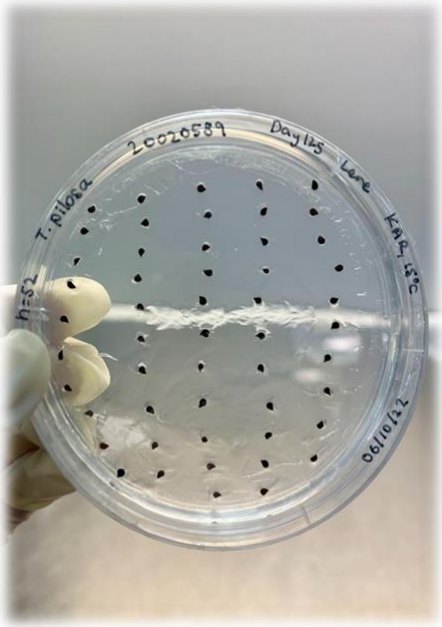
Comparative seed longevity testing on native Western Australian species in ex situ storage conditions



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Declaration

I declare this thesis is my own account of my research and contains as its main content, work which has not been previously submitted for a degree at any tertiary education institution.

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Abstract

Seed banking allows for the maintenance of genetic resources over long periods of time. However, seeds lose their viability over time in storage, and longevity varies among species. A germination trial was conducted on ten species (total of 44 accessions) of understorey herbs/shrubs to determine the viabilities of seed accessions of wild Western Australian species stored in a conservation seed bank (-18˚C and 20% RH). A rapid ageing experiment was conducted in a controlled setting at 45˚C and 60% relative humidity (RH) on selected accessions (total of 24) with similar viabilities within species, to then determine if they were short-lived. Among the six native species that were aged, three species of Asteraceae – *Brachyscome iberidifolia*, *Olearia axillaris* and *Panaetia lessonii* – were found to have accessions with p_{50} values in the very short and short-lived ranges. Longevity varied from very short-lived to medium-lived among all aged seed accessions (0.56 ± 0.45 days to 57.20 ± 1.66 days). Initial seed quality (*K*i) and relative humidity were identified as factors that contributed to differences in seed longevity. Trends within taxonomic groups were identified; species within a genus (*Podotheca*) share similar ranges for longevity, while longevity within families (Asteraceae) tend to be more varied. Seeds of species that are shorter-lived should be monitored and tested regularly – every 5 years – to inform management decisions. Seed collections should be tested for initial viability at the point of banking to ensure that estimates of longevity from future comparative longevity experiments are accurate.

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Introduction

1.1 Background: Biodiversity Conservation

The rate of biodiversity loss has increased on a global and local scale, with more species facing extinction than previously noted (Martyn Yenson et al., 2021). Direct drivers of biodiversity loss include natural disasters such as earthquakes, volcanic eruptions, and weather events, as well as anthropogenic drivers like pollution, deforestation and human-accelerated climate change (Díaz and Malhi, 2022). Land- and sea-use change has been identified as the greatest threat to declines in nature globally, with climate change and direct exploitation also being prominent drivers of decline (Díaz and Malhi, 2022; WWF, 2022).

It is estimated that 2 in 5 of the known plant species worldwide are threatened with extinction in the wild (Royal Botanic Gardens Kew, 2020). Recent assessments show that the current methods of conserving plant diversity have not been sufficient to prevent the sustained decline in biodiversity (Heywood, 2017). Heywood (2017) notes that one of the key issues faced in plant conservation is the lack of baseline data, as our understanding of plant identity, distribution, ecology, demography, dynamics, function and social/economic value is insubstantial.

Australian flora displays a significant level of adaptation, specialisation and endemism, as 84% of vascular plant species do not occur elsewhere (Martyn Yenson et al., 2021). Biodiversity hotspots in particular are noted for their high endemism (Martyn Yenson et al., 2021). To qualify as a biodiversity hotspot, a region must meet the following criteria: it must contain at least 1500 species of endemic vascular plants and have lost at least 70% of its primary native vegetation (CEPF, 2022).

Mediterranean-type climate regions, such as the Southwest Australian Floristic Region (SWAFR), are subject to a unique set of conservation challenges, due to their high plant diversity and endemism (Monks et al., 2018). Of the 8379 described taxa in the SWAFR, 399 are listed as “Threatened” and 154 are “Critically Endangered” (Monks et al., 2018). The IUCN assesses species based on the following factors: geographic range, population size, population declines,

and extinction probability, where a species is placed in a threatened category when a specific threshold is reached (IUCN, 2022). Land-use change and targeted exploitation of species are highlighted as the main threats that increase the extinction risk for plants, as these activities result in smaller population sizes and population declines, as well as smaller geographic ranges (Nic Lughadha et al., 2020). As such, conservation strategies that target priority taxa have been set in place by various governing bodies and authorities across the globe.

Genetic diversity is important to conserving biodiversity, as it influences aspects of survival such as recovery following a disturbance, variation in fitness, species interactions, disease resistance and community structure (van der Merwe et al., 2021). Populations need to be able to evolve and adapt to change to prevent local extinctions from occurring (van der Merwe et al., 2021).

In response to the ongoing decline in biodiversity, ex situ conservation has been recognised as an important aspect of plant conservation strategies, such as species recovery, reintroduction and ecological restoration (Heywood 2017). Ex situ plant conservation strategies are those which involve conserving species away from the site of natural occurrence (Martyn Yenson et al., 2021). This may be done via the collection and storage of whole plants, or germplasm – living plant tissue such as seeds, pollen or plant cells (Martyn Yenson et al., 2021). The most common ex situ conservation options are seed banking, tissue culture, cryopreservation and the use of living plant collections, with seed banks playing a major role in ex situ conservation (Martyn Yenson et al., 2021).

1.2 What is seed banking?

Ex-situ seed banks form a significant component of ex situ plant conservation programs, as collecting and storing seeds at low relative humidity (RH) and temperature provides for the efficient and long-term maintenance of genetic resources for long periods of time, decades or potentially centuries (Walters et al., 2005; Chau et al., 2019). The collection and preservation of viable propagules – in conjunction with in situ conservation – is pivotal to preventing the extinction of plant species, as this provides a backup of genetic diversity for managed habitats (Chau et al., 2019). Seed banking for ex situ conservation is seen as the most appropriate action for most species, as seeds store genetic material and are structured to facilitate survival and longevity in the dry state (Offord et al., 2021). Moreover, seeds are relatively easy to collect, and collections may represent a range of genetic diversity in a species when there is proper sampling of a population for harvest (Li and Pritchard, 2009).

According to Offord et al. (2021), the advantages of seed banking include the ability to store seeds of many species for long periods, as compared to short-term storage in tissue culture. Other advantages of seed banking include low cost, space efficiency, and less resource intensity when compared to other ex situ options such as cryopreservation and tissue culture (Offord et al., 2021). The disadvantages are that seeds of some species are sensitive to desiccation and not suitable for conventional seed banking, and protocols for storing and germinating many Australian species are still being developed, leading to possible loss of genotypes as seed collections in the seed bank begin to age and lose viability (Offord et al., 2021).

Seeds will lose their viability over time in storage, and seed longevity varies among species (Walters et al., 2005). However, it is difficult to determine when seed viability starts to decline in a conservation storage, as this may take a long period of time, i.e., decades or centuries (Merritt et al., 2014). There is recent evidence suggesting that seeds of some taxa may not be storing as well as expected in seed banks (Walters and Pence, 2020). The reduced survival of seeds in the seed bank could have serious implications for plant conservation, restoration, and

agriculture, as seeds are stored with the purpose of preserving genetic diversity of natural populations.

1.3 Factors affecting seed longevity and storage behaviour

Seed storage behaviour is influenced by temperature and seed moisture content, as well as characteristics specific to species (Walters, 2015; Walters et al., 2005). Moisture content refers to the amount of water contained in a seed, and is influenced by seed maturity during collection, post-harvest environmental conditions, as well as seed size and composition (Merritt et al., 2021). The moisture content of seeds and their interactions with other components within the seed may affect their ability to be stored at low temperatures, and high seed moisture content may increase the ageing rate and result in lethal ice formation when seeds are stored at sub-zero temperatures (Tuckett et al., 2010). Seeds are able to gain or lose moisture in relation to temperature and relative humidity in the surrounding air, and eventually reach equilibrium, where the RH of the air at a given constant temperature at the equilibrium point is referred to as the equilibrium relative humidity (eRH) (Merritt et al., 2021).

Sorption isotherms can be used to represent the relationships between moisture content in seeds and relative humidity at constant temperatures, to aid in the understanding of the water potential of seeds (Tuckett et al., 2010). Isotherms tend to follow a sigmoidal shape, with inflection points at roughly 15-20% and 85% equilibrium relative humidity (eRH), which reflects water binding properties at different seed hydration levels (Tuckett et al., 2010).

