Radicle emergence could overestimate the prediction of seed longevity in wild plants

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Abstract

Seed longevity influences the success of *ex situ* storage and preservation of plant genetic diversity and is thus a critical factor in conservation efforts. Rapid seed ageing experiments at high temperature and high humidity have been widely used to classify seed longevity for hundreds of plant species, with potential implications for longevity in *ex situ* conservation. In this approach, radicle emergence (R) is normally used as a measure of the viability of the seeds. However, R could overestimate the level of normal seedling development and, consequently, the perceived longevity of seeds. Here, seed lifespan for 33 alpine species was compared to assess whether germination criteria could affect seed longevity parameters. Seeds were exposed to controlled ageing [45°C, 60% relative humidity (RH)] and regularly sampled for germination assessment as both radicle emergence (R) and radicle plus cotyledon emergence (R + C). The time taken in storage for viability to fall to 50% (p_{50}) was determined using probit analysis, including either R or R + C data. A coefficient of overestimation of seed longevity (OESL, %) was determined. The results highlight significant differences in seed longevity estimates both across species and the germination criteria. For 17 species, seed longevity estimated by R was significantly higher than that estimated using R + C, resulting in large variation in OESL (0.54–9.01 d). The introduction of OESL facilitates effective screening for seed longevity and recovery, enhancing the overall efficiency of conservation strategies for diverse species.

Introduction

During development, orthodox seeds acquire a set of physiological traits of significance to survival and regeneration: the ability to germinate before and after drying and the capacity to survive long periods of air-dry storage (Bewley et al., 2013; Waterworth et al., 2015). However, seed deterioration begins soon after seed dispersal and then during storage. Over time, viability loss is inevitable (Bewley et al., 2013), even under conditions recommended for genebanks, likely as a result of cumulative oxidative stress (Kranner et al., 2006). Uncertainty in how long a seed lot of a species might survive in storage makes it critical to periodically monitor viability during storage (FAO, 2014). It is also helpful to understand inter- and intra-specific differences in seed longevity and to potentially customise monitoring intervals so that seed lots expected to lose viability sooner are tested before those expected to remain viable for longer (Hay and Whitehouse, 2017). To assess relative seed longevity within a reasonable time frame (i.e. weeks to months), samples of seeds are aged under standard, relatively high moisture and temperature conditions (Hay et al., 2019). This has been used to rank relative seed longevity of hundreds of species (Probert et al., 2009; Mondoni et al., 2011; White et al., 2023) and hundreds of accessions destined for genebank storage (Lee et al., 2019).

As seeds age in storage, their ability to produce normal healthy seedlings may decline before the reduction in radicle emergence (Rao et al., 1987; Bradford et al., 1993; Bewley et al., 2013). Therefore, relying solely on radicle protrusion as an estimate of survival after storage might overestimate the seeds' ability to produce a normal healthy seedling (Ellis and Roberts, 1981). According to the guidelines provided by the International Seed Testing Association (ISTA), normal germination, which refers to a seed's ability to develop into a satisfactory plant (ISTA, 2018), is considered the most reliable and recommended test of seed quality over radicle emergence. This principle is also supported by findings in some wild plants. For example, in *Delphinium* and *Salvia* seeds, cotyledon following by radicle emergence was identified as a better indicator of seed viability after storage (Kwong et al., 2001), as well as in *Salix* seeds after various storage treatments, including cryopreservation (Popova et al., 2013). However, in most, if not all, seed longevity studies on wild plants, radicle emergence has been the main criterion used to determine a seed's viability (see e.g. Long et al., 2008;

Kochanek et al., 2009; Probert et al., 2009; Mondoni et al., 2011; Merritt et al., 2014; Bernareggi et al., 2015; Seglias, 2022; Sommerville et al., 2023; White et al., 2023). In this context, germination studies on wild plant species do not adhere to the guidelines on crop species. Different standards have also been applied in other studies, such as those from the European Native Seed Conservation Network (ENSCONET, 2009), the Australia Flora Bank Guidelines (FloraBank, 1999) and the US Seeds of Success program (Bureau of Land Management, 2018). The reasons for having different standards for native seeds as opposed to crops are extensively discussed by Pedrini and Dixon (2020). It is unquestionable that checking normal seedling development is a valuable means of assessing post-storage viability as it is a better indicator of potential seedling establishment. But it is not always feasible to do this for wild plants, in part because what is considered a 'normal seedling' that is able to grow into a healthy plant has not been documented for many wild species. The attrition, or loss, of individuals as germination progresses from radicle emergence to early seedling emergence (i.e. radicle and cotyledon) has the potential to impact the interpretation of seed viability, depending on which parameter is measured. Indeed, there is the possibility of overestimating seed viability when using radicle emergence alone. The extent of this problem may depend on the species and seed age (Ellis and Roberts, 1981; Tarquis and Bradford, 1992; Popova et al., 2013). Of particular concern is any overestimation of seed longevity (OESL) for the most vulnerable plant species, including those already identified as producing short-lived seeds in storage, for example, species from cool wet climates (Probert et al., 2009) or, more specifically, understorey herbs and alpine species (Ali et al., 2007; Mondoni et al., 2011). In this study, we examine the longevity of seeds from 33 alpine species (including two subspecies of one species, *Silene vulgaris*) under standard storage conditions, evaluating both radicle emergence (R) and radicle plus cotyledon emergence (R + C) to provide insights into the potential OESL.

Material and methods

Studied species and seed collection

The studied species inhabit two of the Natura 2000 habitat types: '6230 – Nardus-rich species grasslands', formed on various types of siliceous rocks with acidic pH and '6170 – Alpine and subalpine calcareous grasslands', formed on calcareous bedrocks with alkaline pH (92/43/CEE 'Habitat' Directive classification) (European Commission, 2007).

