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Exploring seed longevity of UK native trees: implications for *ex situ* conservation

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Abstract

UK trees require increased conservation efforts due to sparse and fragmented populations. *Ex situ* conservation, including seed banking, can be used to better manage these issues. We conducted accelerated ageing tests on seeds of 22 UK native woody species, in order to assess their likely longevity and optimize their conservation in a seed bank. Germination at four ageing time points was determined to construct survival curves, and it was concluded that multiple samples within a species showed comparable responses for most species tested, except for *Fraxinus excelsior*. Of all species studied, one could be classified as very short-lived, four as short-lived and 17 as medium, with none exceeding the medium category. The most important finding of this manuscript is that although some taxonomic trends were observed, the results indicate the need for caution when making broad conclusions on potential seed storage life at a species, genus or family level. Longevity predictions were compared to actual performance of older collections held in long-term storage at the Millennium Seed Bank, Kew. Although most collections remain high in viability in storage after more than 20 years, for short-lived species at least, there is some indication that accelerated ageing predicts longevity in seed bank conditions. For species with reduced potential longevity, such as *Fagus sylvatica* and *Ulmus glabra*, additional storage options are recommended for long-term gene banking.

Introduction

Trees and woodlands provide huge benefits to society, supporting our rural economy and biodiversity as well as providing ecosystem services such as flood prevention. Environmental and economic benefits are also complemented by social services that can improve the public's physical health and mental well-being (IPF, 2012). The extent of woodland in the United Kingdom (UK) has increased by 73% since 1947; however, it remains one of the least forested areas in Europe, with just over 13% tree cover (Forest Research, 2018) compared to an average of 38% for the EU (Eurostat, 2015; Defra, 2018). This means that some populations of UK trees are already highly fragmented and at risk of reduced ecological interactions and genetic integrity (Logan et al., 2015).

The effects of increasing population, trade, migration and climate change place greater pressures on ecosystem services and may intensify future effects on biodiversity (UNEP, 2014). In addition, invasive pests and diseases such as Dutch elm disease (*Ophiostoma novo-ulmi*), red band needle blight (caused by *Dothistroma septosporum*) and ash dieback (*Hymenoscyphus fraxineus*) threaten UK woodlands (Woodland Trust, 2011; Defra, 2018). For example, *Fraxinus excelsior* has mortality rates of up to 85% recorded in Europe due to ash dieback (Coker et al., 2019), a species which in Great Britain accounts for 12% of broadleaf woodland with an estimated social and environmental value of £230 million a year (Defra, 2019). Threats to plant diversity are apparent on a global scale and require complementary efforts from both *in situ* and *ex situ* conservation practices. Seed banking, an *ex situ* option which may cost as little as 1% of *in situ* methods, is a space-efficient method to conserve genetic diversity for many seed-bearing species (Li and Pritchard, 2009). In addition, it has the advantage of removing material from the landscape, protecting it from threats.

The UK National Tree Seed Project (UKNTSP) was established in 2013 with the aim of developing an *ex situ* multi-provenance seed collection of UK native trees and shrubs. These collections aim to safeguard genetic diversity and provide a resource for conservation and research. Where possible, collections for each species are made in every Forestry Commission seed zone in which it naturally occurs. Furthermore, where possible the seed from individual mother trees are stored separately, allowing studies at the intra-specific level (Kallow and Trivedi, 2017; Trivedi and Kallow, 2017). These sampling strategies have likely captured 86% of the alleles found in native *Taxus baccata* populations (Gargiulo et al., 2019) and at least 90% of the alleles present in Great Britain for *F. excelsior* (Hoban et al., 2018).

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Recommendations for conventional seed storage, at −20°C after drying to 3–7% moisture content (MC) (fresh weight) (FAO, 2014; MSB, 2015; CPC, 2018), are based on essential concepts of seed and moisture relations, whereby the longevity of orthodox (desiccation-tolerant) seeds increases as temperature and humidity are decreased (Harrington, 1970; Roberts, 1973; Ellis and Roberts, 1980). Recalcitrant seeds are not suitable for conventional storage as they cannot survive enough desiccation to prevent ice crystallization during freezing (Roberts, 1973). Seeds with variable sensitivity to desiccation and cold temperatures are generally termed intermediate (Ellis et al., 1990; Walters, 2015a). Guidelines suggest that stored collections are checked for viability by germination tests shortly after banking and then retested at defined time intervals, usually every 10 years. For collections with unknown germination requirements, viability can be estimated using germination cut tests, with sample sizes considered according to the proportion of full seeds (i.e. with embryos) (Davies et al., 2015a; Hay and Whitehouse, 2017). Storage of short-lived seed collections in the vapour phase of liquid nitrogen storage (∼−186°C) is expected to further extend potential longevity (Walters et al., 2004). Choosing appropriate viability testing intervals is crucial for effective seed collection management. Monitoring should aim to detect viability loss before less than 85% of initial viability remains but should be as infrequent as possible to ensure that seed and staff resources are not wasted (FAO, 2014). This is of importance for wild collections, where losses in viability, and possibly genetic variation may not be recoverable by regeneration or recollection (Roos, 1982). However, the potential lifespan of a seed collection may not be known when decisions about duplication to cryo-storage and retest intervals are made.

