Shrub	Temperate
Acacia falcata	Fab-	Fab-	94.0	67	Scarification	20	Investing	N	G	Shrub	Temperate
Acacia filicifolia	Fab-	Fab-	73.8	77	Scarification	20	Investing	N	G	Tree	Temperate
Acacia holosericea	Fab-	Fab-	481.1*	3	HW, 2 min	20	Investing	N	G	Shrub/tree	Tropical
Acacia lasiocarpa	Fab-	Fab-	116.9	51	HW, 2 min	15	Investing	N	G	Shrub	Temperate
Acacia macradenia	Fab-	Fab-	273.8*	19	HW, 2 min	20	Investing	N	G	Shrub/tree	Subtropical
Acacia maitlandii	Fab-	Fab-	148.0*	38	HW, 2 min	25	Investing	N	G	Shrub	Desert
Acacia neriifolia[a]	Fab-	Fab-	221.4*	25	HW, 2 min	20	Investing	N	G	Shrub/tree	Subtropical
Acacia pendula	Fab-	Fab-	70.0	82	Scarification	20	Investing	N	G	Tree	Grassland
Acacia pycnantha	Fab-	Fab-	85.1	71	Scarification	15	Investing	N	G	Shrub/tree	Temperate
Acacia retinodes	Fab-	Fab-	49.5	98	Scarification	10/22	Investing	N	G	Shrub/tree	Temperate
Acacia rostellifera	Fab-	Fab-	105.0	60	HW, 2 min	15	Investing	N	G	Shrub/tree	Temperate
Acacia saligna	Fab-	Fab-	137.4	43	HW, 2 min	15	Investing	N	G	Shrub/tree	Temperate
Acacia shirleyi	Fab-	Fab-	365.2*	6	HW, 2 min	20	Investing	N	G	Shrub/tree	Subtropical
Acacia urophylla	Fab-	Fab-	71.3	79	HW, 2 min	15	Investing	N	G	Shrub	Temperate
Achnophora tatei[a]	Aster-	Aster-	12.8	156	Nil	5/15	Spatulate	N	G	Herb	Temperate
Actinotus helianthi	Api-	Api-	21.8	141	Nil	15	Rudimentary	E	G	Herb	Temperate

Table 2 continued

Species	Order (ales)	Family (-aceae)	p_{50} (days)	Rank	Seed Pre-treatment	Germ. temp. (°C)	Embryo type	Endo-sperm	Seed disp.	Plant Life-form	Climatic zone
Allocasuarina distyla	Fag-	Casuarin-	295.3	12	Nil	20	Spatulate	N	S	Tree	Temperate
Allocasuarina fraseriana	Fag-	Casuarin-	110.4	56	Nil	15	Investing	N	S	Tree	Temperate
Allocasuarina paludosa[a]	Fag-	Casuarin-	351.2*	8	Nil	20	Spatulate	N	S	Shrub	Temperate
Alphitonia excelsa	Ros-	Rhamn-	44.5*	102	HW, 2 min	20	Investing	N	G	Shrub/tree	Subtropical
Ammobium alatum	Aster-	Aster-	43.8	104	Nil	20	Spatulate	N	G	Forb	Temperate
Andersonia latiflora	Eric-	Eric-	72.2	78	GA$_3$ 1.44 mM	15	Dwarf	E	G	Shrub	Temperate
Angophora euryphylla	Myrt-	Myrt-	289.7*	14	Nil	20	Investing	N	G	Tree	Temperate
Angophora floribunda	Myrt-	Myrt-	64.2	88	Nil	20	Investing	N	G	Tree	Temperate
Anigozanthos manglesii	Commelin-	Haemodor-	68.5	86	100 °C, 3 h	15	Dwarf	E	G	Herb	Grassland
Anthocercis littorea	Solan-	Solan-	49.3	99	GA$_3$ 0.29 mM	15	Linear	E	G	Shrub	Temperate
Aphanes australiana[a]	Ros-	Ros-	38.0	117	Nil	5/15	Investing	N	G	Herb	Temperate
Aphanopetalum clematideum	Saxifrag-	Aphanopetal-	70.7	81	Nil	15	Linear	E	G	Herb/shrub	Grassland
Arthropodium fimbriatum	Lili-	Lili-	23.4	139	4 wks at 40 °C	10/22	Linear	E	G	Herb	Temperate
Atriplex amnicola	Po-	Chenopodi-	116.1	53	GA$_3$ 1.44 mM	15	Linear	E	G	Shrub	Temperate
Atriplex eichleri[a]	Po-	Chenopodi-	28.9	133	Nil	10/22	Peripheral	E	G	Forb	Grassland
Atriplex nummularia	Po-	Chenopodi-	60.5	90	GA$_3$ 1.44 mM	15	Linear	E	G	Shrub	Temperate
Austrostipa elegantissima	Po-	Po-	15.2	152	100 °C, 3 h	15	Lateral	E	G	Herb	Temperate
Banksia attenuata	Prote-	Prote-	210.7*	26	Nil	15	Investing	N	S	Shrub/tree	Temperate
Banksia ericifolia	Prote-	Prote-	250.4	22	Nil	20	Investing	N	S	Shrub	Temperate
Banksia menziesii	Prote-	Prote-	183.6*	33	Nil	15	Investing	N	S	Shrub/tree	Temperate
Banksia prionotes	Prote-	Prote-	243.6*	24	Nil	15	Investing	N	S	Shrub/tree	Temperate
Blandfordia grandiflora	Asparag-	Blandfordi-	12.5	158	Nil	20	Linear	E	G	Forb	Temperate