Fresh mature seeds of the target species were collected based on their occurrence and abundance in these two habitats at the time of natural seed dispersal at the Stelvio National Park (close to Ubraill pass, 46°32′30.1″N, 10°25′46.1″E; 2,565 m a.s.l.) and in Dosdè Valley (46°25′06.5″N, 10°11′59.8″E; 2,225 m a.s.l.) (Sondrio Province, Italy) during August 2015 following the ENSCONET (2009) guidelines for responsible seed collection practices. After collection, seeds were held in the drying room [15% relative humidity (RH), 15°C] at the Plant Germplasm Bank of the University of Pavia (Italy) for 1–2 months until the commencement of the experiments. Seed viability of all species was determined through standard germination testing and 33 species (14 families; Table 1) having highly viable seeds were selected for this study. Seed mass (average seed weight of three replicates of 50 seeds, converted to 1000 seed weight) of each species was

measured and seed morphology type of each species (at least to the genus level) was derived from Martin (1946) (Table 1).

Experimental storage

Seed longevity was determined using a standard rapid ageing protocol (Newton et al., 2009). Samples of seeds from each species were removed from the drying room after 1–2 months and equilibrated to the laboratory environment over 5 d, monitored using a data logger (approximately 20°C; 50% RH, TinyTag DataLogger). Thereafter, seeds of each species were placed separately inside a Petri dish as a single seed layer and sealed in a $300 \times 300 \times 130$ mm electrical enclosure box (Ensto UK Ltd, Southampton, UK), over a non-saturated solution of LiCl (anhydrous, Laboratory Reagent Grade, Fisher Scientific UK Ltd.) at 60% RH (Hay et al., 2008) and placed in an oven (Binder FD53, Tuttlingen, Germany) in the dark at 45°C. One sample of 60 seeds of each species was retrieved from the large sample of seeds in the electrical enclosure box after 0, 1, 3, 5, 7, 10, 15, 20, 30, 40 and 50 d for germination testing. For each species, the seeds were sown as three subsamples of 20 seeds on 1% agar in distilled water containing 0.00072 mol L^{-1} GA$_3$ (Sigma-Aldrich Company Ltd., Dorset, UK) in 55 mm-diameter Petri dishes and incubated at 25°C light (12 h) and 15°C dark (12 h) in a LMS 250A cooled incubator (LMS Ltd., Sevenoaks, UK). Radicle emergence (R) of 1–2 mm in length and subsequent fully opened cotyledonary leaf emergence (R + C) were recorded separately every 5 d for 40 or 70 d after sowing, depending on species and seed quality. Excess water accumulating on the Petri dish lids during the incubation period was removed in order to minimize the risk of damping off.

Data analysis

Probit analysis was performed using the FITNONLINEAR directive in GenStat 19th Edition (VSN International Ltd., Hemel Hempstead, UK) to simultaneously fit the seed viability equation to the data for both the number of seeds showing radicle emergence (R) and the number of seeds going on to produce fully open cotyledons (R + C):

$$v = K_i - (p/\sigma) \qquad (1)$$

where v is the viability according to R or R + C (in normal equivalent deviates, NED) of the seed lot after p days in storage, K_i is the initial viability (NED) of the seed lot and σ is the time (d) for viability to fall by 1 NED (i.e. the standard deviation of the normal distribution of seed deaths over time) (Ellis and Roberts, 1980). In the same analysis, the effects of constraining K_i and/or σ to the same value for the different germination criteria on the residual deviance were compared using approximate F-tests. In addition, estimates were made of the time for viability to fall to 75 or 50% (p_{75} and p_{50}, respectively), again using the two different criteria. The p_{75} was estimated as it is the viability standard for the seed bank at the Department of Earth and Environmental Sciences, University of Pavia; when the viability falls below 75%, recollection of the species/population will be considered, according to conservation priorities.

When it was not possible to constrain the parameters of equation (1) (K_i and σ) to the same values for the two germination criteria (R or R + C) due to a significant increase in residual

Table 1. Details of the species used in the study and seed characteristics, together with the accepted model when comparing the post-storage germination survival curves (equation 1) when the germination criterion was radicle emergence (R) or radicle and cotyledon emergence (R + C)

Plant species	Thousand seed weight (g)	Seed type	Accepted model
Asteraceae			
Antennaria dioica (L.) Gaertn.	0.78	Spatulate	One line
Arnica montana L.	1.52	Spatulate	One line
Hieracium pilosella L.	0.80	Spatulate	Common slope
Leontopodium alpinum Cass.	1.72	Spatulate	One line
Leucanthemopsis alpina (L.) Heywood	0.42	Spatulate	One line
Senecio doronicum (L.) L.	2.84	Spatulate	One line
Solidago virgaurea L.	0.66	Spatulate	Common slope
Campanulaceae			
Campanula barbata L.	0.06	Dwarf	One line
Campanula cochleariifolia Lam.	0.06	Dwarf	Common intercept
Campanula scheuchzeri Vill.	0.06	Dwarf	One line
Phyteuma hemisphaericum L.	0.10	Dwarf	Common slope
Phyteuma orbiculare L.	0.18	Dwarf	Common slope
Caryophyllaceae			
Silene acaulis (L.) Jacq.	0.28	Peripheral	One line
Silene rupestris L.	0.08	Peripheral	Common slope
Silene vulgaris (Moench) Garcke subsp. *glareosa* (Jord.) Marsden-Jones and Turrill	0.90	Peripheral	Common slope
Silene vulgaris subsp. *vulgaris*		Peripheral	Common slope
Gentianaceae			
Gentiana acaulis L.	0.34	Dwarf	Common slope
Gentiana verna L.	0.40	Dwarf	Common slope
Gentianella anisodonta Borbás.	0.14	Dwarf	Common slope
Juncaceae			
Luzula alpino-pilosa (Chaix) Breistr.	1.37	Basal	One line
Onagraceae			
Epilobium fleischeri Hochst.	0.77	Spatulate	Common slope
Orobanchaceae			
Bartsia alpina L.	0.28	Dwarf	One line
Plantaginaceae			
Veronica bellidioides L.	0.11	Dwarf	Common intercept
Poaceae			
Festuca halleri All. subsp. *halleri*	0.34	Basal	One line
Festuca luedii (Markgr.-Dann.) Foggi, Gr. Rossi, Parolo and Wallossek	1.89	Basal	One line
Festuca quadriflora Honck.	0.60	Basal	One line
Helictotrichon versicolor (Vill.) Pilg.	1.70	Basal	Common intercept
Poa alpina L.	0.56	Basal	One line
Poligonaceae			
Oxyria digyna (L.) Hill	0.78	Peripheral	Common intercept
Primulaceae			
Primula farinosa L.	0.05	Dwarf	Common slope