Seed longevity in conventional storage conditions has been published for relatively few temperate tree species exceeding 10 years (Wang, 1974; Simpson et al., 2004; Pritchard et al., 2014). Seeds of *F. excelsior* from Poland can be stored for at least 10 years at −3 to −5°C and 7 to 10% MC (Suszka, 1987, cited in Chmielarz, 2009). Seeds of *Fagus sylvatica* from Denmark showed no significant decline in germination after 8 years of storage at −8°C and MCs of 4.7 and 8.4% (Poulsen and Knudsen, 1999), while others report low storage potential (Pukacka and Ratajczak, 2007). Variation in desiccation sensitivity in seeds of *F. sylvatica* is also widely reported, with some seed lots surviving desiccation to 4.75 and 4% MC (Poulsen, 1993; Poulsen and Knudsen, 1999; Chmielarz, 2007), while other seed lots are sensitive to desiccation below 10% MC (Gosling, 1991; Hong et al., 1998). In Canada, *Betula* and *Pinus* species stored below 9% MC at −20°C had germination percentages up to 85 and 87%, after 28 and 40 years storage, respectively (Simpson et al., 2004).

Accelerated ageing tests (at 45°C and 60% relative humidity (RH)) indicate which seed collections are likely to be comparatively short-lived (Hay and Probert, 2013; Hay and Whitehouse, 2017). Studies allow comparisons based on species, environment and seed traits including climate (Probert et al., 2009; Merritt et al., 2014), elevation (Mondoni et al., 2011; Satyanti et al., 2018), seed mass (Daws et al., 2006; Satyanti et al., 2018), seed maturity and acquisition of desiccation tolerance (Ali et al., 2007). Such studies ultimately reduce the cost of viability monitoring in the long term, as only when collections with the shortest longevity ranking from accelerated ageing start to show a decline, would the next lowest-ranked collections require testing. A modification to the ageing protocol has been developed, using fewer seeds, to screen for short-lived seed lots only (Davies et al., 2016). Mechanisms behind deteriorative repair in seeds may differ with temperature and moisture conditions, with many predictions yet to be confirmed by long-term storage data (Walters, 2015b). Given the absence of sufficient long-term data, we have used accelerated ageing as a reasonable indicator of comparative longevity among collections in long-term storage, as a basis for collection management decisions, including viability monitoring frequency.

The aim of this study was to support the effective management of *ex situ* seed collections of UK woody species by carrying out accelerated ageing tests on at least one seed collection from each of 22 UK native woody species. An additional aim was to investigate variation in estimated longevity between and within seed collections, including possible correlates.

Materials and methods

Species selection

Seeds from 22 UK native woody species were sampled from Millennium Seed Bank (MSB) collections (Table 1). All collections selected had at least 1500 filled seeds (ideally 1500 per tree sampled), verified taxonomy, and were from wild UK material (i.e. not regenerated). Collections were of high viability with known germination requirements (≥85% germination at the last monitoring test), except for *Aria margaretae* (81% germination) and *Juniperus communis* (83% germination). Prior to experimental sampling, collections were dried to equilibrium with 15% RH and 18°C and then stored at −20°C, except for *F. sylvatica* which was dried to 47% RH before storage at −18°C. Seeds of *F. sylvatica* were only partially dried to ensure minimal loss of viability due to desiccation prior to experimentation, as desiccation sensitivity is known to vary between seeds lots.

Thirty-nine samples from 32 collections in total were tested. All seeds used were collected by the UKNTSP between October 2013 and November 2016. For three species (*F. excelsior*, *Alnus glutinosa* and *Ulmus glabra*), multiple geographically separate collections were sampled (see Supplementary Table S1 for further details), and for four species (*Sambucus nigra*, *Carpinus betulus*, *Malus sylvestris* and *Sorbus aucuparia*), multiple trees within a collection were sampled.

Ageing protocol

The ageing protocol used followed the 150-seed comparative longevity protocol – a reduced seed number screening method for identifying short-lived seed conservation collections (Davies et al., 2016). Ageing experiments were carried out on seed samples between 2016 and 2018. Seeds were removed from storage and equilibrated in their storage containers for at least 24 h before opening. A proportional over-sow was calculated, where possible, to account for the varying presence of empty or insect-infested seeds. Where individual trees within one collection had less than 1500 full seeds, samples were taken proportionally across all the trees in that collection above 100 seeds in number (Table 1). Samples were rehydrated at 47% RH and 20°C for 14 days. The RH was controlled by dissolving anhydrous lithium chloride (LiCl, Laboratory Reagent Grade, Fisher Scientific UK Ltd., Leicestershire, UK) in distilled water. After rehydration, samples were transferred to 60% RH and 45°C (ageing conditions). Germination samples of 25 full seeds were taken at days 2 and 30; and 50 full seeds were taken at days 10 and 15. The RH generated by the LiCl solution was tested approximately every 2 weeks using a hygrometer sensor housed in an AW-DIO water activity

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Table 1. Details of the species used in the study.