Table 2 continued

Species	Order (ales)	Family (-aceae)	p_{50} (days)	Rank	Seed Pre-treatment	Germ. temp. (°C)	Embryo type	Endo-sperm	Seed disp.	Plant Life-form	Climatic zone
Borya sphaerocephala	Asparag-	Bory-	24.9	135	GA₃ 0.29 mM	15	Dwarf	E	G	Herb	Temperate
Brachyscome graminea	Aster-	Aster-	32.6	125	Nil	5/15	Spatulate	N	G	Herb	Temperate
Brachyscome rigidula	Aster-	Aster-	18.7	148	Nil	20	Dwarf	N	G	Herb	Temperate
Brachyscome tatei	Aster-	Aster-	30.3	130	Nil	5/15	Spatulate	N	G	Herb	Desert
Brachyscome tenuiscapa var. *pubescens*	Aster-	Aster-	30.9	128	Nil	20	Spatulate	N	G	Forb	Temperate
Burchardia congesta	Lili-	Colchic-	6.9	167	Nil	15	Rudimentary	E	G	Herb	Temperate
Callitris arenaria	Pin-	Cupress-	38.6	116	KAR$_1$ 0.67 µM	15	Spatulate	E	S	Shrub/tree	Temperate
Callitris oblonga subsp. *corangensis*	Pin-	Cupress-	79.0	75	Nil	20	Spatulate	E	S	Tree/shrub	Temperate
Callitris pyramidalis	Pin-	Cupress-	3.0	172	KAR$_1$ 0.67 µM	15	Spatulate	E	S	Shrub	Temperate
Cardamine papillata[a]	Brassic-	Brassic-	10.1	162	Nil	10/22	Bent	N	G	Herb	Temperate
Carex fascicularis	Po-	Cyper-	41.4	110	Nil	27/12	Capitate	E	G	Sedge	Temperate
Cassinia leptocephala	Aster-	Aster-	69.1	85	Nil	20	Dwarf	N	G	Shrub	Temperate
Casuarina glauca	Fag-	Casuarin-	135.8	45	Nil	25	Spatulate	N	S	Tree	Subtropical
Centrolepis aristata	Po-	Centrolepid-	40.6	111	GA₃ 1.44 mM	15	Rudimentary	E	G	Herb	Temperate
Chloris truncata	Po-	Po-	42.2	107	Nil	20	Lateral	E	G	Grass	Temperate
Chloris ventricosa	Po-	Po-	43.9	103	Nil	20	Lateral	E	G	Grass	Temperate
Chrysocephalum apiculatum	Aster-	Aster-	32.9	124	Nil	20	Dwarf	N	G	Herb	Temperate
Clematis linearifolia	Ranuncul-	Ranuncul-	8.0	166	Nil	15	Rudimentary	E	G	Herb/shrub	Temperate
Corymbia calophylla	Myrt-	Myrt-	272.7*	20	Nil	15	Folded	N	S	Tree	Temperate