(*Continued*)

Table 1. (Continued.)

Plant species	Thousand seed weight (g)	Seed type	Accepted model
Rosaceae			
Dryas octopetala L.	0.90	Spatulate	One line
Geum montanum L.	0.86	Spatulate	One line
Saxifragaceae			
Saxifraga stellaris L.	0.03	Dwarf	Independent
Scrophulariaceae			
Pedicularis rostratocapitata Crantz	0.88	Dwarf	One line

deviance, further analyses were implemented. In these cases, the OESL resulting from using R as the germination criterion (instead of R + C) was estimated both in relation to the number of days and percentage using the following equations:

$$OESL_x(d) = p_{50}(R) - p_{50}(R + C) \qquad (2)$$

$$OESL_x(\%) = 100 \times \frac{p_{50}(R) - p_{50}(R + C)}{p_{50}(R + C)} \qquad (3)$$

with x equal to 50 or 75. These calculations were also made in GenStat and hence the standard errors of the estimates of $OESL_x$, based on all the germination data used to fit each survival curve, were also provided.

In addition, when it was not possible to constrain K_i and σ to the same values for the two germination criteria (R or R + C), the two fitted versions of equation (1) were used to estimate the viability using R as the germination criterion as a function of the viability using R + C as the germination criterion, that is,

$$v_R = K_{iR} - \frac{\sigma_{R+C} \times (K_{iR+C} - v_{R+C})}{\sigma_R} \qquad (4)$$

Results

Seed survival curves

Seed viability declined with increasing duration of the ageing treatment in all species (Supplementary Figs S1–S6). For every sampling time and across all species, R was higher (most samples) or equal to the count of R + C (Supplementary Figs S1–S6; Fig. 1). In some cases, the differences were large (e.g. *Silene rupestris*, see Supplementary Fig. S3B), whereas for 16 species, the differences were very small throughout the sampling period and it was possible to fit a single survival curve ('one line') to the R and R + C data without a significant increase in residual deviance ($P > 0.05$); this group includes all Rosaceae, four out of five Poaceae, five out of seven Asteraceae and the single representative species of the Juncaceae, Orobanchaceae and Scrophulariaceae (Table 1). For those species where a single line could not be fitted, a common slope model was the most frequently accepted model (12 species), while the common intercept model was accepted for just four species. Only for *Saxifraga stellaris*, was it not possible to constrain either the slope or the intercept of the R and R + C survival curves.

Estimates and OESL

Some seed lots, most notably those of *Bartsia alpina*, *Gentianella anisodonta* and *Gentiana verna*, had low initial viability, hence estimates for viability to fall to 75 or even 50% were negative (Tables 2–3). Using R as the criterion for germination, the estimate for $p_{50(R)}$ ranged from 1.24 d for seeds of *B. alpina* (Table 2) to 41.71 d for seeds of *Silene vulgaris* ssp. *glareosa* (Table 3).

Since p_{50} is usually used as the relative measure of longevity in comparative studies, we considered the species rankings depending on whether R or R + C was used as the germination criterion. The species with the largest rank shift was *Primula farinosa*, changing from the 8th longest-lived seeds based on R to the 13th shortest-lived seeds when using R + C (Fig. 2). Other species that also fell in the ranking were *Gentiana acaulis* (from 11th to 18th longest-lived) and *Phyteuma orbiculare* (from 22nd to 29th). Species that moved up the ranking included *Campanula barbata*, *Luzula alpino-pilosa* and *Dryas octopetala* (moving from 14th to 9th, 15th to 11th and 21st to 17th, respectively). There were some changes in the rankings and in the shift in rankings depending on the germination criterion when p_{75} rather than p_{50} was used as the measure of longevity (cf. Fig. 2 and Supplementary Fig. 7).

There were 16 species for which the common line model could be accepted for both criteria (Table 1). Conversely, in species where the common slope model or the common intercept model was applicable for both germination criteria, the extent of OESL by using R rather than R + C varied among species, both in terms of percentage and the number of days (Tables 3–5). For example, the $OESL_{50}$ (days) varied widely, ranging from 0.54 d for *Oxyria digyna* to 9.01 d for *Silene rupestris*. Similarly, the $OESL_{50}$ (%) exhibited substantial variation, ranging from 12.51% for *Hieracium pilosella* to 4196% for *Gentiana verna*. Overall, of the 16 seed lots for which estimates of $OESL_{50}$ (%) were determined, 13 (81%) had $OESL_{50}$ values between 10 and 90% with the remaining three above this range (Tables 3–5).

