The influence of cone age on the relative longevity of Banksia seeds

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INTRODUCTION

The genus Banksia (Proteaceae) comprises >170 species (Mast and Thiele, 2007), most of which are endemic to Australia (George, 1981). Banksia species form an important component of vegetation communities throughout Australia, predominantly in the south west of Western Australia. From the perspective of conservation, banksias are important as species form important components of ecological communities protected under this Act. Conservation of threatened plant species should focus primarily on in situ conservation, involving recovery actions aimed at ensuring survival in the natural habitat. Ex situ conservation of plants, as seed, can complement in situ recovery actions. Survival of plants both in situ and ex situ can be heavily dependent on seed quality; seeds must retain viability in situ until suitable conditions are experienced for recruitment, and ex situ until required for recovery actions.

Survival under a given set of temperature and moisture content conditions can be predicted using the seed viability equation

\[ v = K_i - p/\sigma \]

where \( v \) is viability (probits), \( K_i \) is an estimate of initial viability (probits), \( p \) is time (d) and \( \sigma \) is the standard deviation of the normal distribution of seed deaths in time (Ellis and Roberts, 1980). This equation assumes that the slopes of seed survival curves (1/\( \sigma \)) are not affected by either genotype or seed quality; however, some studies have indicated variation in \( \sigma \) related to seed developmental age (Kameswara Rao et al., 1991; Hay and Probert, 1995), genotype (Zanakis et al., 1994; Kochanek et al., 2009) and seed priming (Probert et al., 1991). More commonly, differences in longevity are attributed to differences in \( K_i \) and, as seed deaths follow a normal distribution in time, small differences in percentage germination can have a large effect on \( K_i \) and hence on seed longevity (Ellis and Roberts, 1980).

Serotiny is a plant adaptation where seeds are retained in the canopy for a prolonged period rather than either being dispersed and germinating, or forming a soil seed bank. Plants therefore retain seeds produced in a number of different years as an aerial seed bank (Lamont et al., 1991). Many Banksia species exhibit some degree of serotiny. An index of the degree of serotiny of banksias is calculated as the inverse slope of the linear regression of the percentage of open follicles against cone age and so is a function of both the proportion of seeds and the length of time of retention on the plant (Cowling and Lamont, 1985). Species with a degree of serotiny <4 are considered weakly serotinous,
wetness is not always consistent within a species; for example, serotiny tends to decrease with increasing rainfall (Cowling and Lamont, 1985).

Serotinous seeds gradually lose viability while on the plant. Decreases in seed germination with increasing cone age have been recorded for a number of serotinous Banksia species (e.g. Cowling and Lamont, 1985; Cowling et al., 1987; Enright et al., 1996; Barrett et al., 2005). The rate of viability loss with time on the plant is species specific (e.g. Cowling et al., 1987). Previous Banksia studies examining cone age and seed viability have focused on the viability of seeds at the time of collection, but there is currently no information on how these seeds perform in ex situ storage after collection.

In the present study, we tested two hypotheses. First, that within a species, seeds from cones of different ages lose viability at the same rate (i.e. do not differ in the value of $\frac{1}{\sigma}$), with any differences in longevity being attributable to differences in initial viability ($K_i$). Secondly, that there is a positive relationship between degree of serotiny and the time for viability to fall by one probit ($\sigma$). Three serotinous Banksia species from the northern sandplains of south west Western Australia were used: Banksia attenuata R.Br., a summer flowering, non-resprouting shrub/tree (George, 1981) which has been associated with variable levels of serotiny along a climatic gradient from weak (3.5) to moderate (17.2) (Cowling and Lamont, 1985); B. hookeriana Meisn., a winter flowering, non-resprouting shrub (George, 1981) with relatively strong serotiny (18–25) (Enright and Lamont, 1989; Enright et al., 1998); and B. leptophylla var. melletica A.S.George, a winter flowering, non-resprouting shrub (George, 1999) with strong serotiny (52.9) (Enright and Lamont, 1989).

**MATERIALS AND METHODS**

**Cone collection and seed extraction**

Cones from three species of Banksia L.f. (B. attenuata, B. hookeriana and B. leptophylla var. melletica) were collected in June 2005 and stored at 15°C, 15% relative humidity (RH) until the seeds were extracted. Only infrutescences with developed follicles were collected. Specimens were lodged at the Western Australian Herbarium (B. attenuata, PERTH 07250916; B. hookeriana, PERTH 07249748; and B. leptophylla var. melletica, PERTH 07249683). Cones of B. attenuata and B. hookeriana were divided into age classes using the stem node counting method (Lamont, 1985). This was not possible for B. leptophylla, so cones were divided into three age classes based on follicle colour, and floral remnant persistence and colour: young (follicles red/brown, floral remnants grey with many persistent); old (follicles red/brown, floral remnants grey with few persistent, cones showing signs of weathering); and immature (undeveloped seeds). Only young and old cones were collected.