Early studies on the storage behaviour of agricultural seeds led to the conception of two categories – “orthodox” seeds and “recalcitrant” seeds – with respect to the way seeds react to desiccation and low storage temperatures (Chau et al., 2019). Orthodox seeds are those which not only tolerate desiccation to low moisture contents, but also see a predictable increase in longevity when there is a decrease in moisture content and temperature (Merritt et al., 2021).

They can therefore be stored by conventional seed banking conditions (Merritt et al., 2021; Chau et al., 2019).

Recalcitrant seeds are those which are sensitive to desiccation and do not survive sub-zero temperatures, making them candidates for alternative methods of conservation, such as cryopreservation (Merritt et al., 2021; Chau et al., 2019; Walters et al., 2013). Further research led to a third category, "intermediate," being established, as there is great variation in seed storage behaviours due to the species-specific characteristics (Merritt et al., 2021). Intermediate seeds are generally desiccation tolerant, but not to the extent of orthodox seeds (Merritt et al., 2021). There is now a general consensus that seed storage behaviour is better expressed as a continuum rather than in discrete categories (Merritt et al., 2021; Chau et al., 2019; Walters, 2015).

It is important to note the storage histories of seed collections, as this has an effect longevity. When comparing between long-term storage conditions, seeds dried to equilibrium and then stored under optimal gene banking conditions (low RH and temperature) tend to have greater longevity than seeds that are stored under ambient room conditions (Solberg et al., 2020).

The conventional storage conditions to maintain the viability of seeds are at 20% relative humidity (RH) and -20˚C (FAO, 2014; Walters, 2015). Seed longevity may vary from 50 to 400 years for different species stored under genebank conditions, assuming that their initial storage quality was high (Walters, 2015). Likewise, Chau and colleagues (2019) corroborate this and place the standard of desiccation to 15 ± 3% RH and temperature at -18 ± 3°C.

Although these standards are internationally recognised, most of the theory has been derived from studies on agricultural species, rather than wild species (Hay and Probert, 2013). Despite these protocols being generally appropriate for both wild and crop species, the gaps in knowledge for some wild species may affect management decisions in relation to collection and storage (Hay and Probert, 2013). For example, while studies on seed development in crop species have already established optimal collection periods, the window of opportunity for seed

collection of wild species may be narrow, and indicators of maturity may not be obvious, resulting in varying degrees of seed maturity within an accession (Hay and Probert, 2013). The longevity of seeds of wild species may also vary within a species as a result of population differences or environmental effects (Hay and Probert, 2013). As some seeds of wild species are found to be short-lived in storage, comparative longevity studies are necessary to ensure a greater understanding of seed longevity among different species and seed lots within a species (Hay and Probert, 2013).

1.4 Measuring seed longevity through rapid ageing experiments

Leprince and colleagues (2016) describe the concept of "seed vigour" as an indicator of seed performance in the field. Seed vigour factors in the rate and uniformity of germination and seedling growth, the ability of seedlings to emerge under unfavourable conditions, as well as the retention of these traits after storage (Leprince et al., 2016). In relation to this is seed longevity, defined as the ability of seeds in storage to remain viable for long periods of time in a dry and inactive state (Leprince et al., 2016).

Seeds do not exhibit signs of ageing until a specific threshold of time is reached, after which their viability declines (Walters, 2015). Walters (2015) defines seed longevity as the period in which seeds remain asymptomatic, and there are no detectable changes in seed viability. There are currently no good tools to detect changes in a population of seeds in storage while they remain in the asymptomatic phase, as viability appears to be maintained up until the threshold (Walters, 2015).

The pattern for seed survivability in ex situ storage is most commonly described as a sigmoidal/s-shaped curve, which is influenced by the normal distribution of viability for a seed lot and the environment seeds are exposed to (Ellis and Roberts, 1980; Chau et al., 2019). The survival curve may also include a period of stability where there is little loss in viability before the sigmoidal

stage, but a steep decline in viability is inevitable nevertheless (Chau et al., 2019). Hence, the role of seed conservationists is to detect when viability drops, so that seeds may be used practically before they reach the end of their longevities in storage (Chau et al., 2019). There is a wide range of longevity for seeds of different species, and also among individual seeds within a seed lot (Lee et al., 2019).

Measuring longevity by directly testing the germination of seeds stored in conservation banking conditions alone may not be efficient, as it may take many years before we begin to see a decline in viability (Walters et al., 2005; Merritt et al., 2014). Studying the comparative longevity of species via rapid ageing experiments in a controlled environment serves as an alternative method of testing for declines in viability (Merritt et al., 2014).

To understand how seeds age in both short-term and long-term storage conditions, seed viability is often modelled by calculating the time taken for viability to decline to 50%, which is known as the p_{50} value, that is used to rank species and compare longevities (Merritt et al., 2021; Newton et al., 2009).

Newton and colleagues (2009) have outlined the protocols used for testing seed viability in rapid ageing experiments, used by the Millennium Seed Bank Partnership (MSBP) to compare longevities of different species stored in the seed bank, where a single seed survival curve is generated to allow for comparison of p_{50} values among species. Seeds selected should be from large collections and have a viability above 85%, with known germination requirements (Newton et al., 2009). Seeds are rehydrated and then aged with the use of solutions of lithium chloride (LiCl) in an enclosed box to achieve the required relative humidity (Newton et al., 2009). At the rehydration stage, consisting of 14 days (subject to seed size), the LiCl solution is used to bring the relative humidity to 47% and the temperature is set to 20°C, while at the ageing stage, RH is 60% and the temperature is set at 45°C (Newton et al., 2009).

During the ageing process, one sample of seeds (~50 seeds per sample) should be removed at set intervals (e.g., 1, 2, 5, 9, 20, 30, 50, 75, 100 and 125 days) and plated akin to a germination

test (Newton et al., 2009). The germination test should run for 42 days until there have been 14 days without any germination, after which a cut test should be performed, so as to exclude the empty seeds (Newton et al., 2009). Seed viability should be plotted with this data against the number of days aged, to create the aforementioned seed survival curve, analysed with a probit analysis to fit the following equation outlined by Ellis and Roberts (1980): $\boldsymbol{v = K_i - p/\sigma}$.

Here, v is the viability in probits after p days of ageing, K_i is the y-intercept and a measure of initial seed quality, and σ is the time taken for viability to fall by 1 probit, and the p_{50} value can be calculated using the following equation $p_{50} = K_i$ x σ (Ellis and Roberts, 1980). Species are ranked by the time taken for viability to decline to 50% (p_{50}) or by the standard deviation of the frequency distribution of seed deaths in time (σ) (Merritt et al., 2014; Newton et al., 2009).

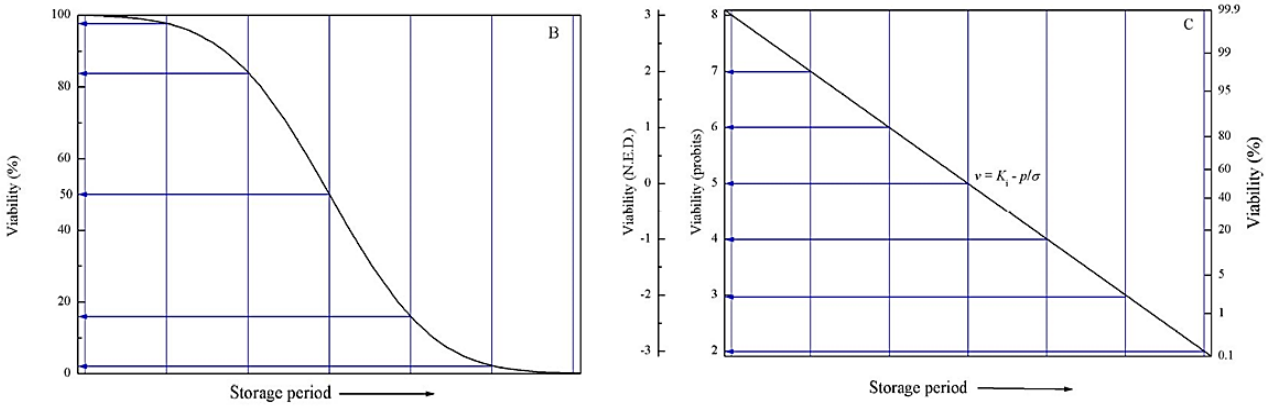

Fig. 1: Normally distributed seed survival curve (**B**) and transformed values for a probit analysis (**C**) (reproduced with permission from Hay, 2004).