Interspecies variation in seed longevity

When R + C was used as the germination criterion, p_{50} ranged between −0.25 d (i.e. initial fitted viability was less than 50%) for *Gentianella anisodonta* and 33.98 d for *Silene vulgaris* subsp. *glareosa* (Tables 2–5, though both species are reported in Table 3; Fig. 2). There were some taxonomic trends in longevity, for example, the four *Silene* taxa all had p_{50} estimates >20 d; other species with p_{50} estimates >10 d belonged to the Asteraceae. The
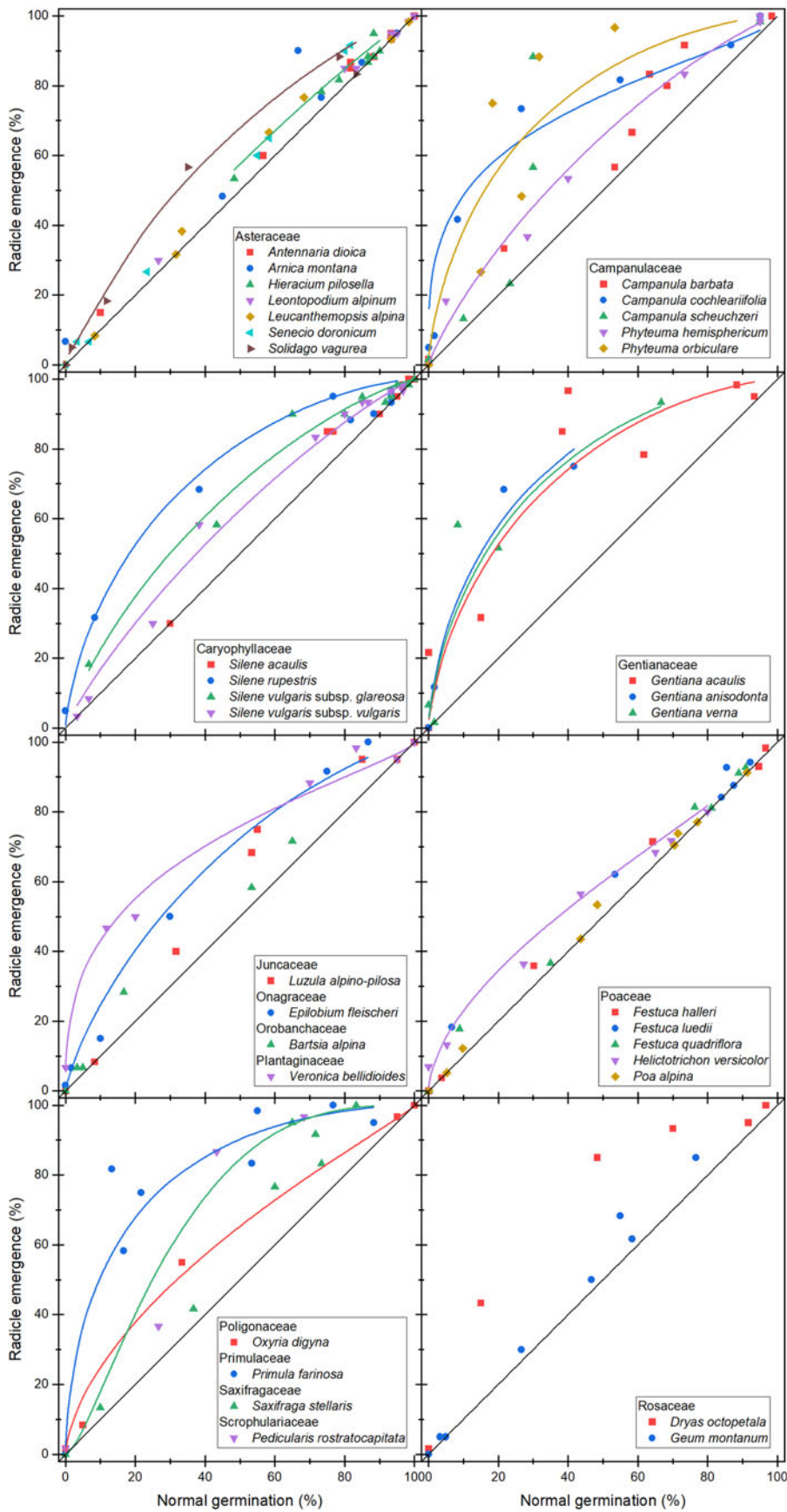
Figure. 1. Observed and fitted (equation 4) relationships between radicle (R) and radicle plus cotyledon emergence (R + C) for seeds of 33 alpine species from 14 different families, as indicated. Seeds were aged for different periods of time at 45℃ and 60% RH. The black line is the line of equality (i.e. R = R + C); the curved lines show the difference between the fitted survival curves for R and R + C for the respective species where it was not possible to constrain to a common line for R and R + C.

Table 2. Estimates (±SE) of seed longevity parameters, K_i and σ (equation 1) and the time for viability to fall to 75 and 50% (p_{75} and p_{50}) from laboratory seed storage experiments in which seeds were stored at 60% RH and 45°C with samples taken at regular intervals for a germination test