Table 2 continued

Species	Order (ales)	Family (-aceae)	p_{50} (days)	Rank	Seed Pre-treatment	Germ. temp. (°C)	Embryo type	Endo-sperm	Seed disp.	Plant Life-form	Climatic zone
Corymbia clarksoniana	Myrt-	Myrt-	58.1*	92	Nil	20	Folded	N	S	Tree	Subtropical
Corymbia eximia	Myrt-	Myrt-	287.1*	15	Nil	20	Investing	N	S	Tree	Temperate
Corymbia hamersleyana	Myrt-	Myrt-	394.9*	5	Nil	25	Folded	N	S	Tree	Desert
Corymbia opaca	Myrt-	Myrt-	276.1*	18	Nil	25	Folded	N	S	Tree	Desert
Corymbia terminalis	Myrt-	Myrt-	58.2*	91	Nil	20	Folded	N	S	Tree	Tropical
Cryptandra arbutiflora	Ros-	Rhamn-	193.4	30	HW, 2 min	15	Spatulate	E	G	Shrub	Temperate
Cyperus ixiocarpus[a]	Po-	Cyper-	183.1	34	Nil	35	Capitate	E	G	Herb	Desert
Daviesia cordata	Fab-	Fab-	39.0	115	HW, 1 min	15	Folded	N	G	Shrub	Temperate
Dichopogon capillipes	Asparag-	Asparag-	19.1	147	Nil	15	Peripheral	E	G	Herb	Temperate
Dillwynia sieberi	Fab-	Fab-	146.4	39	Scarification	20	Bent	N	G	Shrub	Temperate
Dioscorea hastifolia	Dioscore-	Dioscore-	24.9	136	Nil	15	Capitate	E	G	Herb/shrub	Temperate
Diplopeltis huegelii	Sapind-	Sapind-	114.1*	54	HW, 1 min	15	Peripheral	N	G	Shrub	Temperate
Dodonaea falcata	Sapind-	Sapind-	316.6*	10	Scarification	20	Folded	N	G	Tree	Grassland
Dodonaea hackettiana	Sapind-	Sapind-	111.6	55	HW, 1 min	15	Bent	N	G	Shrub/tree	Temperate
Dodonaea heteromorpha	Sapind-	Sapind-	365.0	7	Scarification	20	Folded	N	G	Shrub	Grassland
Dodonaea procumbens	Sapind-	Sapind-	106.1	59	Scarification	10/22	Bent	E	G	Shrub	Grassland
Dodonaea stenozyga[a]	Sapind-	Sapind-	56.4	94	HW, 1 min	15	Bent	N	G	Shrub	Temperate
Dodonaea viscosa subsp. *spatulata*	Sapind-	Sapind-	280.0	17	Scarification	20	Folded	N	G	Shrub	Temperate
Doryanthes excelsa	Asparag-	Doryanth-	24.1	137	Nil	15	Capitate	E	G	Shrub	Temperate
Dracophyllum secundum	Eric-	Eric-	47.7	100	Nil	20/5	Dwarf	E	G	Shrub	Temperate
Epilobium gunnianum	Myrt-	Onagr-	42.5	106	Nil	15	Investing	N	G	Herb	Temperate
Eucalyptus crebra	Myrt-	Myrt-	108.6*	58	Nil	20	Folded	N	S	Tree	Subtropical
Eucalyptus gomphocephala	Myrt-	Myrt-	190.2*	32	Nil	15	Folded	N	S	Tree	Temperate