For all three species, the number of follicles (open and closed) was counted on 30 cones of each age class, with the exception of ≥10-year-old B. hookeriana for which ten cones were assessed due to a lack of material. The cones were burnt with a gas torch until follicle rupture, soaked in water for 6–8 h, then dried (15°C, 15% RH) to release the seeds. Seeds were extracted in May to July 2006 and stored (15°C, 15% RH) until use. The numbers of firm, insect-damaged and decayed seeds were counted. The number of aborted seeds was estimated as the difference between the potential seed number (two per follicle) and the sum of the firm, insect-damaged and decayed seeds. The degree of serotiny was calculated from the inverse slope of the regression of the percentage of closed follicles against cone age, excluding insect-damaged follicles from the calculation (Cowling and Lamont, 1985).

**Seed ageing**

The relative longevity of seeds for each species and cone age class was determined using a comparative longevity test as described by Probert et al. (2009). Lithium chloride concentrations were adjusted at the respective temperatures to give the desired RH conditions. Preliminary experiments using B. leptophylla var. melletica indicated that the seeds were long lived, with $p_{50}$ (the time for viability to fall to 50%) estimated in excess of 200 d when aged at 45°C and 60% RH. In order to reduce the ageing period, the RH and temperature were raised to 50°C and 63% RH. These conditions aimed to hold moisture content at the same level whilst reducing $p_{50}$ to approx. 90 d. Seeds were pre-equilibrated at 15°C and 44% RH for at least 2 weeks before being moved to the ageing conditions of 50°C and 63% RH. Pre-equilibration minimizes changes in seed moisture content at the start of the ageing treatment (Kochanek et al., 2009). Seed ageing commenced in April and May 2007. Seed samples (B. attenuata, 20–27 seeds per sample; B. hookeriana, 24–27 seeds per sample from 1- to 9-year-old cones and 10–11 seeds per sample from ≥10-year-old cones; B. leptophylla var. melletica, 36–56 seeds per sample) were removed periodically (each 6–14 d, except seeds from B. hookeriana ≥10-year-old cones which were sampled at 49 and 77 d), over a period of 126 d. Samples were germinated in an incubator at 15°C, with a 12 h photoperiod, on 7.5 g L⁻¹ agar plates. Seeds were classified as germinated when the radicle grew to at least 5 mm, and testing continued either until seeds had germinated or until no germination was seen for a period of 1 month.

**Statistical analysis**

Follicle data were assessed using analysis of variance followed by the Tukey–Kramer test using Statview for Windows Version 5 (SAS Institute Inc., Cary, NC, USA). Longevity was analysed by plotting the final germination against time in the ageing conditions using probit analysis in Genstat Version 10 (VSN International Ltd, Hemel Hempstead, UK) to derive initial viability ($K_i$) in normal equivalent deviates (NEDs); where NED viability = probit viability – 5 and $\sigma$, the standard deviation of the normal distribution of seed deaths in time. Using the seed viability equation, $p_{50}$ was calculated.

In order to compare the values of $\sigma$ (and hence $p_{50}$) from this study with others (Long et al., 2008; Probert et al., 2009), estimates of $\sigma$ at 45°C and 60% RH were made.
RESULTS

Banksia leptophylla var. melletica produced between six and 68 follicles per cone, with an average of 34 follicles per cone, but there was no difference between the young and old cone age classes (Fig. 1A). The other two species had fewer follicles per cone, and the maximum number of follicles in an individual cone was 30 in B. attenuata and 24 in B. hookeriana. Differences between age classes were minor, and B. attenuata 1-year-old cones contained fewer follicles (ten) than the ≥6-year-old cones (15), and 2- to 3-year-old B. hookeriana cones contained fewer follicles (eight) than 6- to 7-year-old cones (12) (Fig. 1B, C).

The number of open follicles increased with cone age (Fig. 1). The degree of serotiny was very strong (40.4) for B. hookeriana, and 37 % for 1-year-old B. attenuata cones, but was low (<10 %) for B. leptophylla var. melletica and other B. attenuata age classes. Seed decay was observed infrequently, and only in the older cone age classes. Banksia hookeriana had the highest level of decayed seeds at 10 % of the seeds from ≥10-year-old cones.