In a rapid ageing study done on wild alpine species in Italy, Mondoni and colleagues (2011) have outlined categories of relative longevity, which help to delineate what p_{50} values are associated with short-lived and long-lived species respectively, when aged at 45 °C and 60 % relative humidity. p_{50} values less than or equal to 1 day are considered "very short" in term of longevity, while p_{50} values greater than 1 day but less than or equal to 10 days are considered "short" (Mondoni et al., 2011). p_{50} values greater than 100 days to less than or equal to 1000 days are

considered “long” and values greater than 1000 days are considered “very long” (Mondoni et al., 2011). Values between 11 to 100 denote “medium” longevity (Mondoni et al., 2011).

Data from rapid ageing experiments can be used to study the relationships between seed longevity and seed characteristics such as morphological, physical and physiological traits, as well as relationships between longevity and habitat conditions (Merritt et al., 2014).

1.5 Relationships between seed longevity and seed characteristics, climate and taxonomy

Rapid ageing experiments have identified species within families that have relatively long-lived and short-lived seeds. Seed longevity in storage is generally greater for species from hot, dry regions and shorter for those from cool, dry alpine regions (Chau et al, 2019). Chau and colleagues (2019) mention that there is less data regarding variation of seed longevity in a conservation storage for species from hot, wet regions.

Similarly, Probert and colleagues’ (2009) study also showed that species from cool and wet environments were shorter-lived in storage as compared to their hot and dry counterparts. Probert and colleagues (2009) found that there was no correlation between seed mass and longevity, as well as lipid content and longevity. Likewise, Walters and colleagues’ (2005) study corroborates this, but indicates a slight correlation between lipid content and longevity, although this relationship does not persist when families with a wide range of compositions and longevities, such as Asteraceae and Fabaceae, are viewed individually.

There has been a high incidence of recalcitrant seeds in tropical ecosystems, and freeze sensitivity is thought to be more prevalent in seeds from tropical regions (Chau et al., 2019). There is a prevalence of long-lived seeds in Australian species, in relation to the seeds of species native to other continents and regions around the world (Probert et al., 2009; Merritt et al., 2014).

In Australia, the families Casuarinaceae, Fabaceae, Myrtaceae, Rhamnaceae and Sapindaceae comprise of some species with long-lived seeds, while Asteraceae, Brassicaceae, Poaceae and Orchidaceae comprise of species with short-lived seeds (Merritt et al., 2021). The variation in longevity (p_{50}) is however wide-ranging and species-specific, as these plant families may also comprise of species that are exceptions to this pattern (Merritt et al., 2021).

Probert and colleagues' (2009) study, which tested a broad range of orders and families across continents and climate ranges, backs up the idea that there are certain taxonomic groupings which have characteristic longevities. This is also highlighted in Merritt and colleagues' (2021) findings, that the Myrtaceae and Fabaceae are generally long-lived.

There is also a relationship between seeds with endosperm and shorter lifespans, while non-endospermic seeds tended to have longer lifespans (with p_{50} values of 20.3 days and 65.7 days respectively) (Probert et al., 2009).

Forbis et al. (2002) studied the evolution of embryo size in angiosperms and gymnosperms. They describe the seeds of ancestral angiosperms to be albuminous – filled mostly by the endosperm, with the embryo making only a small part of the seed volume (Forbis et al., 2002). In contrast, the ancestors of more derived angiosperm groups had exalbuminous seeds – dominated mostly by the embryo and lacking endosperm (Forbis et al., 2002). This may help to explain the findings of Probert et al. (2009), which suggest that longevity in seeds and the development of larger embryos may have evolved as a response to a drier climate as compared to the moist forest habitats of early endospermic angiosperms. The evolution of a larger embryo was associated with an increase in germination rate and a decrease in dormancy, which could be an adaptation to specific habitat conditions as well as adult longevity (Vandelook et al., 2012).

Tuckett and colleagues' (2010) study however, found that in species of Hydatellaceae, a family now regarded as basal angiosperms, species were not short-lived, and had p_{50} values in the medium longevity range (24.2 days for *Trithuria austinensis* and 44.3 days for *Trithuria submersa*), higher than three out of four of the other Western Australian vernal pool species

tested. More research on early divergent angiosperms is required to further test if they are short-lived in storage (Tuckett et al., 2010).

The study conducted by Mondoni et al. (2011) deviates from Probert and colleagues' (2009) findings, as it was found that the structure of seeds was not significant in accounting for variation in seed longevity for alpine and lowland species used in the study. The longevity of non-endospermic seeds was overestimated, and the longevity of endospermic seeds was underestimated (Mondoni et al., 2011).

Mondoni and colleagues (2011) also found that the model from Probert et al. (2009) did not explain much of the variation in p_{50} for lowland seeds. However, a similar relationship between p_{50} and mean annual temperature was observed in both studies, with p_{50} accounting for 40.2% of the variance in the study by Mondoni et al. (2011), suggesting that seed longevity increases with higher mean annual temperatures. The relationship between p_{50} values and rainfall was shown to be weak, as p_{50} accounted for less than 10% of the variance for mean total annual rainfall, and greater than 20% of the variance for mean daily rainfall during the reproductive period Mondoni et al. (2011). The study by Merritt et al. (2014) deviates from these findings, as seeds from areas with higher rainfall were seen to be longer-lived. The difference between these two studies may be explained by the use of species primarily from warm temperate, Mediterranean and arid environments in the study by Merritt and colleagues (2014), as compared to the wetter habitats of alpine species in Mondoni and colleagues' (2011) study.

Mondoni et al. (2011) conclude that seeds of alpine species, which grow in cool and wet habitats, may not have experienced selection pressure for resistance to ageing as compared to lowland species that grow in warmer and drier habitats, and are therefore short-lived in storage.

Merritt and colleagues (2014) found that seed dispersal syndrome was an important factor in predicting seed longevity. Seed dispersal was classified as either geosporous; where seeds are annually released into the soil seed bank, or serotinous; where seeds are retained on the parent plant, forming a canopy seed bank (Merritt et al., 2014). Serotinous species were seen to be

longer-lived than geosporous species, although the degree of serotiny among species did not seem to have a significant relationship when compared against seed longevity, as taxa from *Corymbia* and *Eucalyptus* were amongst the longer-lived species in the study but are considered to be weakly serotinous (Merritt et al., 2014). Similarly, in the study on three *Banksia* species by Crawford et al. (2011), time taken for viability to fall by 1 probit was similar among all species, and differences in longevity was attributed to initial seed viability, despite the differences in serotiny for all three species. It is likely that morphological traits which are common among serotinous species may have contributed to their longer lifespans, such as large embryos in relation to seed size and small/lack of endosperm, making serotiny a good indicator for longevity in storage under seed banking conditions (Merritt et al., 2014).

Embryo types were also considered in the study by Merritt et al. (2014), which showed that seeds with an embryo to seed ratio (E:S) of 0.8 to 1.0, also known as folded embryos, were much longer lived than other embryo types. Seeds with large spatulate embryos (E:S of 1.0) and those with investing embryos (E:S of 0.7 to 1.0) were also long-lived, although not to a considerable degree in relation to other embryo types (Merritt et al., 2014). These three embryo types were common in serotinous species and those with physical dormancy (Merritt et al., 2014).

1.6 Aims of project

The matter of seed viability and potential longevity for seeds of wild species stored in conventional conservation storage is still not well understood, with only a few recent studies undertaken on Australian species (Probert et al., 2009; Hay et al., 2010; Tuckett et al., 2010; Crawford et al., 2011; Merritt et al., 2014; Satyanti et al., 2018; Dalziell et al., 2019). To inform the curation of seeds of Western Australian species stored in Kings Park's conservation seed bank, this project aimed to:

1) Identify potentially short-lived species, using a chronosequence approach whereby multiple seed accessions stored for different periods of time were tested for viability.

2) Use rapid ageing as a tool to determine the potential future longevity of seed accessions determined to have similar viability across the chronosequence.

Materials and methods

2.1 Study species

Ten species were chosen for germination testing, based on prior research identifying potentially short-lived seeds, i.e. species from Asteraceae and herbaceous understorey species (Merritt et al., 2014). These were: *Brachyscome iberidifolia*, *Hyalosperma cotula*, *Myriocephalus gueriniae*, *Olearia axillaris*, *Olearia pimeleoides*, *Panaetia lessonii*, *Pithocarpa cordata*, *Podotheca angustifolia*, *Rhodanthe sterilescens* and *Trachymene pilosa*. All species are understorey herbs or shrubs, from the plant family Asteraceae with the exception of *Trachymene pilosa*, from Arialaceae (Barrett and Tay, 2018; Western Australian Herbarium, 2022). A total of 44 accessions were used.