Taxon	K_i (NED)	σ (days)	p_{75} (days)	p_{50} (days)
Asteraceae				
Antennaria dioica	2.12 ± 0.14	9.89 ± 0.82	14.2 ± 0.7	21.0 ± 0.9
Arnica montana	2.22 ± 0.16	5.87 ± 0.48	9.1 ± 0.5	13.0 ± 0.5
Leontopodium alpinum	2.52 ± 0.22	10.33 ± 1.17	19.0 ± 1.0	26.0 ± 1.4
Leucanthemopsis alpina	1.43 ± 0.14	4.82 ± 0.43	3.7 ± 0.4	6.9 ± 0.4
Senecio doronicum	1.10 ± 0.12	4.48 ± 0.39	1.9 ± 0.4	4.9 ± 0.3
Campanulaceae				
Campanula barbata	1.33 ± 0.18	7.07 ± 0.97	4.7 ± 0.8	9.4 ± 0.8
Campanula scheuchzeri	1.51 ± 0.33	2.34 ± 0.39	2.0 ± 0.5	3.5 ± 0.4
Caryophyllaceae				
Silene acaulis	2.38 ± 0.22	10.86 ± 1.34	18.5 ± 1.1	25.9 ± 1.5
Juncaceae				
Luzula alpino-pilosa	1.83 ± 0.18	4.47 ± 0.44	5.2 ± 0.4	8.2 ± 0.4
Orobanchaceae				
Bartsia alpina	0.44 ± 0.09	2.83 ± 0.24	–[a]	1.2 ± 0.2
Poaceae				
Festuca halleri subsp. *halleri*	2.05 ± 0.08	1.92 ± 0.08	2.6 ± 0.1	3.9 ± 0.1
Festuca leudii	1.83 ± 0.23	3.81 ± 0.52	4.4 ± 0.5	7.0 ± 0.5
Festuca quadriflora	1.90 ± 0.18	3.32 ± 0.31	4.1 ± 0.3	6.3 ± 0.3
Scrophulariaceae				
Pedicularis rostratocapitata	0.83 ± 0.33	2.12 ± 0.59	0.3 ± 0.6	1.8 ± 0.5
Rosaceae				
Dryas octopetala	2.15 ± 0.55	2.61 ± 0.67	3.9 ± 0.7	5.6 ± 0.6
Geum montanum	0.68 ± 0.13	5.62 ± 0.68	0.1 ± 0.7	3.8 ± 0.5

The parameters shown are the results of fitting a model with the same estimates for K_i and σ ('common line') when the germination criterion was radicle (R) or radicle plus cotyledon emergence (R + C) (Supplementary Figs S1–S6).
[a]Estimate was negative (initial viability <75%).

relative rankings in longevity were slightly changed when the measure of seed longevity was p_{75} rather than p_{50} (Supplementary Fig. S7). For example, *Primula farinosa* was ranked 8th and 21st according to $p_{50(R)}$ and $p_{50(R + C)}$ (Fig. 2) but 10th and 27th according to $p_{75(R)}$ and $p_{75(R + C)}$ (Supplementary Fig. S7).

Discussion

In this study, we used both radicle emergence (R) and subsequent cotyledon emergence (R + C) as criteria for assessing germination in 33 species after different experimental storage periods at 45°C and 60% RH, to understand their relative seed longevity. Since radicle emergence normally occurs before cotyledons appear, the position of the survival curves for R + C are shifted to the left (i.e. to shorter storage periods) than those for R (Supplementary Figs S1–S6). This shift was negligible for about half the species so that a single survival curve could be fitted to the R and R + C data without a significant increase in residual deviance (Table 2). Consequently, for these species, germination criterion did not affect the prediction of seed longevity, that is,

either R or R + C can be used as a proxy of germination in seed longevity studies. However, for the other species, a single line model could not be fitted. Rather, a common slope or a common intercept model could be accepted, depending on the species (Table 1). For one species, neither of these constrained models could be accepted, that is, the accepted model was one with independent estimates for K_i and σ for the two germination criteria. Given these results, the OESL from using R rather than R + C showed considerable variation in terms of days and as a percentage.