Seed survival curves described the reduction in seed germination as time in the ageing environment progressed (Fig. 3). Analysis of deviance showed that survival curves for all cone age classes within each species could be described by regressions with a common slope (1/s) but with different values for K_0 (P = 0.05). Where it was statistically acceptable to constrain age classes to a common line (B. attenuata 2- to 3- and 4- to 5-year-old cones; B. hookeriana 2- to 3- and 4- to 5-year-old cones; and B. hookeriana 6- to 7- and 8- to 9-year-old cones; P = 0.05), the equation for this line was used to describe survival (Table 1, Model 1; Fig. 3). The 1-year-old data set for B. hookeriana could be constrained to that of the 6- to 7- and 8- to 9-year-old cones (P = 0.05); however, it is displayed separately (Fig. 3C). When analysing all three species together, it was statistically acceptable to fit a common value of 29.1 to σ (P = 0.05; Table 1, Model 2).

Initial viability generally decreased with cone age, but remained high (K_i > 90 % viability when converted from NED to %) for most Banksia species and cone age class combinations (Fig. 3C; Table 1, Model 1). The ≥10-year-old cones from B. hookeriana were the only group below 90 % viability (64 %). A decrease in time taken for viability to reach 50 % (p_50) was associated with the reduction in K_i with cone age (Table 1). Banksia leptophylla var. melletica

![Fig. 1. Number of closed or open follicles per cone (mean ± s.e.) for three species of Banksia (A, B. leptophylla var. melletica; B, B. attenuata; and C, B. hookeriana) in relation to cone age class. Different letters indicate significant differences in total follicles (P = 0.05).](https://academic.oup.com/aob/article-abstract/107/2/303/188286)
The degree of serotiny varied between the Banksia species. Banksia hookeriana was strongly serotinous, B. attenuata was moderately serotinous and B. leptophylla var. melletica intermediate between the two. Cowling and Lamont (1985) suggested that there is a trend of decreasing serotiny along a climatic gradient of increasing rainfall. In B. attenuata this was supported by the population examined here having the same degree of serotiny (7.9) as a Hill River population (5.2) growing in an area with similar annual rainfall (Cowling and Lamont, 1985). Also, the estimated degree of serotiny for B. leptophylla var. melletica (25.1) was considerably less than another population of B. leptophylla (52.9) collected from a lower rainfall zone (Enright and Lamont, 1989). This trend did not hold for B. hookeriana where the degree of serotiny (40.4) was far higher than that recorded for another population (18.3) in the same rainfall zone (Enright and Lamont, 1989); however, it is possible that there are local differences in rainfall or differences in the depth of the water table at these sites.

Initial viability ($K_i$) of seeds decreased as cone age increased, as previously reported (Cowling and Lamont, 1985; Cowling et al., 1987; Enright et al., 1996; Barrett et al., 2005). This was associated with a reduction in the length of time that seeds survived in the rapid ageing tests. Time for viability to fall by one probit ($\sigma$) was very similar for the three Banksia species in this study, even though they contrasted in serotiny. Consequently, differences in longevity ($p_{50}$) between the species and age classes were attributed only to differences in $K_i$. Similarities in $\sigma$ between congeneric species occur in other families (Hay et al., 2006; Kochanek et al., 2009), although differences in $\sigma$ between populations of the same species have been recorded (Kochanek et al., 2009). Only one collection from one population was tested for each species in this study, so, before conclusions about the entire Banksia genus can be made, it would be prudent to examine longevity within species further (e.g. B. attenuata along a climatic and serotiny gradient), as well as broadening the species and genera examined to encompass a wider range of degree of serotiny.

Seed immaturity at the time of collection resulted in the 1-year-old cone age class for B. hookeriana having a lower $K_i$ than might otherwise be expected. Banksia hookeriana has a long flowering period, peaking in winter but extending from late April to October (George, 1981), so the actual age of any individual cone could have ranged from 8 to 14 months, with those produced during the peak flowering
period ranging between 11 and 13 months old. This long period over which fruit were formed may explain why initial collection limits (Way, 2003; Menges et al., 2004) to ensure safe collecting limits (Way, 2003). These collections should be made within safe collecting limits (Way, 2003; Menges et al., 2004) to ensure that the amount of seeds removed from a population will not adversely affect long-term survival of the population. An important aim of \textit{ex situ} seed collections is to provide material for species recovery by establishing new populations, reintroducing populations or augmenting existing populations (Menges et al., 2004). Genetic variation forms the building blocks for creating new populations and maintaining genetic diversity.