The selected species are native to Western Australia. Six of the study species – *B. iberidifolia*, *H. cotula*, *O. axillaris*, *P. cordata*, *P. angustifolia* and *T. pilosa* – are native to the southwest of Western Australia and sourced from the Kings Park and Bold Park bushland, where their distribution is either widespread or scattered (Barrett and Tay, 2018). The Kings Park and Bold Park bushlands are two of the largest urban remnant bushlands in the Swan Coastal Plain, covering 400 hectares and 442 hectares respectively (Government of Western Australia, 2022a). The main plant communities are limestone heathland, Banksia woodland and low moist areas with *Banksia ilicifolia* at Kings Park, and Tuart-Banksia woodlands and limestone heathlands at Bold Park (Government of Western Australia, 2022a; Government of Western Australia, 2022b). Seeds of dominant species have been collected from these bushlands over the last twenty years to be used by the Kings Park Science team for bushland restoration and research.

M. gueriniae is mostly found across Murchison in the Midwest of Western Australia, with some populations in the Swan Coastal Plain, the Wheatbelt and Carnarvon (Western Australian Herbarium, 2022). *O. pimeleoides* is mainly found within the Midwest and the Wheatbelt, with some populations east of Kalgoorlie, and in the southern part of Carnarvon (Western Australian

Herbarium, 2022). *P. lessonii* is widely distributed across the SWAFR, with populations present from Carnarvon to Esperance, and also along the Swan Coastal Plain (Western Australian Herbarium, 2022). *R. sterilescens* is mainly found in the Midwest, Pilbara and Carnarvon (Western Australian Herbarium, 2022).

2.2 Key components of experiment

There were two key components to the experiment: a germination trial and a rapid ageing experiment. All experiments were undertaken in the laboratory at Kings Park and Botanic Garden, Perth, WA. Prior to experimentation, all seeds had been stored at -18˚C within hermetically sealed aluminium foil bags in the seed bank at Kings Park. However, seeds of different accessions had varied storage history due the changing seed banking practices and facilities over time.

Seeds were stored in glass jars under ambient conditions prior to 1990, and from 1990 – 1997 the jars were stored in an airconditioned room at c. 23˚C. In 1997 seeds were transferred to hermetically sealed aluminium foil bags and stored in chest freezers at -18˚C. In 2005, a controlled environment drying room was constructed, and all seeds were dried at 15˚C and 15% RH prior to storage in laminated foil bags at -18˚C, and from 2016 all seeds have been stored in a walk-in freezer room at -18˚C.

Due this varied storage history, particularly the drying environment, upon removal from the seed bank, seeds were first measured to determine equilibrium relative humidity (eRH) with the use of a hygrometer (Rotronic Instruments UK Ltd, Crawley, UK). The average seed mass was also determined for each accession. X-rays (Faxitron Multifocus, Arizona) were taken to determine if embryos were present, and seeds were only selected for subsequent germination testing and experimentation if they were filled (i.e. contained an apparently viable embryo).

2.3 Seed mass

X-rayed seeds that had apparent embryos were used for mass calculation. Seed masses (mg) were calculated by weighing three measures of ten seeds for each accession, then taking the average of the three measures.

2.4 Germination trial (Chronosequence)

A chronosequence of multiple accessions of the same species from similar locations across different collection years were used in this experiment to assess how storage history influences viability.

For each accession used, four replicates of 25 seeds were plated onto Petri dishes. Most species were plated onto Petri dishes containing solidified water-agar medium (0.7% w/v). For species from Asteraceae, seeds were plated on water-agar containing gibberellic acid (GA_3, 1mM). For *Trachymene pilosa*, seeds were plated onto water-agar containing karrikinolide (KAR_1, 1µM). Petri dishes were incubated in a plant growth chamber at 15˚C to germinate under a 12/12 hour light/dark cycle. Germination was scored regularly (twice weekly) for six weeks, with seeds deemed to have germinated upon radicle emergence of > 2 mm. A cut test was performed at the end of the germination trial (~42 days) on any non-germinated seeds to determine their viability – seeds were deemed viable if healthy embryos were present, and decayed seeds were deemed non-viable.

2.5 Rapid ageing experiment (comparative longevity testing)

Based on the results from the germination trial, a number of species that showed similar final germination across multiple accessions were selected for rapid ageing experiments to further characterise future storage potential. The following species were used for comparative longevity testing: *Brachyscome iberidifolia*, *Hyalosperma cotula, Olearia axillaris*, *Panaetia lessonii*, *Podotheca angustifolia* and *Trachymene pilosa*.

For each accession used, ten samples of 53 seeds were placed in mesh bags and rehydrated at 47% RH by suspending above a non-saturated lithium chloride (LiCl) solution in an air-tight electrical enclosure box and placed at 20˚C in an incubator over a period of 14 days (Newton et al., 2009). The rehydration solution was prepared by adding 385g of LiCl per litre of reverse osmosis water (Newton et al., 2009). Samples were then placed at 60% RH in a second electrical enclosure box, in a drying oven at 45˚C for ageing (Newton et al., 2009). The ageing solution was prepared by adding 300g of LiCl per litre of reverse osmosis water (Newton et al., 2009). The relative humidity inside the box was monitored over the ageing period using a data logger (T-TEC, Australia), to ensure a constant environment was maintained. A sample of seeds for each accession was removed at regular intervals (e.g. 2, 5, 10, 20, 30, 40, 50, 75, 100, and 125 days) and plated up as per a germination test.

2.6 Statistical analysis

Germination data from the chronosequence experiment were analysed using R version 4.1.0 (R Core Team, 2021) in *RStudio* version 1.4.1106 (Rstudio Team, 2021). Final germination response after ~4 weeks (28-35 days) were analysed using a generalised linear model with an inbuilt "logit" link function, with binomial errors. Pairwise comparisons of each accession within a species were made using a Holm's adjusted pairwise T-test, using the package *modelbased*. Graphs for the germination data were created in SigmaPlot for Windows version 11 (2008, systat software inc.).

Analysis for the rapid ageing experiment was performed using Genstat for Windows 22nd edition (VSN International, 2022) and graphs were created in OriginPro8 (version 8, 2010, OriginLab Corporation, Northampton, MA, USA). Four-week germination data from the chronosequence (~28-35 days) was used as a point of initial percentage germination in the rapid ageing analysis.

For the rapid ageing experiment, a probit analysis was used to estimate the time taken for viability to decline to 50% (p_{50}) under storage conditions, as well as the intercept (*K*i) and slope ($1/\sigma$) of the seed survival curve. The seed survival curve was generated by plotting percentage germination against the ageing period, to fit the viability equation:

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

where *v* is the viability of the seed collection in storage after *p* days, *K*i is a measure of initial seed viability and σ (sigma) is the time taken for viability to fall by 1 probit. (Ellis and Roberts, 1980; Newton et al., 2009). The p_{50} was used to rank and compare species longevities and was calculated via the equation: $p_{50} = Ki \times \sigma$ (Newton et al., 2009).

Results

3.1 Relative humidity and seed mass

The relative humidity (RH) for accessions of *B. iberidifolia* ranged from 35.4% in the youngest accession to 51.2% in the 2000 accession. RH for the 2004 accession was not available. The average seed mass per accession (for ten seeds) was varied, ranging from 1.37mg to 3.40mg (**Table 1**). The RH for accessions of *H. cotula* were similar, ranging from 17.8% to 22.9%. The average seed mass per accession ranged from to 0.77mg to 1.03mg (**Table 1**). The RH for accessions of *M. gueriniae* were more varied, ranging from 23.4% in the youngest accession to 72.3% in the oldest accession. The average seed mass per accession ranged from to 20.33mg to 36.77mg (**Table 1**). The RH for accessions of *O. axillaris* ranged from 18.3% to 43.1%. The average seed mass per accession was quite similar, ranging from to 2.03mg to 3.13mg (**Table 1**). The RH for accessions of *O. pimeleoides* were also similar, ranging from 32.1% to 33.5%. The average seed mass per accession was varied, ranging from to 5.37mg to 24.83mg (**Table 1**).

The RH for accessions of *P. lessonii* ranged from 34.4% to 60.2%. The average seed mass per accession was similar, ranging from 0.31mg to 0.37mg (**Table 1**). The RH for accessions of *P. cordata* ranged from 18.6% in the youngest accession to 47.2% in the 1999 accession. The average seed mass per accession ranged from 0.77mg to 0.98mg (**Table 1**). The RH for accessions of *P. angustifolia* ranged from 23.4% in the youngest accession to 40.7% in the oldest accession. The average seed mass per accession was similar, ranging from 3.23mg to 3.80mg (**Table 1**). The RH for accessions of *R. sterilescens* ranged from 29.6% in the youngest accession to 45.5% in the 1999 accession. The average seed masses were similar, ranging from 2.43mg to 22.67mg (**Table 1**). The RH for accessions of *T. pilosa* were varied, ranging from 15.3% in the youngest accession to 49.5% in the oldest accession. The average seed mass per accession was similar, ranging from 8.77mg to 16.63mg (**Table 1**).

Table 1: Table of ten study species (total of 44 accessions used), with accession numbers, year of collection, relative humidity (%) and average seed mass (mg).