Differences in the survival curves depending on germination criteria have long been recognized, for example, by Rao et al. (1987) for lettuce seeds, with the curve for normal germination [seeds that produced healthy seedlings *sensu* ISTA (2018)] being shifted to shorter storage periods than the survival curve for total germination (normal plus abnormal seedlings). Furthermore, Ellis and Roberts (1981) described how all seeds go through a 'defective' phase, as the last stage before they lose the ability to germinate, and Ellis et al. (1985) provided advice on evaluation of seedlings when testing the viability of seeds stored in a genebank. Nevertheless, most of the recent literature
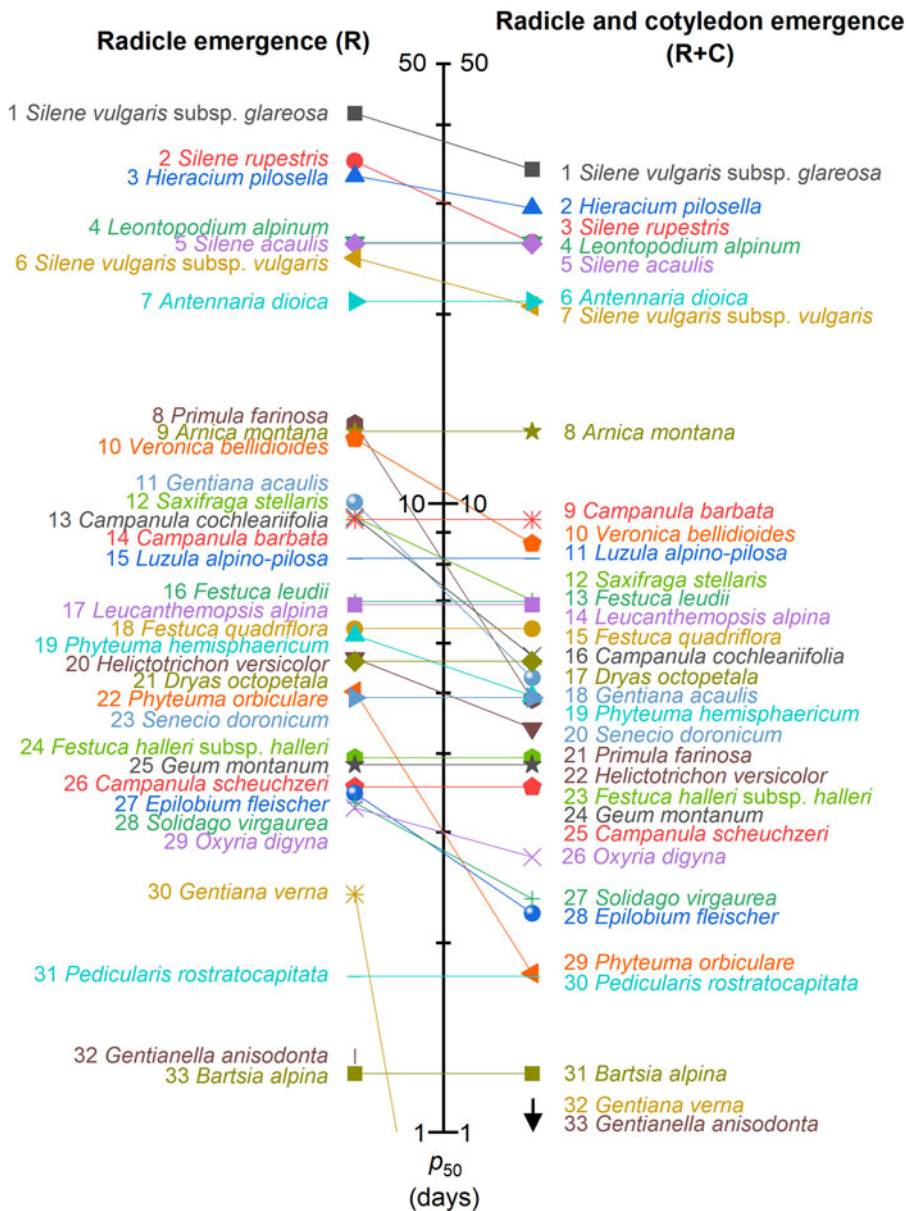
Figure. 2. Species ranking for relative seed longevity depending on whether the germination criterion is radicle emergence (R) or radicle plus cotyledon emergence (R + C). The y-axis scale shows p_{50}, the time for viability to fall to 50% when seeds are stored at 45°C and 60% RH (Tables 2–5). The scale is logarithmic. The numbers next to the species name refer to the ranking. The p_{50} estimates for *Gentiana verna* and *Gentianella anisodonta* when R + C was the criterion were <1 (see the black arrow).

exploring correlates of seed longevity in wild species has relied on R as a measure of seed viability (e.g. Probert et al., 2009; Mondoni et al., 2011; Merritt et al., 2014; Bernareggi et al., 2015; Seglias, 2022; Sommerville et al., 2023; White et al., 2023), indicating a possible OESL for some species. Adoption of R is easier when dealing with wild species since it is often unknown what would be considered abnormal, that is, a seedling that is not able to develop into a mature, reproductive plant. Nonetheless, this bias in the estimation of longevity could mistakenly target species for more frequent viability monitoring and vice versa and alter the perception of successful *ex situ* conservation of wild species if the ability to regenerate plants from seeds is not better assessed. This concern seems particularly relevant given the growing role of seed banks and other collections/stocks in the provision of propagation material for habitat restoration and species recovery (White et al., 2023). For example, Mondoni et al. (2011) reported that seeds of alpine plants have very short, short or medium longevity, based on seed storage experiments with R as the germination

criterion. According to our results, seed longevity for seedling growth of several alpine species may be lower than previously reported. For example, two species (*Veronica bellidioides* and *Primula farinose*) previously classified as having medium longevity (10 d < p_{50} ≤ 100 d; Mondoni et al., 2011) according to $p_{50(R)}$ should be reclassified as short-lived (1 d < p_{50} ≤ 10 d) according to $p_{50(R + C)}$. Similarly, and more alarmingly, seeds of two other species (*Gentiana verna* and *Gentianella anisodonta*) classified as having short seed longevity according to $p_{50(R)}$ were found to be very short-lived (p_{50} ≤ 1 d) based on $p_{50(R + C)}$. *Ex situ* storage of short-lived seeds such as those of alpine species may, therefore, be more problematic than currently thought.

The range in p_{50} estimates observed for these taxa are similar to those observed by Probert et al. (2009) and Mondoni et al. (2011), for seeds stored under identical conditions with radicle emergence (R) as the germination criterion. Both these previous studies emphasized a correlation between climate and seed longevity, with seeds from species originating from cooler, wetter

Table 3. Estimates (±SE) of seed longevity parameters, K_i and σ (equation 1), the time for viability to fall to 75 and 50% (p_{75} and p_{50}) from laboratory seed storage experiments, and the overestimation of p_{75} and p_{50} (OESL) when the germination criterion used was radicle emergence (R) rather than radicle plus cotyledon emergence (R + C) (equations 2 and 3)