The conversion and assumptions described above can be used to compare the longevity from this study with those of Probert et al. (2009) as the same relative moisture contents were used. Equivalent $p_{50}$ values for the longest-lived age categories for the three species were: \textit{B. attenuata}, 154 d; \textit{B. hookeriana}, 170 d; and \textit{B. leptophylla} var. \textit{mellitica}, 234 d. These longevity values were relatively high compared with the results of Probert et al. (2009), fitting their predictions that non-endospermic seeds and seeds from warm, arid environments are longer lived than those from cool, moist environments. The $p_{50}$ values for the \textit{Banksia} species in this study were combined with $p_{50}$ values for other serotinous Australian species of the genera \textit{Allocasuarina}, \textit{Calothamnus}, \textit{Eucalyptus}, \textit{Hakea}, \textit{Melaleuca} and \textit{Regelia} (Probert et al., 2009) to produce a mean of 292 d. The longevity of these serotinous species was higher than the mean $p_{50}$ of 39 d derived for non-serotinous species of Australian genera (\textit{Atriplex}, \textit{Chenopodium}, \textit{Oxychloris}, \textit{Podotheca}, \textit{Solanum}, \textit{Swainsona} and \textit{Waitzia}) (Probert et al., 2009). The strong correlation between seed longevity from rapid ageing tests and longevity for many plant families under gene bank conditions (Probert et al., 2009) indicates that the seeds of the three \textit{Banksia} species in this study would be long lived when stored under these conditions.

There is a positive relationship between longevity obtained from comparative ageing studies (45 °C, 60 % RH) and soil seed bank persistence for a range of European and Australian weed species (Long et al., 2008), thus the $p_{50}$ values $>50$ d should indicate that seeds of \textit{Banksia} and other serotinous species have long-lived soil seed banks. Available soil seed bank data for serotinous \textit{Banksia} (Weiss, 1984; Cowling et al., 1987) and \textit{Eucalyptus} (Wellington and Noble, 1985; Yates et al., 1995) indicate that they are short lived in the soil because, after seed release, most often triggered by fire, seeds germinate as soon as enough water is available. If the seed bank concept is broadened to encompass canopy seed banks for serotinous species then the prediction based on $p_{50}$ holds, as \textit{Banksia} would be considered to have a long-lived canopy seed bank.

When seeds are to be collected for \textit{ex situ} conservation purposes, collection quality (viability and potential longevity), quantity and genetic diversity are of paramount importance (Way, 2003). These collections should be made within safe collecting limits (Way, 2003; Menges et al., 2004) to ensure that the amount of seeds removed from a population will not adversely affect long-term survival of the population.
blocks from which these populations will be built, thus *ex situ* collections should retain enough diversity to enable the established populations to withstand future selection pressures. Higher diversity levels will improve the chance of successful species recovery. In the *Banksia* collections studied, the youngest mature cohort would be targeted to maximize the quantity, viability and potential longevity of a seed collection. A substantial proportion of the total seeds on a plant would normally be contained within the youngest cohort (Cowling *et al.*, 1987; Lamont and Barker, 1988), therefore care should be taken to avoid exceeding safe collection limits when seed yield is considered across all cohorts. This recommendation does not take into account the genetic diversity of the collection.

The genetic diversity of *B. hookeriana* seeds increases as more cone age classes are sampled (Barrett *et al.*, 2005). It would therefore be prudent when undertaking *Banksia* seed collections, where the genetic diversity of the seed collection is important (e.g. *ex situ* conservation or restoration), to collect from a wide range of cone ages. This has practical implications for *ex situ* collections. Although this study found the rate of seed ageing of different cone age classes to be the same, if seeds from a range of cone age classes are stored together older seeds would be lost from the collection sooner than young seeds due to differences in $K_i$. Ideally, seeds from identifiable cone age classes should be stored separately so the viability for each class could be monitored over time.

The time taken for viability to fall by one probit ($\sigma$) was the same irrespective of cone age. *Banksia* seeds in cones on the parent plant appeared to be inherently long lived, with 50% of remaining seeds predicted to be viable after 13 years. Increased levels of serotiny did not equate to higher seed longevity within the *Banksia* species studied; however, seeds from Australian serotinous species do appear to be longer lived than those of non-serotinous species. This may be an artefact of the life history of species tested. If species reliant on a persistent soil seed bank for survival were to be considered, such as monocarpic fire ephemerals (species which live for only one season then remain as seed, dormant in the soil until the next fire event), a different pattern may emerge.

In conclusion, the viability and hence longevity of seeds of these three *Banksia* species declined as cone age increased. The rate of ageing ($1/\sigma$) did not differ within or between the collections tested. Contrary to expectations, no relationship was found between comparative longevity and degree of serotiny. In general, seeds of serotinous plant species appear to be longer lived those of than non-serotinous plant species, but this relationship now needs further investigation. In banksias, viable seed yield is likely to be highest in the youngest mature cohort and there may be a temptation for seed collectors to focus on collecting these cones; however, it is recommended that collections are made from across cone ages to maximize the potential genetic diversity of a collection because genetic diversity is the building block which underpins successful species recovery.

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LITERATURE CITED