Species	Accession #	Collection Year	Relative Humidity (%)	Average seed mass (mg)
Brachyscome iberidifolia	19820473	1982	43.3	1.70
	20000426	2000	51.2	2.03
	20020768	2002	43.8	3.40
	20040734	2004	N/A	1.60
	20171454	2017	35.4	1.37
Hyalosperma cotula	20060615	2006	22.9	0.77
	20080060	2008	17.8	0.93
	20100160	2010	19.0	1.03
Myriocephalus gueriniae	19910056	1991	72.3	33.00
	19950793	1995	45.6	26.30
	19960490	1996	48.2	20.33
	20040839	2004	46.4	36.53
	20171638	2017	23.4	36.77
Olearia axillaris	19920844	1992	43.1	2.03
	20061736	2006	18.3	2.67
	20100811	2010	18.9	2.63
	20140749	2014	21.9	3.13
	20150561	2015	37.2	3.00
Olearia pimeleoides	19870494	1987	33.5	5.60
	19890210	1989	32.6	24.83
	20040809	2004	32.1	5.37

Table 1 continued

Panaetia lessonii	20010288	2001 (a)	34.4	0.31
	20010648	2001 (b)	40.5	0.31
	20030441	2003	60.2	0.36
	20040681	2004	41.8	0.37
Pithocarpa cordata	19920728	1992	37.8	0.77
	19990321	1999	47.2	0.88
	20010248	2001	42.8	0.85
	20061245	2006	22.5	0.86
	20110387	2011	18.6	0.98
Podotheca angustifolia	20040253	2004	40.7	3.77
	20061247	2006	33.2	3.80
	20080478	2008	28.7	3.23
	20120326	2012	23.4	3.37
Rhodanthe sterilescens	19890010	1989	39.1	17.17
	19921153	1992	45.3	22.67
	19990139	1999	45.5	2.43
	20171377	2017	29.6	19.03
Trachymene pilosa	20020589	2002	49.5	8.77
	20040574	2004	40.8	12.70
	20061202	2006 (a)	24.6	13.90
	20061215	2006 (b)	34.9	10.00
	20080458	2008	20.8	11.60
	20150067	2015	15.3	16.63

3.2 Germination trial (Chronosequence)

Germination for accessions of *B. iberidifolia* ranged from 71 ± 4.43% to 82 ± 4.16 %, with the 2002 accession having the highest germination at 82 ± 4.16% (**Fig. 2 (A)**; **Table 2**). The oldest accession, collected in 1982, did not germinate. The difference among the accessions that germinated was not statistically significant (p = 0.4809). Final germination was similarly high in all three accessions of *H. cotula*, with the 2006 and 2010 accessions reaching 95 ± 5% and 95 ± 3% germination respectively, and the 2008 accession reaching 96 ± 2.31% germination (p = 0.9281) (**Fig. 2 (B)**; **Table 2**). The oldest accession of *M. gueriniae*, collected in 1991, did not germinate (**Fig. 2 (C)**; **Table 2**). Final germination percentages ranged from 4 ± 1.63% to 78 ± 6.83%, with the 1995 accession outperforming all other collection years. The younger accessions for this species, made in 2004 and 2017, had noticeably lower germination, at 4 ± 1.63% and 7 ± 2.52% respectively. The difference among accessions was statistically significant (p < 0.001). A post hoc pairwise comparison revealed significant differences between the 1995 accession and all other accessions – 1995, 1996, 2004 and 2017 – where germination occurred (p < 0.001 for each comparison). The oldest accession of *O. axillaris*, collected in 1992, did not germinate (**Fig. 2 (D)**; **Table 2**). Final germination was similar across the 2014, 2006 and 2010 accessions, at 84 ± 1.63%, 86 ± 5.29% and 88 ± 4.32% respectively. The youngest accession in 2015 had the lowest germination at 39 ± 5.97%. The difference among accessions was statistically significant (p =< 0.001). A post hoc pairwise comparison revealed significant differences between the 2015 and all other accessions – 2006, 2010 and 2014– where germination occurred (p < 0.001 for each comparison). The 1987 and 1989 accessions of *O. pimeleoides* did not germinate (**Fig. 2 (E)**; **Table 2**). Final germination for the 2004 accession was 84 ± 1.63%. No significant difference was observed among accessions (p = 1). Final germination for *P. lessonii* ranged from 51 ± 3% in 2003 to 77 ± 1.91% in 2001(a), with a noticeable difference between both accessions made in 2001, as 2001(a) had the highest germination, and 2001(b) reached 52 ± 4.90% germination (**Fig. 2 (F)**; **Table 2**). The difference among accessions was statistically significant (p < 0.001). A post hoc pairwise comparison revealed that the 2001(a) accession was statistically different when

compared to the 2001(b), 2003 and 2004 accessions ($p = 0.001$, $p = 0.001$ and $p = 0.003$ respectively). Final germination for *P. cordata* ranged from 25 ± 4.43% in the oldest accession, collected in 1992, to 94 ± 2% in the youngest accession, collected in 2011 (**Fig. 2 (G)**; **Table 2**). The difference among accessions was statistically significant ($p < 0.001$). A post hoc pairwise comparison revealed that the 1992 accession was statistically different when compared to all other accessions ($p < 0.001$ for each comparison). Final germination at four weeks reached 100 ± 0% in all accessions of *P. angustifolia* ($p = 1$) (**Fig. 2 (H)**; **Table 2**). Final germination for *R. sterilescens* ranged from 75 ± 5.51% in the youngest accession (2017), to 94 ± 1.15% in the 1992 accession (**Fig. 2 (I)**; **Table 2**). The difference among accessions was statistically significant ($p < 0.001$). A post hoc pairwise comparison revealed that the 1992 accession was statistically different when compared to the 1989, 1999 and 2017 accessions ($p = 0.004$, $p < 0.001$ and $p = 0.003$ respectively). Final germination was similarly high in all accessions of *T. pilosa*, reaching 100 ± 0% in all accessions aside from 2004 where germination was at 98 ± 2% ($p = 1$) (**Fig. 2 (J)**; **Table 2**).

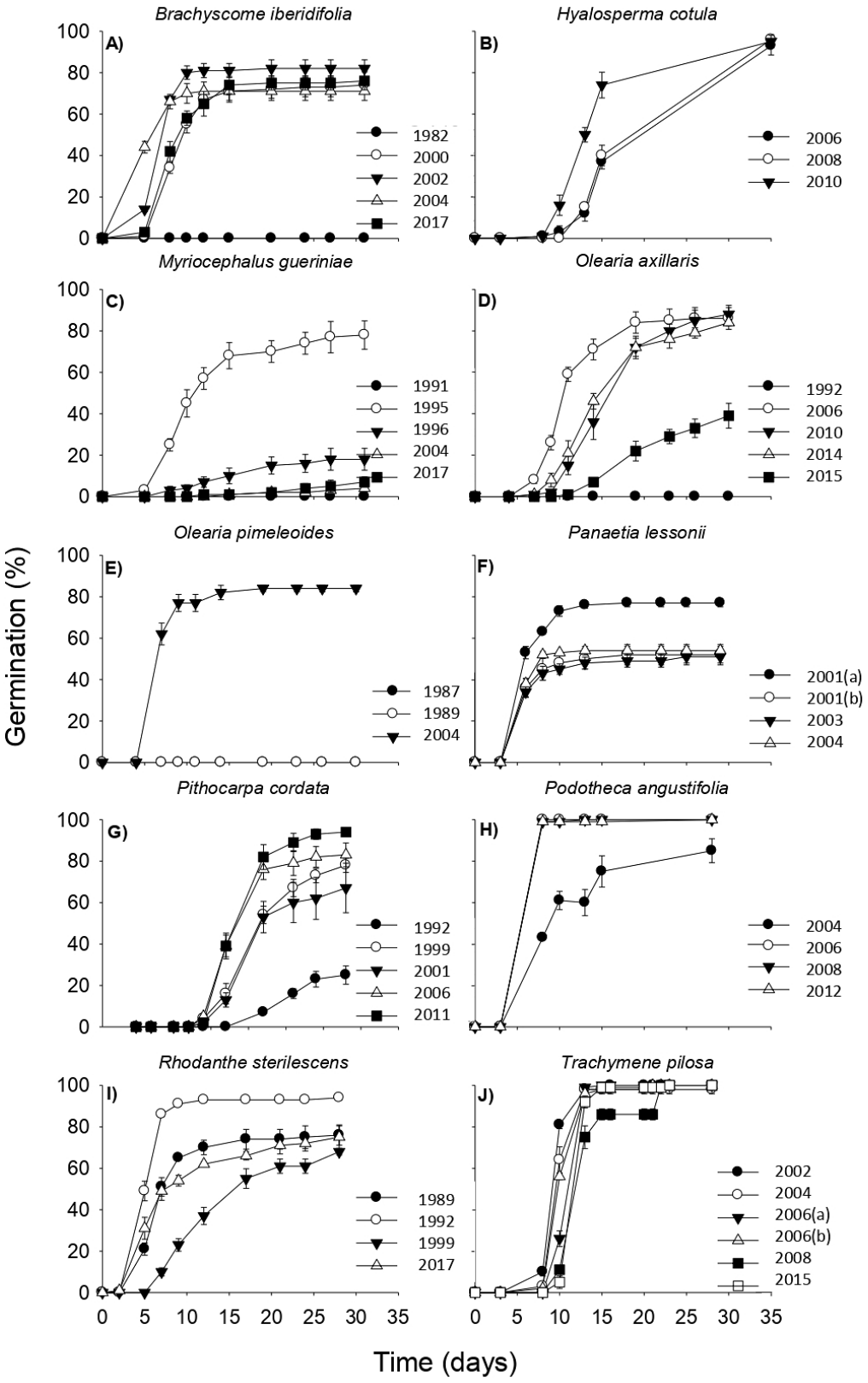

Fig. 2: Cumulative germination (%) over four weeks (28-35 days) for each accession across the chronosequence. Data points represent means ± standard errors.