Table 2 continued

Species	Order (ales)	Family (-aceae)	p_{50} (days)	Rank	Seed Pre-treatment	Germ. temp. (°C)	Embryo type	Endo-sperm	Seed disp.	Plant Life-form	Climatic zone
Eucalyptus leucophloia	Myrt-	Myrt-	88.5*	70	Nil	25	Folded	N	S	Tree	Tropical
Eucalyptus leucophylla	Myrt-	Myrt-	193.2*	31	Nil	20	Folded	N	S	Tree	Tropical
Eucalyptus loxophleba	Myrt-	Myrt-	270.7*	21	Nil	15	Folded	N	S	Tree	Temperate
Eucalyptus populnea	Myrt-	Myrt-	41.4*	109	Nil	15	Folded	N	S	Tree	Subtropical
Eucalyptus pruinosa	Myrt-	Myrt-	136.7*	44	Nil	20	Folded	N	S	Tree	Tropical
Eucalyptus tereticornis	Myrt-	Myrt-	98.8*	64	Nil	15	Folded	N	S	Tree	Subtropical
Eucalyptus wandoo	Myrt-	Myrt-	336.1*	9	Nil	15	Folded	N	S	Tree	Temperate
Ficinia nodosa	Po-	Cyper-	36.6	118	Nil	20	Dwarf	E	G	Rush	Temperate
Gastrolobium capitatum	Fab-	Fab-	104.2*	61	HW, 1 min	15	Bent	N	G	Shrub	Temperate
Geum urbanum	Ros-	Ros-	53.3	97	Nil	20	Spatulate	N	G	Herb	Temperate
Gnephosis eriocarpa[a]	Aster-	Aster-	16.1	150	Nil	5/15	Spatulate	N	G	Forb	Desert
Gompholobium polyzygum	Fab-	Fab-	194.5*	29	HW, 1 min	25	Bent	N	G	Shrub	Desert
Gossypium robinsonii	Malv-	Malv-	200.9*	27	HW, 1 min	25	Folded	E	G	Shrub	Desert
Grevillea robusta	Prote-	Prote-	14.7	153	Nil	20	Investing	N	G	Tree	Subtropical
Grevillea wickhamii	Prote-	Prote-	93.2*	68	KAR$_1$ 0.67 µM	25	Investing	N	G	Shrub/tree	Desert
Haemodorum spicatum	Commelin-	Haemodor-	45.9	101	Nil	15	Rudimentary	E	G	Herb	Temperate
Hakea amplexicaulis	Prote-	Prote-	69.2	84	Nil	15	Investing	N	S	Shrub	Temperate
Hakea francisiana	Prote-	Prote-	116.3	52	Nil	10/22	Investing	N	S	Shrub/tree	Grassland
Hakea petiolaris[a]	Prote-	Prote-	181.8	35	Nil	15	Investing	N	S	Shrub/tree	Temperate
Hakea prostrata	Prote-	Prote-	109.2	57	Nil	15	Investing	N	S	Shrub	Temperate
Hardenbergia comptoniana	Fab-	Fab-	142.5*	42	HW, 1 min	15	Bent	N	G	Shrub	Temperate
Hibiscus splendens	Malv-	Malv-	84.9	72	Scarification	20	Folded	E	G	Tree/shrub	Temperate
Hydrocotyle callicarpa	Api-	Aral-	12.7	157	GA$_3$ 0.29 mM	15	Linear	E	G	Herb	Temperate