Species	Family (-aceae)	Collections sampled	Trees sampled	Max p_{50} (days) ± SE	Range p_{50} (days)	Longevity category	Germination conditions	1000 Seed weight (g)	Endosperm present
Sambucus nigra	Adox-	1	3	11.3 ± 0.50	7	Medium	15°C, 112 days; 5°C, 175–186 days; 15/5°C	3.535	1
Alnus glutinosa	Betul-	3	3[a]	15.8 ± 1.51	1	Medium	25°C	1.469	1
Betula pendula	Betul-	1	1	23.0 ± 1.16		Medium	25°C	0.282	1
Betula pubescens	Betul-	1	1	23.5 ± 2.60		Medium	30°C	0.424	1
Carpinus betulus	Betul-	1	3	6.50 ± 1.02	3	Short	5°C	42.33	1
Lonicera periclymenum	Caprifol-	1	1	13.4 ± 0.64		Medium	5°C	4.990	1
Euonymus europaeus	Celastr-	1	1	4.30[b]		Short	15°C, 112 days; 5°C, 140 days; 15°C	41.00	1
Juniperus communis	Cupress-	1	1	31.8 ± 16.1		Medium	15°C, 112 days; 5°C, 140 days; 15°C, 112 days; 5°C	10.34	1
Fagus sylvatica	Fag-	1	1	<2.0[b]		Very short	5°C	287.3	1
Fraxinus excelsior	Ole-	7	7[a]	21.8 ± 1.83	14	Medium	15°C, 112 days; 5°C, 140 days; 15°C	54.25	1
Pinus sylvestris	Pin-	1	1[a]	12.9 ± 1.27		Medium	20°C	6.440	1
Aria arvonicola	Ros-	1	1	11.0 ± 0.68		Medium	5°C, 69–78 days; 10°C	15.52	0
Aria edulis	Ros-	1	1[a]	27.2 ± 2.11		Medium	5°C, 140 days; 10°C	15.79	0
Aria margaretae	Ros-	1	1[a]	7.80 ± 2.12		Short	5°C	16.61	0
Aria spectans	Ros-	1	1	27.9 ± 2.07		Medium	5°C, 141–154 days; 10°C	12.70	0
Hedlundia cuneifolia	Ros-	1	1	34.0 ± 30.8		Medium	5°C, 76–78 days; 10°C	11.38	0
Karpatiosorbus bristoliensis	Ros-	1	1	40.0 ± 9.51		Medium	5°C, 55–58 days; 10°C	9.347	0
Malus sylvestris	Ros-	1	2	26.2 ± 1.44	1	Medium	5°C, 140 days; 20/10°C	24.40	0
Sorbus aucuparia	Ros-	1	3	35.3 ± 5.34	8	Medium	5°C	3.405	0
Torminalis glaberrima	Ros-	1	1	15.9 ± 1.17		Medium	5°C	23.96	0
Acer campestre	Sapind-	1	1	11.0 ± 0.70		Medium	5°C	88.60	1
Ulmus glabra	Ulm-	3	3	3.60 ± 1.57	3	Short	20°C	6.416	1

Taxonomy follows WCVP (2020). Seed trait data are presented as mean 1000 seed weight (g) and endosperm presence (present = 1, not present = 0). Species are ranked by the maximum (Max) p_{50} ± standard error (SE) and assigned to approximate longevity categories of very short, short or medium as appropriate. All tests were set on 1% agar with a 12/12 h light/dark cycle and alternating temperatures coinciding with a warmer temperature in the light period. For *E. europaeus* and *F. excelsior*, covering structures were removed prior to placing seeds in germination conditions. For *H. cuneifolia* and *K. bristoliensis*, the seed coat was removed in the final step.

[a]Sampled from multiple trees within a collection, including two collections of *A. glutinosa* and one collection of *F. excelsior*.

[b]Too few data points for reliable probit analysis.

probe, used in conjunction with a HygroPalm 3 display unit (Rotronic Instruments UK Ltd., Crawley, UK) and remained within 2% of the desired RH. The germination conditions used (Table 1) followed standard MSB procedures (Davies et al., 2015a,b). Cut tests were performed on ungerminated seeds at the end of the test to confirm they were not viable. Empty and infested seeds were excluded from germination percentage calculations.

Probit analysis

Probit analysis was carried out following the methodology of Davies et al. (2016). Cumulative germination percentages were plotted against germination time and sigmoidal seed survival curves were fitted by Probit analysis using Genstat for Windows, Version 14.2 (VSN International Ltd., Hemel Hempstead, UK), following Roberts (1973) and using the improved viability equation (Ellis and Roberts, 1980):

$$v = K_i - \left(\frac{p}{\sigma}\right)$$

where v is the viability (normal equivalent deviates, NED) of seeds after p days in storage; K_i is a measure of the initial viability (NED) and σ is the time for viability to decline by 1 NED. Probit analysis was also used to estimate time for viability to fall to 50% (p_{50}) (Hay et al., 2014). Zero germination values subsequent to the first zero recorded in ageing tests were excluded from the probit analysis. Data for the first sampling point were excluded where germination increased by more than 10% at the second sampling point.

Species were ranked by p_{50} and assigned to the notional longevity categories of very short-lived ($p_{50} \leq 2$ days), short-lived ($p_{50} > 2$ and ≤ 10 days) and medium ($p_{50} > 10$ and ≤ 100 days). Categories were based on Mondoni et al. (2011), except very short was adjusted from $p_{50} < 1$ to <2 days based on current sampling procedure and ability to make predictions of <2 days. Where p_{50} could not be calculated due to all viability being lost before 10 days, p_{50} was reported as <2 days for tables and a nominal value of 1 day was used for linear and regression analysis. Analysis of deviance was used to compare seed survival curves and an F-test used to test for significance when constraining survival curve data for multiple seed lots to a common intercept and slope (K_i and σ, respectively). Residual deviance was tested at tree, collection, genus and family level, where suitable samples were available for comparison. Samples which could be constrained to a common slope are presented (as single lines) in (Fig. 1).