3.3 Rapid ageing experiment (comparative longevity)

Longevity was varied across all 24 accessions, ranging from 0.56 ± 0.45 days to 57.20 ± 1.66 days (**Table 2**). p_{50} values for *B. iberidifolia* ranged from 4.67 ± 0.86 days in the 2004 accession to 14.61 ± 1.16 days in the 2017 accession. Initial seed quality (*K*i) ranged from 0.50 ± 0.11 to 0.89 ± 0.09 in the 2004 and 2017 accessions respectively (**Table 2**; **Fig. 3**). p_{50} values for *H. cotula* were similar, ranging from 41.88 ± 1.80 days to 42.81 ± 1.76 days. *K*i values were also similar across accessions, ranging from 2.17 ± 0.21 to 2.39 ± 0.23 (**Table 2**; **Fig. 3**). p_{50} values for *O. axillaris* ranged from 2.94 ± 0.437 days in the 2014 accession, to 7.79 ± 0.746 days in the 2006 accession. *K*i values ranged from 0.656 ± 0.112 to 1.011 ± 0.124 in the 2014 and 2006 accessions respectively (**Table 2**; **Fig. 3**). p_{50} values for *P. angustifolia* were similar, ranging from 41.01 ± 1.56 days to 50.91 ± 1.80 days. *K*i varied across accessions, ranging from 2.61 ± 0.25 to 6.24 ± 1.16 (**Table 2**; **Fig. 3**). p_{50} values for *P. lessonii* ranged from 0.56 ± 0.45 days in the 2004 accession to 4.98 ± 0.62 days in the 2001(a) accession. *K*i values ranged from 0.10 ± 0.11 to 0.70 ± 0.10 in the 2003 and 2001(a) accessions respectively (**Table 2**; **Fig. 3**). p_{50} values for *T. pilosa* were mostly similar, ranging from 27.2 ± 1.20 days to 50.2 ± 1.66 days, with the 2006(a) accession having the smallest p_{50}. *K*i values varied, ranging from 2.24 ± 0.25 to 5.10 ± 0.72 (**Table 2**; **Fig. 3**).

Table 2: Initial percent germination, initial seed quality (*K*i) and time taken for viability to decline to 50% (p_{50}) for all 24 accessions of rapidly aged species.

Species	Accession #	Collection Year	Initial germination (%)	SE	*K*i	SE	*p*50	SE
Brachyscome iberidifolia	20000426	2000	74	3.83	0.70	0.12	5.34	0.73
	20020768	2002	82	4.16	0.86	0.11	9.90	0.97
	20040734	2004	71	4.43	0.50	0.11	4.67	0.86
	20171454	2017	76	4.32	0.89	0.09	14.61	1.16
Hyalosperma cotula	20060615	2006	95	5.00	2.39	0.23	42.74	1.73
	20080060	2008	96	2.31	2.17	0.21	41.88	1.80
	20100160	2010	95	3.00	2.39	0.23	42.81	1.76
Olearia axillaris	20061736	2006	86	5.29	1.01	0.12	7.79	0.75
	20100811	2010	88	4.32	1.00	0.17	6.04	0.68
	20140749	2014	84	1.63	0.66	0.11	2.94	0.44

Table 2 continued

Panaetia lessonii	20010288	2001(a)	77	1.91	0.70	0.10	4.98	0.62
	20010648	2001(b)	52	4.90	0.17	0.11	1.01	0.60
	20030441	2003	51	3.00	0.10	0.11	0.90	1.05
	20040681	2004	54	2.00	0.14	0.11	0.56	0.45
Podotheca angustifolia	20040253	2004	100	0.00	3.09	0.27	50.91	1.80
	20061247	2006	100	0.00	2.61	0.25	41.01	1.56
	20080478	2008	100	0.00	5.35	0.56	57.20	1.66
	20120326	2012	100	0.00	6.24	1.16	49.59	1.66
Trachymene pilosa	20020589	2002	100	0.00	5.10	0.72	48.47	1.46
	20040574	2004	98	2.00	2.86	0.26	46.83	1.73
	20061202	2006(a)	100	0.00	2.24	0.25	27.20	1.20
	20061215	2006(b)	100	0.00	3.04	0.33	43.39	1.54
	20080458	2008	100	0.00	3.60	0.34	50.20	1.66
	20150067	2015	100	0.00	4.24	0.45	49.51	1.55

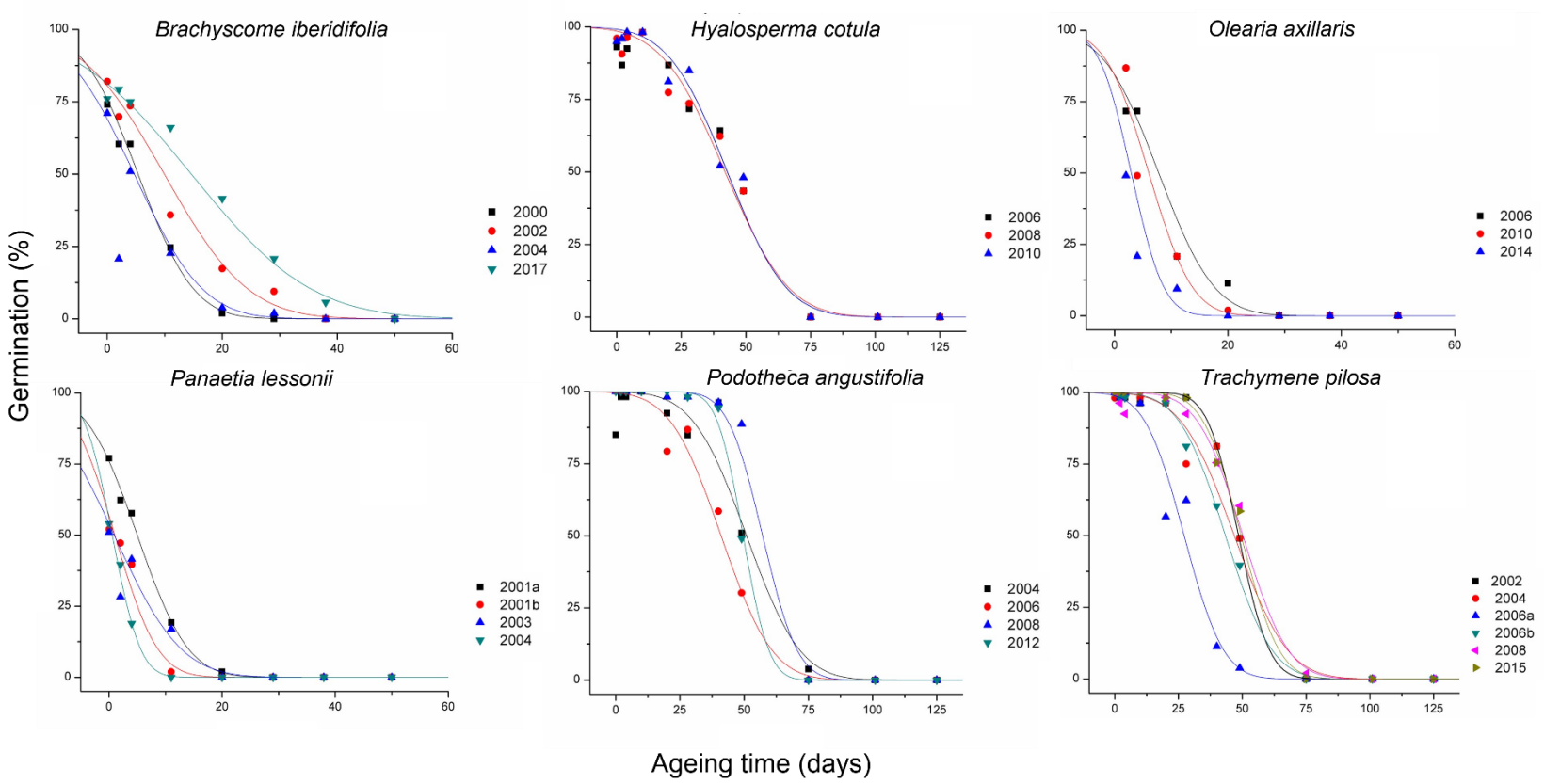

Fig. 3: Seed survival curves fitted for probit analysis, where germination (%) is measured against the ageing time (days) for accessions of rapidly aged species.