Table 2 continued

Species	Order (ales)	Family (-aceae)	p_{50} (days)	Rank	Seed Pre-treatment	Germ. temp. (°C)	Embryo type	Endo-sperm	Seed disp.	Plant Life-form	Climatic zone
Indigofera monophylla	Fab-	Fab-	100.6*	63	HW, 1 min	25	Bent	E	G	Shrub	Desert
Isotoma hypocrateriformis	Aster-	Campanul-	26.9	134	GA₃ 0.29 mM	15	Micro	E	G	Herb	Temperate
Jacksonia aculeata	Fab-	Fab-	101.5*	62	HW, 1 min	25	Bent	E	G	Shrub	Desert
Jacksonia chappilliae[a]	Fab-	Fab-	134.7*	46	Scarification	20	Bent	N	G	Shrub	Temperate
Juncus amabilis	Po-	Junc-	35.0	121	Nil	10/22	Broad	E	G	Sedge	Temperate
Juncus australis	Po-	Junc-	41.6	108	Nil	5/15	Broad	E	G	Sedge	Temperate
Juncus holoschoenus	Po-	Junc-	21.7	142	Nil	15	Broad	E	G	Herb	Temperate
Juncus planifolius	Po-	Junc-	35.9	120	Nil	15	Broad	E	G	Rush	Temperate
Juncus usitatus	Po-	Junc-	79.0	76	Nil	27/12	Broad	E	G	Rush	Temperate
Kennedia prostrata	Fab-	Fab-	144.4	40	HW, 1 min	15	Bent	E	G	Shrub	Temperate
Levenhookia pusilla	Aster-	Stylidi-	29.6	132	GA₃ 0.29 mM	15	Micro	E	G	Herb	Temperate
Lomatia myricoides	Prote-	Prote-	8.5	165	Nil	20	Investing	N	G	Tree/shrub	Temperate
Lycopus australis	Lami-	Lami-	31.8	127	Nil	20/5	Spatulate	N	G	Herb	Temperate
Microlaena stipoides	Po-	Po-	40.0	112	GA₃ 0.29 mM	15	Lateral	E	G	Herb	Temperate
Mirbelia dilatata	Fab-	Fab-	486.0*	2	HW, 30 s	15	Folded	N	G	Shrub	Temperate
Mirbelia rubiifolia	Fab-	Fab-	143.5	41	Scarification	20	Bent	N	G	Shrub	Temperate
Montia fontana[a]	Caryophyll-	Portulac-	11.8	159	Leaching	5/15	Peripheral	E	G	Herb	Temperate
Ornduffia albiflora	Aster-	Menyanth-	3.7	171	GA₃ 2.89 mM	15	Linear	E	G	Herb	Temperate
Ozothamnus diosmifolius	Aster-	Aster-	14.5	154	Nil	20	Dwarf	N	G	Shrub	Temperate
Pandorea pandorana	Lami-	Bignoni-	4.4	170	Nil	20	Investing	N	G	Climber	Temperate
Patersonia sericea	Asparag-	Irid-	39.8	114	Nil	20	Linear	E	G	Herb	Temperate
Phyllangium distylis[a]	Gentian-	Logani-	16.7	149	Nil	10/22	Spatulate	E	G	Herb	Grassland
Phyllanthus calycinus	Malpighi-	Phyllanth-	23.6	138	GA₃ 0.29 mM	15	Linear	E	G	Shrub	Temperate

Table 2 continued

Species	Order (ales)	Family (-aceae)	p_{50} (days)	Rank	Seed Pre-treatment	Germ. temp. (°C)	Embryo type	Endo-sperm	Seed disp.	Plant Life-form	Climatic zone
Plantago drummondii	Lami-	Plantagin-	6.8	168	Nil	10/22	Spatulate	E	G	Herb	Desert
Plectranthus parviflorus	Lami-	Lami-	34.5	122	Nil	25	Spatulate	N	G	Shrub	Temperate
Poa hiemata	Po-	Po-	11.8	160	Nil	20	Lateral	E	G	Grass	Temperate
Podolepis neglecta	Aster-	Aster-	15.6	151	Nil	20	Dwarf	N	G	Herb	Temperate
Podolepis robusta	Aster-	Aster-	8.9	163	Nil	20	Spatulate	N	G	Herb	Temperate
Ptilotus latifolius	Caryophyll-	Amaranth-	30.0	131	Nil	10/22	Peripheral	E	G	Forb	Desert
Pultenaea villosa	Fab-	Fab-	131.4	47	Scarification	20	Bent	N	G	Shrub	Temperate
Pycnosorus globosus	Aster-	Aster-	36.1	119	Nil	20	Dwarf	N	G	Herb	Subtropical
Regelia ciliata	Myrt-	Myrt-	157.4*	37	Nil	15	Spatulate	N	G	Shrub	Temperate
Rhagodia preissii	Po-	Chenopodi-	54.6	95	Nil	10/22	Peripheral	E	G	Shrub	Desert
Rhodanthe chlorocephala subsp. rosea[a]	Aster-	Aster-	69.3	83	Nil	15	Investing	N	G	Herb	Subtropical
Rorippa eustylis[a]	Brassic-	Brassic-	11.7	161	Nil	10/22	Bent	N	G	Herb	Desert
Senecio linearifolius[a]	Aster-	Aster-	8.8	164	Nil	5/15	Spatulate	N	G	Shrub	Temperate
Senna artemisioides subsp. oligophylla	Fab-	Fab-	309.0*	11	HW, 2 min	25	Investing	E	G	Shrub	Desert
Senna glutinosa subsp. glutinosa	Fab-	Fab-	284.9*	16	HW, 2 min	25	Investing	E	G	Shrub	Desert
Siegfriedia darwinioides	Rhamn-	Rhamn-	200.0	28	HW, 2 min	15	Spatulate	E	G	Shrub	Temperate
Solanum cinereum	Solon-	Solan-	84.9	72	SW	25	Linear	E	G	Shrub	Subtropical
Solanum orbiculatum	Solon-	Solan-	97.4	65	KAR_1, 0.67 μM	13/26	Linear	E	G	Shrub	Grassland
Solenogyne bellioides	Aster-	Aster-	56.7	93	Nil	20	Spatulate	N	G	Herb	Temperate
Spergularia brevifolia	Caryophyll-	Caryophyll-	43.7	105	Nil	5/15	Peripheral	E	G	Herb	Grassland
Spyridium globulosum	Ros-	Rhamn-	158.1	36	HW, 2 min	15	Spatulate	E	G	Shrub	Temperate