Determination of seed characteristics

The weight of air-dried (15% RH at 18°C) seeds was calculated by weighing 5 lots of 50 seeds for each collection in the study. The presence or absence of endosperm was assigned according to Martin (1946) classifications. Species within the Rosaceae family (*Malus* and *Sorbus*), which have only a very thin lining of fleshy endosperm, are included as non-endospermic.

All statistical analysis comparing p_{50} to seed characteristics was undertaken in the R statistical platform using ANOVA, T-tests and linear regression as appropriate (R Core Team, 2019).

Conventional storage data

Conventional storage data from seed conservation collections were extracted for 16 species which matched our study species either at the species or genus level (Table 3). The collections presented are independent of those experimentally tested above and were selected based on the length of time they had been banked at the MSB (ideally longer than 10 years), which ranged from 8.5 to 28.2 years. All collections were dried to equilibrium with 15% RH and 15–18°C before storage at −20°C according to standard procedures at the time. A significant decline in viability was monitored in conventional storage by comparing the initial (highest) germination result with the most recent retest.

The value of normal deviate Z was calculated using a two-tailed test (Ellis and Roberts, 1985), where a correction factor is applied to enable the normal distribution to be used in the analysis:

$$Z = \frac{(p_1 - p_2)}{\sqrt{\bar{p}\,(100 - \bar{p})\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

where p_1 is $((100 \times g_1)/(n_1 - 0.5))$; p_2 is $((100 \times 2)/(n_2 + 0.5))$; $\bar{p}$ is the mean value of p_1 and p_2; g_1 and g_2 are the numbers of germinated seeds; and n_1 and n_2 are the numbers of full seeds sown (where $n > 19$) for the initial and most recent retest, respectively. The decline was considered significant ($P < 0.05$), where $Z > 1.64$ and the lowest result <95%. Where possible, storage data were analysed by probit analysis (to calculate p_{50} in years).

Plant health

The material of *F. excelsior* was held under a Defra plant health licence and handled according to Defra plant health guidelines.

Results

Viability and p_{50} summary

Survival curves were fitted (where data allowed), for 20 of the 22 species (Table 1 and Fig. 1). Based on p_{50} estimates, one species (*F. sylvatica*) was ranked as very short-lived, four species (*U. glabra*, *Euonymus europaeus*, *C. betulus* and *A. margaretae*) as short-lived and 17 species as medium longevity (Table 1). No collections were ranked above medium longevity. Calculated initial viability (K_i) was above 1.04 NED (85% germination) for 30 samples studied. Low initial viability was indicated in eight tree samples from six species (including *F. sylvatica*, *U. glabra* (x3), *A. margaretae* and *F. excelsior* (x2)). These are considered to have undergone ageing before sampling day 2. The slope (σ) ranged from less than −0.19, for *S. nigra*, *C. betula* and *Acer campestre*, to greater than −0.06, for *Karpatiosorbus bristoliensis* and *J. communis*.

Samples from three species, *F. sylvatica*, *U. glabra* (two collections) and *E. europaeus*, had only two points available for model fitting, with zero germination recorded at the second sampling point. An increase in germination between the 2- and 10-day samples was observed for six samples, and consequently, the 2-day sample was excluded for *Betula pubescens* and *J. communis*, which showed the largest increase in germination (>10%). Considering the limited degrees of freedom, data were not excluded for *Torminalis glaberrima* & *Aria edulis*, *A. glutinosa* and *Betula pendula*, where the increase was relatively minor and the difference in p_{50} estimates would be small (<1 day). The calculated p_{50} for *Hedlundia cuneifolia* may also be slightly underestimated, due to the increase in germination between the 10- and