Discussion

The aims of this study were to identify short-lived species and assess potential future longevity of accessions with similar viability from the chronosequence. The data from this study showed that some seeds of wild Western Australian species were short-lived on a species level, while other accessions had lower values for longevity (p_{50}) within a species due to having lower initial viability (Ki).

4.1 Germination trial (chronosequence)

In some cases, the data showed that seeds from the conservation storage did not follow the expected trend; some older accessions within a species had better final germination than that of younger accessions. Among accessions of *M. gueriniae* and *O. axillaris,* the younger collections were seen to have significantly lower final germination percentages than accessions made as far back as 20-30 years ago (**Fig. 2**).

The 1991 accession of *M. gueriniae* had the highest relative humidity (**Table 1**) when compared to the rest of the accessions – both within the species and among other species used in this study. This accession did not germinate (**Fig. 2**), and it is likely that the high relative humidity may have contributed to the deterioration of the seed lot, as we know that excessive amounts of moisture negatively impact the longevity of seeds (Tuckett et al., 2010; Merritt et al., 2021).

4.2 Rapid ageing experiment (comparative longevity testing)

The results from the ageing experiment showed that species within the family Asteraceae had varying levels of longevity. Among the five native Western Australian daisies studied, three species – *Brachyscome iberidifolia*, *Olearia axillaris* and *Panaetia lessonii* – had accessions that fell in the range of 'very short' or 'short-lived' (Mondoni et al., 2011) (**Fig. 3**; **Table 2**). *P. lessonii* was the shortest-lived among species tested, with three of the accessions having the lowest p_{50} values across all aged accessions (**Table 2**).

When x-raying seed collections prior to experimentation, some species – notably *B. iberidifolia* – had more instances of unfilled or partially filled seeds within the accession (i.e. no apparent embryo or embryo not fully developed), suggesting that seed lot quality may have negatively impacted longevity. Despite having relatively high initial germination from the chronosequence experiment (**Fig. 2 (A)**)*,* accessions of *B. iberidifolia* were short-lived and had lower initial viability (**Fig. 3**; **Table 2**). This is also apparent among the aged accessions of *O. axillaris* (**Fig. 2 (D)**; **Fig. 3**; **Table 2**).

A majority of the rapidly aged accessions were found to have lower initial viability (**Table 2**). *P. angustifolia* and *T. pilosa* (Araliaceae) were found to have similar ranges of medium longevity within species ($100 > p_{50} > 10$) (Mondoni et al., 2011). Despite this, different accessions within species were found to have varying *K*i values (**Table 2**).

The 2002 and 2015 accessions of *T. pilosa* had the same final germination and similar longevities despite the difference of over ten years in storage (**Fig. 2 (J)**;**Table 2**). The 2015 accession had a lower relative humidity than that of the 2002 accession – and the lowest relative humidity across all accessions in the chronosequence. The difference in initial seed quality (*K*i) between the two accessions may explain why the 2002 accession has maintained viability better in storage, despite the high relative humidity. Accessions of *H. cotula*

maintained similar longevities regardless of their collection year, with similar initial viability as well (**Table 2**).

There were more instances of abnormal germination across the rapidly aged species over time (**Fig. 4**). Abnormal germination was characterised by weak or deformed structures, and/or emergence of the cotyledons without the appearance of the radicle (De Barro, 2008). Seedlings with abnormal germination are unable to develop into normal plants when grown under favourable conditions (De Barro, 2008).

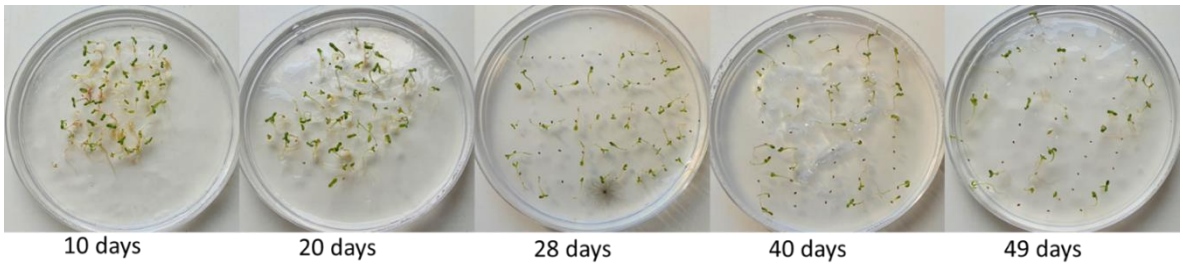

Fig. 4: Germinated plates of *H. cotula* seedlings across five intervals of rapid ageing. Photo credit: Dr David Merritt.

It is possible that factors such as the time of harvest or seed handling protocols for some of these accessions may not have been optimal, leading to the various levels of seed quality (Hay and Probert, 2013; Cochrane et al., 2021). As we do not have good records of how seeds were handled prior to storage, and the drying room (i.e. controlled environment used to dry seeds to 15% RH prior to storage) at Kings Park was not operational until 2005, many of the older accessions of seeds used in this study were not dried to international standards (15% RH and 15˚C) up until this point (Merritt et al., 2021). Changes in practices and facilities over the years are common in seedbanks globally, and it is important to consider these factors when assessing the data for older seed collections.

4.3 Longevity of Australian species

Seed longevity is known to vary between species by over four orders of magnitude (Probert et al., 2009; Mondoni et al., 2011; Merritt et al., 2014). When compared to species on a global scale (~460 species), seeds of Australian flora have been identified as being longer-lived, with over 15% of the native Australian species studied by Merritt et al. (2014) having p_{50} values greater than 200 days. Of all the tested species, only Australian flora has had p_{50} values greater than 200 days (Merritt et al., 2014). The longest-lived species tested is *Calothamnus rupestris* (p_{50} of 771 days), a serotinous woody shrub belonging to the family Myrtaceae, that has non-endospermic seeds (Merritt et al., 2014). Similarly, of the 195 species tested in the study by Probert and colleagues (2009), the Australian species were among those with longer lifespans, with an average p_{50} value of 202.7 days, compared with values of 25.2, 28.8, and 38.6 days for seeds from Chile, England, and the USA respectively (Merritt et al., 2014). In a study done on Western Australian aquatic, vernal pool species however, the p_{50} values ranged from 5.83 days in *Glossostigma drummondii* to 44.34 days in *Trithuria submersa*, falling in the short-lived and medium-lived categories (Tuckett et al., 2010). In another study on aquatic Australian species, it was found that the average longevity for *Nymphaea violacea* was similar to that of Tuckett and colleagues' (2010) findings, at 22.2 days (Dalziell et al., 2019). This variation between longevities may be explained by the difference in the environmental conditions of the respective habitats of species used in these experiments – hot and dry terrestrial conditions in contrast to temporary wetlands and aquatic environments (Tuckett et al., 2010; Dalziell et al., 2019).

4.4 Longevity within taxonomic groups

While longevity for Asteraceae varied from the very-short to medium range in this study, greater variation in p_{50} values was observed for species within Asteraceae in other studies: 105.0 days in *Cotula coronopifolia* and 25.9 days in *Waitzia nitida*, with a mean p_{50} of 52.6 days (Probert et al., 2009), and 7.1 ± 0.85 days in *Taraxacum alpinum* and 76.3 ± 2.87 days in *Centaurea maculosa* (Mondoni et al., 2011). Likewise, variation within other plant 33 families was also observed: 65.1 days in *Brassica napus* and 22.2 days in *Lepidium montanum* with a mean p_{50} of 43.6 days (Brassicaceae) (Probert et al., 2009), and 5.2 ± 0.60 days in *Festuca violacea* and 37.8 ± 1.34 days in *Sesleria pichiana* (Poaceae) (Mondoni et al., 2011).