Table 2 continued

Species	Order (ales)	Family (-aceae)	p_{50} (days)	Rank	Seed Pre-treatment	Germ. temp. (°C)	Embryo type	Endo-sperm	Seed disp.	Plant Life-form	Climatic zone
Streptoglossa macrocephala	Aster-	Aster-	89.2	69	Nil	25	Investing	N	G	Herb/shrub	Desert
Stylidium affine	Aster-	Stylidi-	32.9	123	GA$_3$ 2.89 mM	15	Dwarf	E	G	Herb	Temperate
Stylidium graminifolium[a]	Aster-	Stylidi-	22.8	140	Nil	20	Dwarf	E	G	Herb	Temperate
Swainsona extrajacens	Fab-	Fab-	81.2	74	Scarification	10/22	Bent	E	G	Forb	Desert
Telopea speciosissima	Prote-	Prote-	5.8	169	Nil	25	Investing	N	G	Shrub	Temperate
Thysanotus patersonii	Asparag-	Asparag-	13.2	155	4 wks at 40 °C	5/15	Linear	E	G	Herb	Temperate
Trachymene incisa	Api-	Arali-	39.8	113	Nil	25	Rudimentary	E	G	Herb	Temperate
Tristania neriifolia	Myrt-	Myrt-	21.4	143	Nil	20	Spatulate	N	G	Tree/shrub	Temperate
Trymalium ledifolium	Ros-	Rhamn-	448.5*	4	HW, 2 min	15	Spatulate	N	G	Shrub	Temperate
Velleia trinervis	Aster-	Goodeni-	70.9	80	Nil	15	Spatulate	E	G	Herb	Temperate
Vittadinia cuneata	Aster-	Aster-	20.7	145	Nil	20	Spatulate	N	G	Herb	Temperate
Wurmbea biglandulosa	Lili-	Colchic-	53.5	96	Nil	15	Dwarf	E	G	Herb	Temperate
Wurmbea dioica[a]	Lili-	Colchic-	32.3	126	Nil	15	Dwarf	E	G	Herb	Temperate
Xanthorrhoea gracilis	Asparag-	Xanthorrhoe-	65.7	87	Nil	15	Linear	E	G	Shrub	Temperate
Xanthorrhoea preissii[a]	Asparag-	Xanthorrhoe-	61.1	89	Nil	15	Linear	E	G	Shrub/tree	Temperate
Xerochrysum bracteatum[a]	Aster-	Aster-	21.2	144	Nil	20	Spatulate	N	G	Herb	Temperate
Xerochrysum subundulatum	Aster-	Aster-	20.6	146	Nil	20	Spatulate	N	G	Herb	Temperate

Seed longevity is expressed as p_{50} (the time to 50 % viability loss) for seeds aged at 45 °C and 60 % RH, or 60 °C and 60 % RH. For comparative purposes the p_{50} values presented for species aged at 60 °C and 60 % RH (indicated by an asterisk) are an estimate of the p_{50} at 45 °C, based on the measured p_{50}. Seed longevity for each species is ranked as 1–172, with 1 being the longest-lived species. Seed pre-treatment refers to that treatment required to break dormancy. Germination temperature (Germ. Temp.) refers to the constant or daily alternating (12/12 h) temperature regime used for germination testing. Seed embryo type was classified according to Martin (1946). Seeds were classified as *E* endospermic, *N* non-endospermic, *Seed Disp.* seed dispersal syndrome *G* as geosporous (release of mature seeds into the soil seed bank) or *S* serotinous (retention of mature seeds in the plant canopy).

[a] Species for which seeds were aged at both 45 and 60 °C

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