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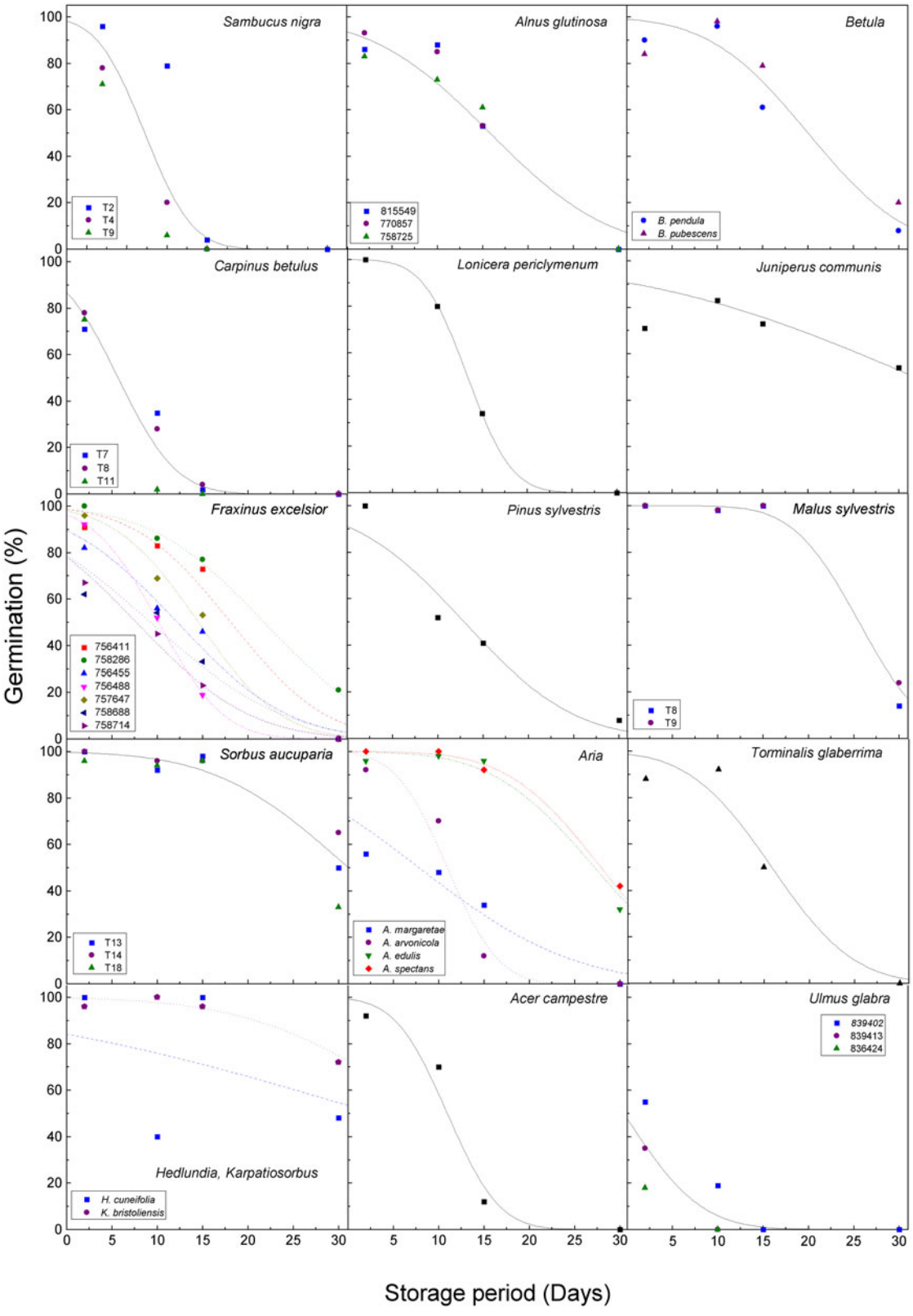


Fig. 1. Survival curves fitted by probit analysis for seed lots (aged at 45°C and 60% RH). Common slopes are presented (as single lines) where possible (Table 2). Symbols represent individual trees (*T*), collections or species. For details on collection locations for *A. glutinosa*, *F. excelsior* and *U. glabra*, see collection numbers in Supplementary Table 1.

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15-day samples. Despite this, the calculated p_{50} remains one of the highest in the study, though the standard error is very high (34.0 ± 30.8 days).

Slope comparisons and F-tests

Individual tree samples within collections were investigated for 11 samples from four species. No significant difference in residual deviance was found at a tree level, allowing common slopes to be fitted for all collections investigated (Table 2 and Fig. 1). Estimates of p_{50} differed by 1 and 2 days for *M. sylvestris* (two trees) and *C. betulus* (three trees) and to 7 and 8 days for *S. nigra* (three trees) and *S. aucuparia* (three trees), respectively.

Common slopes were determined for multiple collections of two species, *U. glabra* (three collections) and *A. glutinosa* (three collections) (Table 2 and Fig. 1) but could not be determined for *F. excelsior* (seven collections), which differed in p_{50} by 14 days ($F_{12,14} = 7.996$) (Table 2 and Fig. 1). *F. excelsior* slopes remained significantly different when K_i's below 85% (1.04 NED) were excluded (four collections) ($F_{6,8} = 8.9$).

At a genus level, slopes of four species of *Aria* were found to differ significantly, with a range in p_{50} of ~20 days. p_{50} ranged from 8 to 11 days for *A. margaretae* and *Aria arvonicola* and to 28 days for *A. edulis* and *Aria spectans* ($F_{6,8} = 8.9$) (Table 2 and Fig. 1). Both species of *Betula* could be constrained to a common slope and are plotted as a single line in Fig. 1.

Within Betulaceae, *Alnus* and *Betula* samples could be constrained to a common slope, with a combined p_{50} of 20 days. However, *Carpinus* could not, with a significantly shorter p_{50} of 6 days ($F_{14,14} = 6.247$) (Table 2 and Fig. 1). Rosaceae was not compared at a family level as collections varied significantly at a genus level.

Geographical location and seed characteristics

No significant correlation was found between *F. excelsior* longevity and geographical location of seed collections (see Supplementary Table S1 for locations) using a simple linear regression ($F_{1,5} = 1.988$, $P = 0.2176$ by latitude; $P = 0.9371$ by longitude), though a trend towards lower p_{50} estimates as the latitudinal gradient increased was observed ($R^2 = 0.2845$ by latitude).

A simple linear regression showed seed mass (1000 seed weight) did not correlate with longevity ($F_{1,30} = 2.2712$, $P = 0.1423$, $\log_{10}$), though *F. sylvatica*, which had the smallest p_{50} estimate (<2 days) also had the largest 1000 seed weight of 287 g. A two sample *T*-test showed that endospermic seeds were significantly shorter-lived than non-endospermic seeds ($t_{30} = -2.5332$, $P = 0.0168$) (Fig. 2, $\log_{10}$ scale).