In contrast, similar ranges in longevity were reported for species within a genus. In Probert and colleagues' (2009) study, *Podotheca gnaphalioides* was found to have medium longevity, with a similar p_{50} value to the accessions of *P. angustifolia* (**Table 2**) used in this study, at 43.8 days. Likewise, across species of Primulaceae, longevities ranged from short-lived to long-lived (4 to 140 days), while species within the genus *Primula* were found to have similarly short-lived longevities (6 to 22 days) (Probert et al., 2009). Within the family Gentianaceae, some species were significantly longer-lived (38 and 37 days in *Blackstonia perfoliata* and *Centaurium erythraea* respectively) than the short-lived range for species within the genus *Gentiana* (8 to 13 days) (Probert et al., 2009). It is likely that these taxonomic trends are a result of similar seed structure and habitats among species of a single genus (Probert et al., 2009).

In the case of most of the species used in this study, the differences in p_{50} values present across species (**Table 2**) are hence not likely to have been largely impacted by differences in the habitats of collection sites, as eight out of ten of the study species occur along the Swan Coastal Plain, in a similar Mediterranean climate, and all ten of the species can be found in sandy soil types (Barrett and Tay, 2018; Western Australian Herbarium, 2022). Further

experimentation could be done on taxonomically related species (i.e. at the genus level) to determine if the variations in p_{50} values are due to seed structure differences or habitat conditions.

4.5 Limitations

While the experiment was successful in identifying short-lived and medium-lived seeds in the Kings Park seed bank, it was not without its limitations. One of the drawbacks of this experiment is that there are no germination data for the initial viability of the species used in this study. Seeds were not tested for their viability at the point of collection, hence limiting the ability to calibrate the process for rapid ageing experimentation in the future.

Rapid ageing experiments are also not able to predict ageing in real time, as the data from ageing is only able to rank which accession is more likely to last longer in storage in relation to another via the p_{50} values.

Whilst we have chosen to analyse final germination data after 4 weeks (28-35 days), it is possible that further analysis of the germination curves, including the calculation of time taken to reach 50% germination, may be useful additional information from these data (e.g. see Dalziell et al., 2022).

As noted in the introduction, other studies have also included relationships between seed longevity and factors such as the presence of endosperm, seed dispersal methods, embryo size ratios and the effects of elevation, temperature and rainfall in the native habitat of study species. Further analysis could be done to confirm if the variation in p_{50} values across the study species was unaffected by habitat conditions or climatic factors in the respective distributions of the study species – i.e. comparing the climatic factors of the collection sites in the Pilbara and Midwest regions with that of the Perth Metropolitan area, in relation to the longevity of each species. Analysis could also be done to determine if seed mass is a factor

that correlates to longevity. In some prior studies, seed mass was shown to have no significant correlation to longevity, despite this being an apparent trend in crop species (Proberts et al., 2009; Walters et al., 2005). In contrast to this, in studies of Australian species, seed mass had a positive correlation to longevity (Merritt et al., 2014; Satyanti et al., 2018). In alpine Australian flora, seeds with lower seed mass were longer-lived than those of higher seed mass (Satyanti et al., 2018).

Seeds of Asteraceae are commonly geosporous and non-endospermic (Hirst et al., 2021; Western Australian Herbarium, 2022). As prior studies show that serotiny may indicate longer-lived species, and that it is linked to having a larger embryo and no endosperm (Crawford et al., 2011; Merritt et al., 2014), analysis on these variables could further aid in the understanding of factors affecting longevity of the species used in this study.

4.6 Longevity studies inform seed banking/germplasm conservation practices

In the context of improving seed banking practices, it is important to study seed longevity, as this data can inform management decisions, such as when seed collections need to be replenished to safeguard the viability of these collections (Hay et al., 2022).

In order to maximise their longevity, seeds of short-lived species (*B. iberidifolia, O. axillaris* and *P. lessonii* (**Table 2**)) should be assigned priority for post-harvest handling and storage (Merritt et al., 2014; Li and Pritchard, 2009). Post-harvest handling follows immediately after the collection of seeds. Seeds should be kept at cool and dry temperatures while being transported, kept in shady conditions and ventilated, and damp seeds should be spread out to dry naturally (Cochrane et al., 2021). The moisture content of seeds should be monitored after collection, as high temperatures and moisture levels will cause seeds to age rapidly (Merritt et al., 2021). Seed moisture content can be measured non-destructively via the use

of a hygrometer, to get the eRH (%) reading of a sample of seeds (Merritt et al., 2021) and a temperature data logger may be used to monitor conditions during post-harvest (Cochrane et al., 2021).

For short-lived species, the frequency of monitoring while in storage may need to be altered depending on the viability of the accession. Initial germination tests should be conducted upon acquisition, and collections should be tested regularly – every 5 years if they are short-lived and 10 years if they are long-lived (Merritt et al., 2021). As frequent testing may be costly and subject to staff and resource availability (Merritt et al., 2021), alternative methods of storage can be recommended for species that are shown to be shorter-lived.

Li and Pritchard (2009) found in the review of seed longevities by Probert et al. (2009), that 26% of the orthodox seed collections showed significant declines in viability after 20 years, under seed banking conditions of 15% RH and -20 °C. Li and Pritchard (2009) also found that the estimated half-lives of seeds kept for an average of 38 years under ∼5 °C, and ∼25 years at −18 °C was greater than 100 years for only 22% of the 276 species used in the study by Walters et al. (2005). Likewise in this study, among the older seed collections (16-20 years in storage), half of the species that were rapidly aged had shorter half-lives (*B. iberidifolia, O. axillaris* and *P. lessonii* (**Table 2**)).

Tuckett et al. (2010) suggest that cryopreservation – defined as the storage of seeds in liquid nitrogen at ultra-low temperatures – can be used to extend longevity in orthodox seeds which have shorter lifespans and high conservation value. Cryopreservation is defined as the storage of germplasm at very low temperatures with either liquid nitrogen or its vapour to preserve living tissue (Funnekotter et al., 2021). Cryopreservation, like seed banking, is a low maintenance and space efficient option for long-term storage of germplasm, particularly for germplasm that cannot be considered for dry storage at -20˚C (Funnekotter et al., 2021). Cryopreservation reduces the need to conduct regular monitoring of viability or subculturing

tissue cultures, and also reduces the space required to maintain such collections, but requires considerable labour, time and knowledge to develop a successful protocol, which is likely to be species specific (Funnekotter et al., 2021). Orthodox seeds may be stored in liquid nitrogen without chemical cryoprotection, due to their natural low moisture contents (Funnekotter et al., 2021). Seeds are recommended to be equilibrated at 25-35% RH at temperatures of 5-20°C before storage in liquid nitrogen (Funnekotter et al., 2021). Once equilibrated, seeds are to be places into a cryovial and immersed in liquid nitrogen without any further treatment required (Funnekotter et al., 2021). Sub-samples of seeds should be retrieved from the cryovial without a few days for viability testing to ensure that the effects of temperature change were not damaging to the seeds (Funnekotter et al., 2021). When seeds are to be retrieved, they should be thawed for around 20 minutes at ambient room temperature with the use of a dry box of silica gel, to prevent water from entering the cryovials in the event of ice forming (Funnekotter et al., 2021). This method of cryopreservation may also be applied to non-orthodox seeds if they are tolerant to desiccation and initial freezing, but short-lived in conventional storage conditions (Funnekotter et al., 2021).

Tissue culture is another alternative storage method for short-lived seeds and desiccation sensitive species (Sommerville et al., 2021). Tissue culture is best suited as a technique for species that are rare, have high conservation value, or are considered to be “problematic”, as it is often a last resort due to the need for specialised consumables, equipment, staff, and laboratory facilities (Sommerville et al., 2021).

Conclusion

Seed banking for ex situ conservation of Australian species has been found to be appropriate in many cases, due to the long-lived nature of seeds from hot and dry regions around the continent. In this study, a number of wild Western Australian species were shown to be shorter lived: *B. iberidifolia* ranging from 4.67 ± 0.86 to 14.61 ± 1.16 days*, O. axillaris* ranging from 2.94 ± 0.44 to 7.79 ± 0.75 days and *P. lessonii* ranging from 0.56 ± 0.45 to 4.98 ± 0.62 days (**Table 2**). Longevity varied across the aged species in this study, from being very short-lived to medium-lived. Species within a genus are likely to share similar ranges for longevity (*Podotheca*), while the ranges within families tend to be more varied (Asteraceae). Factors that affect seed longevity should be studied more in depth to gain a better understanding of how different taxa will respond to storage conditions. Seed accessions with low p_{50} values should be tested regularly (e.g. every 5 years) to monitor their remaining storage potential and inform management decisions for seed banking. To achieve the most success from future comparative longevity experiments (i.e. more accurate estimates of longevity), acquired seeds should be tested for initial viability at the point of banking. Seeds that are considered to be orthodox may still show significant declines in viability during storage. For seeds that are problematic in seed banking conditions – both orthodox and intermediate type storage – cryopreservation may be an alternative storage option.

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