Family, Species	Criterion	K_i (NED)	σ (days)	OESL (days)	p_{75} (days)	OESL$_{75}$ (%)	p_{50} (days)	OESL$_{50}$ (%)
Asteraceae								
Hieracium pilosella	R	1.77 ± 0.08	18.71 ± 1.22	3.7 ± 1.2	22.8 ± 1.7	21.9 ± 7.6	33.2 ± 1.3	12.5 ± 4.2
	R + C	1.58 ± 0.06	18.71 ± 1.22		16.3 ± 1.2		29.5 ± 1.2	
Solidago virgaurea	R	1.58 ± 0.08	2.11 ± 0.08	1.0 ± 0.2	1.9 ± 0.2	107.4 ± 26.7	3.3 ± 0.1	42.3 ± 7.9
	R + C	1.11 ± 0.08	2.11 ± 0.08		1.0 ± 0.1		2.4 ± 0.1	
Campanulaceae								
Phyteuma hemisphaericum	R	2.04 ± 0.19	3.03 ± 0.26	1.2 ± 0.5	4.3 ± 0.6	42.3 ± 20.0	6.2 ± 0.3	24.8 ± 10.6
	R + C	1.63 ± 0.15	3.03 ± 0.26		2.8 ± 0.5		4.9 ± 0.3	
Phyteuma orbiculare	R	1.55 ± 0.35	3.24 ± 0.64	3.2 ± 1.0	2.1 ± 0.9	n.a.	5.0 ± 0.6	180 ± 123
	R + C	0.55 ± 0.29	3.24 ± 0.64		–[a]		1.8 ± 0.7	
Caryophyllaceae								
Silene rupestris	R	3.52 ± 0.19	9.96 ± 0.50	9.0 ± 1.0	26.7 ± 1.8	46.7 ± 6.2	35.0 ± 0.7	34.7 ± 4.4
	R + C	2.61 ± 0.11	9.96 ± 0.50		20.0 ± 1.4		26.0 ± 0.7	
Silene vulgaris subsp. *glareosa*	R	2.90 ± 0.44	14.36 ± 2.29	7.7 ± 4.0	29.3 ± 5.7	31.8 ± 18.9	41.7 ± 3.1	22.7 ± 12.8
	R + C	2.37 ± 0.28	14.36 ± 2.29		26.1 ± 5.8		34.0 ± 2.7	
Silene vulgaris subsp. *vulgaris*	R	1.98 ± 0.08	12.42 ± 0.49	4.0 ± 1.0	15.6 ± 1.0	32.5 ± 9.7	24.6 ± 0.8	19.3 ± 5.4
	R + C	1.66 ± 0.08	12.42 ± 0.49		12.7 ± 1.0		20.6 ± 0.7	
Gentianaceae								
Gentiana acaulis	R	1.93 ± 0.39	5.19 ± 0.96	4.7 ± 1.7	6.6 ± 2.0	266 ± 270	10.0 ± 1.3	89.7 ± 47.4
	R + C	1.02 ± 0.34	5.19 ± 0.96		1.8 ± 1.5		5.3 ± 1.1	
Gentiana verna	R	1.01 ± 0.35	2.36 ± 0.53	2.3 ± 0.9	0.8 ± 0.8	n.a.	2.4 ± 0.6	4196 ± 64,165
	R + C	0.02 ± 0.39	2.36 ± 0.53		–[a]		0.1 ± 0.7	
Gentianella anisodonta	R	0.88 ± 0.14	1.50 ± 0.16	1.6 ± 0.3	0.3 ± 0.2	n.a.	1.3 ± 0.2	n.a.
	R + C	−0.17 ± 0.16	1.50 ± 0.16		–[a]		–[b]	
Onagraceae								
Epilobium fleischeri	R	1.70 ± 0.19	2.03 ± 0.17	1.2 ± 0.4	2.1 ± 0.4	141.6 ± 76.8	3.5 ± 0.3	54.7 ± 20.3
	R + C	1.10 ± 0.18	2.03 ± 0.17		0.9 ± 0.3		2.2 ± 0.2	
Primulaceae								
Primula farinosa	R	2.06 ± 0.33	6.49 ± 1.12	8.5 ± 1.9	8.4 ± 2.0	1695 ± 5953	13.4 ± 1.5	174.2 ± 72.7
	R + C	0.75 ± 0.29	6.49 ± 1.12		0.5 ± 1.6		4.9 ± 1.2	

Seeds were stored at 60% RH and 45°C with samples taken at regular intervals for a germination test. The parameters shown are the results of fitting a model with the same estimate for σ ('common slope') when the germination criterion was radicle (R) or radicle plus cotyledon emergence (R + C) (Supplementary Figs S1, S2 and S4–S6).
[a]Estimate was negative (initial viability <75%).
[b]Estimate was negative (initial viability <50%).
n.a., calculation could not be performed due to the negative estimate of p_{50} or p_{75}.

habitats ranked lower in terms of seed longevity. Our results here, however, again serve to emphasize that there is also variation in seed longevity even when seeds are collected from localities with very similar climates. Thus, more research is clearly needed to further tease out the genetic and environmental controls – and their interaction – on seed longevity.

We introduced a coefficient, denoted as OESL, for species exhibiting the difference of seed longevity based on germination criteria (i.e. R *vs* R + C). This coefficient provides a deeper understanding of the criticality in assessing the R + C criterion over R. It can be used to prioritize species that require R + C as the germination criterion in seed storage studies. Indeed, even a slight

Table 4. Estimates (±SE) of seed longevity parameters, K_i and σ (equation 1), the time for viability to fall to 75 and 50% (p_{75} and p_{50}) from laboratory seed storage experiments, and the overestimation of p_{75} and p_{50} (OESL) when the germination criterion used was radicle emergence (R) rather than radicle plus cotyledon emergence (R + C) (equations 2 and 3)