Conventional storage data

Germination performance of older collections held in long-term storage of the same species (or genus for *Aria*) as those in the study is presented for 16 species of UK native trees (Table 3). *S. nigra*, *A. campestre*, *H. cuneifolia* and *K. bristoliensis* are not represented due to insufficient data or collections of suitable age. Retests were conducted approximately every 10 years with limited test sizes (9–55 seeds) and a maximum of four test intervals. Initial germination was at least 85% for all but *J. communis*, *Fagus sylvatica* and *Pinus sylvestris,* with 62, 80 and 60%, respectively. Significant germination decline was detected (by *Z*-test) for three species: *B. pubescens*, *F. sylvatica* and *U. glabra*. *Fagus sylvatica* has a calculated p_{50} of 13 years under conventional storage conditions. Reliable estimates of p_{50} cannot yet be calculated for the remaining species due to insufficient decline in the collections to date.

Discussion

In this study, we carried out accelerated ageing experiments and fitted survival curves (where possible) for 22 species of UK native trees and shrubs representing 18 genera and 11 families (Table 1 and Fig. 1). Estimates of p_{50} showed that of all species studied, one could be classified as very short-lived, four as short-lived and 17 as medium, with none exceeding the medium category. This range in p_{50} is comparable to that found by Mondoni et al. (2011) for alpine species (4.7–37.5 days), suggesting that the UK woody species studied also have seeds of short to medium longevity in air-dry storage. Interestingly, it is generally predicted that species from cool wet environments will be more likely to have short-lived seeds (Probert et al., 2009), to which our results add support. For those species with the shortest longevity predictions, such as *F. sylvatica* and *U. glabra*, we recommend retest intervals at least every 5 years and recollection where necessary as well as further research into alternative storage methods such as cryo-storage. Successful cryo-storage has already been reported for seeds of a number of temperate tree species, including *A. glutinosa* (Chmielarz, 2010a), *C. betulus* (Chmielarz, 2010b), *B. pendula* (Chmielarz, 2010c), *U. glabra* (Chmielarz, 2010d), *F. excelsior* (Chmielarz, 2009), *M. sylvestris* (Michalak et al., 2015) and seeds and embryonic axes of *F. sylvatica* (Chmielarz, 2007; Nuc et al., 2016).

A known restriction of the reduced seed number longevity protocol is having too few data points for probit analysis or reduced accuracy due to fewer sampling points (Davies et al, 2016). This was the case for samples from three species (*F. sylvatica*, *U. glabra* and *E. europaeus*) where rapid viability decline resulted in only two data points. Although such examples will lead to an increase in standard errors and a reduction in accuracy of the fitted survival curve, comparative ranking into approximate longevity categories and appropriate management decisions could be implemented. Caution is advised however when comparing species data based on relatively few samples, such as for *S. nigra*.

Across previous studies, multiple factors have been shown to influence, or correlate with, estimated longevity of seeds, with the potential to be used as predictors of longevity in storage. These include seed mass, endosperm presence/absence, maternal environment, habitat type, dormancy mechanisms and seed oil content (Martin, 1946; Hong et al., 1998; Walters et al., 2005; Probert et al., 2009; Kochanek et al., 2010; Long et al., 2015). In this study, we found no evidence of a correlation between longevity and seed mass; however, we did find that those species which had no (or very thin) endosperm generally had a higher p_{50} estimate (i.e. greater potential longevity) (Fig. 2). Both Merritt et al. (2014) and Probert et al. (2009) found similar patterns across the Australian and global species sets studied, respectively, although Mondoni et al. (2011) found no relationship between endosperm presence and estimated longevity in Italian alpine species. The exact physiological reason for this relationship is uncertain, and it has been suggested that it may link with embryo size rather than endosperm presence/absence *per se* (Probert et al., 2009). It should also be noted that within this study, those species with no endosperm are predominantly Rosaceae species, specifically those which were formerly within *Sorbus* (Sennikov and

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Table 2. Comparison of fitted slopes

Comparison	Species name	Number of samples	Degrees of freedom (N, D)	*F*	Significant ($P < 0.05$)	$p_{50} \pm$ SE
Tree level	*Sambucus nigra*	*3*	*4, 4*	5.055	NS	7.3 ± 0.50
	Carpinus betulus	*3*	*4, 5*	5.019	NS	5.7 ± 0.50
	Malus sylvestris	*2*	*2, 4*	0.113	NS	25.6 ± 1.0
	Sorbus aucuparia	*3*	*4, 6*	0.548	NS	31.1 ± 1.8
Species level	*Fraxinus excelsior*	*7*	*12, 14*	7.996	Sig.	13.4 ± 0.50
	Ulmus glabra	*3*	*4, 2*	3.340	NS	−0.4 ± 1.50
	Alnus glutinosa	*3*	*4, 6*	5.055	NS	15.9 ± 0.70
Genus level	*Aria* spp.	*4*	*6, 8*	8.900	Sig.	18.6 ± 0.90
	Betula pendula and *pubescens*	*2*	*2, 3*	1.495	NS	20.0 ± 0.78
Family level	*BETULACEAE – Alnus*, *Betula*	*5*	*8, 9*	0.734	NS	18.3 ± 0.55
	BETULACEAE – Alnus, *Betula*, *Carpinus*	*8*	*14, 14*	6.247	Sig.	13.6 ± 0.41

An approximate *F*-test (*F*-values shown) was used to test for a significant (Sig.) increase ($p < 0.05$) in residual deviance when survival curves were constrained to a common slope at a tree, species, genus or family level. Common slopes are presented (as single lines) where not significant (NS), as shown in Fig. 1.

Table 3. Details of collections held in long-term storage of the same species (or genus for *Aria* sp.) as experimental collections in the study

Serial No.	Species	Geographical location (UK)	Date collected	Initial germination (%)	Last germination (%)	Age (years)	*Z*	p_{50} years
123774	*Alnus glutinosa*	England:South Yorkshire	14-Oct-97	98	98	19.7		
86446	*Betula pendula*	England:Dorset	10-Sep-90	93	92	27.9		
88266	*Betula pubescens*	Scotland:Highlands	06-Nov-90	93	80	24.0	*1.8*	
87214	*Carpinus betulus*	England:West Sussex	10-Oct-90	100	84	27.2	[a]	
87465	*Lonicera periclymenum*	England:Shropshire	17-Sep-90	98	98	27.2		
408042	*Euonymus europaeus*	England: Oxfordshire	17-Oct-07	86	86	8.50		
130682	*Juniperus communis*	England:Lancashire	18-Sep-98	62	62	19.1		
87225	*Fagus sylvatica*	England:East Sussex	10-Oct-90	80	12	25.8	*4.96*	13.2 ± 2.01
65148	*Fraxinus excelsior*	England:Greater London	12-Nov-86	96	96	21.8		
126498	*Pinus sylvestris*	Scotland:Highlands	01-Jan-98	69	60	19.5	[a]	
97318	*Aria lancastriensis*	England:Cumbria	06-Sep-92	100	100	25.0		
131885	*Aria rupicola*	Northern Ireland: Londonderry	17-Oct-98	100	98	19.9		
130659	*Malus sylvestris*	England:Cheshire	20-Sep-98	100	100	19.0		
76618	*Sorbus aucuparia*	England:Devon	14-Sep-89	100	97	28.2		
123752	*Torminalis glaberrima*	England:Greater London	20-Oct-97	87	87	20.1		
126605	*Ulmus glabra*	England:Lancashire	26-May-98	96	72	20.3	*2.64*	

Species are ordered alphabetically by family and species, as shown in Table 1 and Fig. 1. Initial germination percentage (%) represents the highest result for each collection. Age (in years) is calculated from the date of storage to the date of the last test. Significant germination decline is indicated by $Z > 1.64$ ($P < 0.05$).
[a]Data not suitable for *Z*-test ($n < 20$ sown in initial test).

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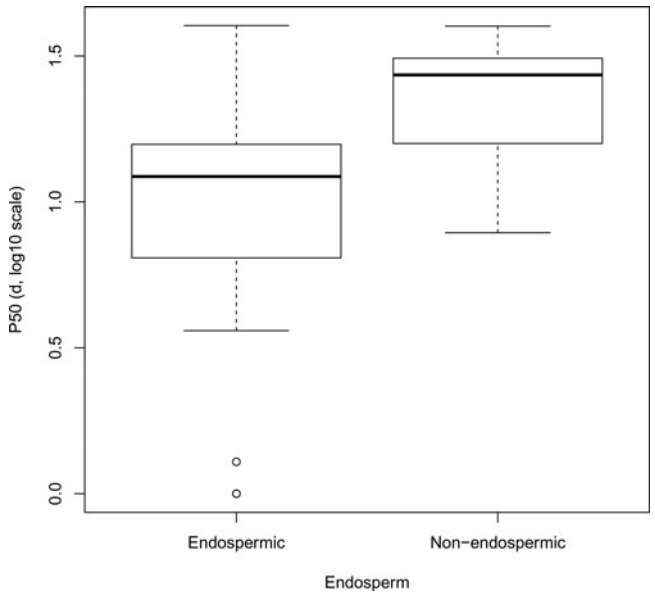


Fig. 2. Box plots comparing p_{50} for endospermic and non-endospermic seeds.

Kurtto, 2017), so it does not represent a taxonomically diverse sub-sample.

In this study, we found no evidence for variation in longevity estimates between individual trees from the same populations for the four species studied (Table 2). Previous studies have found that factors such as different maternal environments (Kochanek et al., 2010), seed maturity and post-harvest handling can influence longevity (Hay and Probert, 1995; Hay et al., 2006; Ali et al., 2007; Kalemba et al., 2009) and so could cause differences between individuals within a population. These impacts were likely minimized for collections in this study, as seed collections for individuals within each population were made on the same day and processed in the same manner. In addition, geolocation data for the maternal trees sampled within each population indicated that the maximum linear distance between any two individuals from the same population was 465 m (183 m average) which is well within both insect (e.g. Oddou-Muratorio et al., 2005) and wind pollination distances (Robledo-Arnuncio and Gill, 2005) and likely within the same patch of woodland subject to the same environmental variations. Moreover, evidence from genetic studies into UK woody species has generally found little genetic variation within populations (e.g. Logan et al., 2015; Gargiulo et al., 2019) which could be a factor. However, the sample sizes tested per population here are small (2–3).