Family, Species	Criterion	K_i (NED)	σ (days)	p_{75} (days)	OESL$_{75}$ (days)	p_{50} (days)	OESL$_{50}$ (days)	OESL$_{50}$ (%)
Campanulaceae								
Campanula cochleariifolia	R	1.89 ± 0.15	5.02 ± 0.41	6.1 ± 0.5	2.4 ± 0.4	9.5 ± 0.5	3.8 ± 0.6	66.0 ± 11.7
	R + C	1.89 ± 0.15	3.03 ± 0.32	3.7 ± 0.3		5.7 ± 0.3		
Plantaginaceae								
Veronica bellidioides	R	2.22 ± 0.20	5.72 ± 0.64	8.8 ± 0.7	2.8 ± 0.7	12.7 ± 0.9	4.0 ± 1.0	46.8 ± 14.0
	R + C	2.22 ± 0.20	3.90 ± 0.50	6.0 ± 0.5		8.6 ± 0.6		
Poaceae								
Helictotrichon versicolor	R	1.13 ± 0.08	5.01 ± 0.32	2.3 ± 0.3	0.5 ± 0.2	5.7 ± 0.3	1.3 ± 0.3	29.1 ± 8.2
	R + C	1.13 ± 0.08	3.88 ± 0.28	1.8 ± 0.2		4.4 ± 0.2		
Poligonaceae								
Oxyria digyna	R	2.43 ± 0.19	1.35 ± 0.10	2.4 ± 0.2	0.4 ± 0.1	3.3 ± 0.2	0.5 ± 0.2	19.8 ± 7.2
	R + C	2.43 ± 0.19	1.13 ± 0.08	2.0 ± 0.1		2.7 ± 0.1		

Seeds were stored at 60% RH and 45°C with samples taken at regular intervals for a germination test. The parameters shown are the results of fitting a model with the same estimate for K_i ('common intercept') when the germination criterion was radicle (R) or radicle plus cotyledon emergence (R + C) (Supplementary Figs S2–S5).

difference in seed longevity between R and R + C is significant for short-lived species in comparison with medium or long-lived seeds. These subtle distinctions do not hold the same significance for long-lived seeds. In general, OESL (%) is more useful than OESL (days). For example, *Epilobium fleischeri* seeds can be classified as short-lived, with a p_{50} of 3.5 d for R and 2.2 d for R + C, while *Silene vulgaris* subsp. *glareosa* seeds fall into the medium-lived category, with a longevity of 41.7 d for R and 33.9 d for R + C. Considering OESL in days, the choice of germination criterion has a greater impact for the medium-lived species compared with the short-lived species (OESL of 7.7 and 1.2 d, respectively; Table 3). However, when examining the OESL coefficient (%), a different picture emerges, with short-lived seeds revealing a 54.7% overestimation, while that for the medium-lived seeds being just 22.7%. Hence, this coefficient plays a crucial role in prioritizing species within seed banks, allowing us to determine the most suitable criterion for each species. This approach enables effective screening of species, facilitating the selection of the most appropriate germination criterion, thereby enhancing the overall efficiency and precision of seed storage studies. We have focused on OESL$_{50}$ since p_{50} has become our standard measure of longevity (Walters et al., 2005; Hay et al., 2019). If the R and R + C survival curves do not have the same slope, the divergence is greater when viability has fallen to 50% than it is when viability has fallen to just 75% (Tables 4 and 5). Accordingly, there were some changes in the relative rankings of p_{75} seed longevity

among the taxa considered here, depending on whether the germination criterion was R or R + C (Supplementary Fig. S7). This also needs to be considered if the seed bank recollection standard is 75%.

Finally, the OESL resulting from using R instead of R + C has implications on understanding the ageing process itself. Depending on the germination criteria, for some species the ageing process resulted in the same proportion of abnormal seedlings over the course of viability loss of the seed lot (i.e. common slope model; Table 3), whereas for others, it appears that an increasing proportion of the population of seeds can accumulate damage that is phenotypically expressed before they lose the ability to germinate (R) (i.e. common intercept model; Table 4). Furthermore, the differences between R and R + C during seed ageing may be not only species-specific, but also affected by other factors, such as conservation conditions. Rao et al. (1987) noted that a common slope model is acceptable in some storage conditions and a common intercept model is acceptable for others, with no apparent trend according to moisture content or temperature, or 'rate' of ageing.

Our results illustrate the need to be more discerning when deciding whether a seed is viable or not when conducting seed storage experiments for wild species in general and, perhaps, alpine species in particular. We introduced a coefficient, denoted as OESL (%), which facilitates effective screening, enabling the selection of the most appropriate germination criterion for

Table 5. Estimates of seed longevity parameters for *Saxifraga stellaris* (Saxifragaceae)

Criterion	K_i (NED)	σ (days)	p_{75} (days)	OESL$_{75}$ (days)	OESL$_{75}$ (%)	p_{50} (days)	OESL$_{50}$ (days)	OESL$_{50}$ (%)
R	2.10 ± 0.22	4.53 ± 0.50	6.5 ± 0.5	4.1 ± 0.9	178.3 ± 97.1	9.5 ± 0.5	2.5 ± 0.8	35.8 ± 14.6
R + C	0.69 ± 0.26	6.94 ± 1.03	2.3 ± 0.8			7.0 ± 0.6		

Estimates (±SE) were made of K_i and σ (equation 1), the time for viability to fall to 75 and 50% (p_{75} and p_{50}) from laboratory seed storage experiments, and the overestimation of p_{75} and p_{50} (OESL) when the germination criterion used was radicle emergence (R) rather than radicle plus cotyledon emergence (R + C) (equations 2 and 3). Seeds were stored at 60% RH and 45°C with samples taken at regular intervals for a germination test. For this species, it was not possible to constrain either K_i or σ without a significant increase in residual deviance (Supplementary Fig. S4).

potential seedling emergence post-storage. Such considerations should enhance the overall conservation efficiency. This might be particularly important for valuable collections of rare and endangered species, especially those with short-lived seeds. Whilst R may continue to be the preferred criterion in germination tests for seed storage experiments for practical reasons, it may be necessary to use a more stringent 'viability' standard (the parameter that germination is estimating), than that typically used (e.g. 75%; ISTA, 2018), to ensure that the genetic potential of the desired ≥75% of individuals is realized.

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