At the population level, differences in estimated longevity were not found for *U. glabra* or *A. glutinosa* but were found between the seven *F. excelsior* populations tested (Table 2), with collections in both short and medium longevity categories. Previous accelerated ageing studies have also found evidence of variation between populations in expected seed longevities, for example, Mondoni et al. (2011) reported significant differences in estimated longevity between different populations of the same species collected from different altitude zones. However, the geographical factors investigated here could not explain the differences in predicted longevity between *Fraxinus* populations nor could excluding those collections with low initial viability. Despite this, there was a general trend for a reduction in p_{50} as the latitude of collection increased over the 5.6 degrees of latitude studied. The variation observed could also be due to different maternal environments or environmental influences beyond those investigated here. For example, embryo maturity at the point of collection may also influence storage potential of *F. excelsior* seeds (see Chmielarz, 2009) particularly given that the seeds are classified as having morphophysiological dormancy (Wager, 1996). Moreover, genetic evidence suggests that although generally there is little differentiation in neutral genetic diversity among British *F. excelsior* populations, there are unique haplotypes in Scotland and a gradient of allelic diversity from western Scotland to eastern England (Sutherland et al., 2010); thus, genetic variation could have an influence. It is possible that similarities in estimated longevity between the *U. glabra* and *A. glutinosa* populations were due to limited geographic spread. For example, the three *U. glabra* populations spanned only 2.4 degrees (decimal) of latitude across southern England into the Midlands where conditions are relatively similar. Thus, it is possible that if populations of *U. glabra* and *A. glutinosa* from further north and west were to be tested then differences in longevity may be evident.

From a taxonomic perspective, within this study, we found variation between genera in species estimated longevity patterns. For example, both species of *Betula* (*B. pendula* and *B. pubescens*) had similar p_{50} estimates and could be fitted to a common slope, whereas the four species within the genus *Aria* could not, placing them into different estimated longevity categories (Tables 1 and 2). *Aria* along with *Karpatiosorbus* and *Hedlundia* were all formally subgenera within *Sorbus*, which have recently been raised to the genus rank (Sennikov and Kurtto, 2017). The whole group are interesting taxonomically in that speciation is believed to be currently ongoing, driven by apomixis as well as hybridization (Hamston et al., 2018). The three ancestral species, *S. aucuparia*, *A. edulis* and *T. glaberrima* all ranked within the medium longevity category but varied in estimated p_{50} from 15.9 to 35.3. The taxonomic relationships between the other species tested are unclear (Hamston et al., 2018), so at this time, it is not possible to speculate as to taxonomic based trends in p_{50} within this complex.

In order to validate some initial conclusions from this study, longevity predictions were compared to the actual performance of collections held in storage, of which all except *E. europaeus* were banked under conventional storage conditions prior to 2000 (Table 3). Storage data show that many of the species studied can survive without significant viability decline for at least 20 years (Table 3). Collections showing decline after 20 years of storage include *F. sylvatica* and *U. glabra* which were also the two shortest-lived species as predicted by accelerated ageing tests in this study. *U. glabra*, which has declined from 100 to 72% after 20 years of conventional storage, is reported as short-lived (Hong et al., 1998). Seeds of *F. sylvatica* are known to have low storage potential, which is correlated with an increase in the presence of reactive oxygen species and lipid hydroxyperoxides, indicative of damage to the membranes within the seeds (Pukacka et al., 2003; Pukacka and Ratajczak, 2007). Seed maturity, locality and post-harvest handling are likely key factors for maximizing potential longevity for this species (Chmielarz, 2007; Kalemba et al., 2009).

Two species of *Betula* (*B. pendula* and *B. pubescens*) were ranked as medium longevity in the accelerated ageing study (Table 1), while under conventional conditions only *B. pubescens* is showing decline (from 93 to 80% germination after 24 years), with *B. pendula* remaining high in viability (92% germination) after nearly 28 years (Table 3). Within the literature, *Betula* (and *Alnus*) species are considered short-lived (Hong et al., 1998), although

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conventional storage data from Canadian tree seeds, including *Betula* species show good survival for up to 40 years (Simpson et al., 2004). Other species ranked as short-lived in this ageing study, including *E. europaeus* and *C. betulus*, are currently retaining high viability after 8.5 and 27.2 years in conventional storage, respectively. Additional monitoring is advisable for *E. europaeus* and *C. betulus*, which are ranked as short-lived under accelerated ageing conditions. A recent review by Colville and Pritchard (2019) found that for five species which had had both artificial ageing experiments and testing in cold storage, there was no difference in the comparative ranking of life span. However, evidence from Nagel et al. (2019) studying wheat genotypes suggests the mechanisms of seed ageing differ under conventional and accelerated ageing situations. Our results, although limited by the potential variation between collections, suggest that accelerated ageing can be a useful method, along with other seed traits, to identify species whose seeds are likely to be relatively short-lived in storage.

Given these findings, while *ex situ* seed collections form an important resource for conservation and scientific research, particularly given the plant health threats currently facing our woody flora, it does highlight the need to focus on multiple complementary conservation initiatives. For the UK woody flora, this should include other *ex situ* methods such as living collections and clone banks, alongside *in situ* methods such as gene conservation units, as recently highlighted in the Strategy for UK Forest Genetic Resources (Trivedi et al., 2019). This paper also supports the value of accelerated ageing tests for aiding decisions in appropriate monitoring and handling of seed collections in long-term *ex situ* collections. Increased efforts to understand the mechanisms behind seed ageing will also help to optimize storage procedures for all species (Walters et al., 2010; Ballesteros and Walters, 2011). It is important that current knowledge and assumptions are continuously tested, and data are made available to others.

Supplementary material. To view supplementary material for this article, please visit: https://doi.org/10.1017/S0960258520000215.

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