Viability of ʻōhai *Sesbania tomentosa* seeds after 3 decades of ambient conditions

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Abstract The plant species *Sesbania tomentosa* (ʻōhai; Fabaceae) is endemic to the Hawaiian Islands, federally listed as endangered in the USA and has been proposed for categorization as Vulnerable on the IUCN Red List. In 2021, c. 12,000 seeds from 12 seed lots collected during 1990–1992 from across the Hawaiian Islands were discovered in ambient herbarium conditions (55% relative humidity (RH) at 20 °C). International gene bank standards suggest drying seeds in equilibrium with 15% RH and stored at −18 °C. To investigate seed viability, we mechanically scarified then sowed 15 seeds from each accession at daily alternating regimes of 12 h light and 12 h dark at temperatures of 25/15 °C, respectively. Germination was observed after 7 days and ended after 34 days. Mean final germination was 88.9 ± SD 0.1% (range 73–100%). Each seed lot was accessioned into the National Tropical Botanical Garden's Seed Bank and Laboratory. In seeds with a water-impermeable seed coat (i.e. physical dormancy), such as *S. tomentosa*, seeds can desorb but not absorb water. Therefore, if the seeds were initially dried, although exposed to high RH for up to 32 years, seed equilibrium RH may have remained low, which may in part explain the observed high germinability. This study holds significance for managers who are working to conserve this endangered Hawaiian species and suggests that even suboptimal conditions may still yield highly viable seeds several decades into the future.

Keywords Desiccation, ex situ plant conservation, herbarium, physical dormancy, seed banking, seed longevity, *Sesbania tomentosa*, water-impermeable seed coat

Plants are going extinct tens to hundreds of times faster than the background rate (Humphreys et al., 2019), and Hawaiʻi has the largest number of recorded plant extinctions (Humphreys et al., 2019; Wood et al., 2019). This is a result of climate change, invasive species and other anthropogenic effects (Sakai et al., 2002). The need for effective plant conservation is more urgent than ever, and seed banking has been shown to be the most cost-effective and efficient means of ex situ conservation of plant germplasm (Guerrant et al., 2004; Cochrane et al., 2007). Under conventional seed banking standards, seeds are desiccated to 10–25% relative humidity (RH), hermetically sealed, and then stored at −18 ± 3 °C (FAO, 2014). Under these conditions, seed lifespan is predicted to be extended decades to centuries (Ellis & Roberts, 1980; Walters et al., 2005).

Ambient herbarium conditions are inappropriate for long-term seed storage and retention of high germinability (e.g. Ellis & Roberts, 1980). Nonetheless, herbaria have been suggested as overlooked repositories of viable seeds (Godefroid et al., 2011), and a global priority list of de-extinction candidate species using herbarium specimens was recently created (Albani Rocchetti et al., 2022a). Germination trials using seeds stored at ambient herbarium conditions have been undertaken with varying success. *Schiedea kauaiensis* (Caryophyllaceae) germinated after 3 years at ambient herbarium conditions (Wolkis et al., 2022). Common and rare species of Fabaceae germinated after 13 (five species of *Acacia*; Doran et al., 1983), 50 (*Prosopis juliflora*; Ffolliot & Thames, 1983) and 97 (*Astragalus neglectus*; Bowles et al., 1993) years, and *Bupleurum tenuissimum* (Apiaceae) germinated after 144 years in ambient herbarium conditions (Godefroid et al., 2011). However, no seeds germinated in a study of *Metrosideros polymorpha* (Myrtaceae) harvested from herbarium specimens 4–38 years old (Wolkis & Deans, 2019).

Sesbania tomentosa Hook. & Arn. (Fabaceae), or ʻōhai in ʻŌlelo Hawaiʻi (the Hawaiian language), is federally listed as endangered in the USA. This shrub, endemic to the Hawaiian Islands, is primarily adapted to coastal and dry lowland areas (Wagner et al., 2005; Cole & Morden, 2021; USFWS, 2023). It has been proposed for categorization as Vulnerable on the IUCN Red List as there are < 1,000 individuals (D1) and there is an estimated ongoing decline of 10% in the next 10 years (C1) (Rønsted, proposed). *Sesbania tomentosa* is listed as orthodox in the seed information database (SER et al., 2023), and likely orthodox in the Hawaiʻi Seed Bank User's Guide (Hawaiʻi Seed Bank Partnership, 2023) based on an ex situ longevity assessment of 295 species in the Hawaiian flora (Chau et al., 2019). In 2021, a sealed 4-l glass jar containing silica gel crystals (a commonly used desiccant) indicating ambient RH (c. 55%) and 12 lots of *S. tomentosa* seeds collected during 1990–1994, with passport data, was discovered in herbarium PTBG (the Herbarium of the National Tropical Botanical Garden; NTBG). We assessed the germinability of each seed lot to investigate longevity and to determine whether accessioning each lot into the NTBG Seed Bank and Laboratory was warranted.

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As the seeds were stored in a jar and not physically attached to specimens, we did not consider the decision framework for harvesting seeds from herbarium specimens (Albani Rocchetti et al., 2022b). For each accession, one replicate of 15 randomly selected seeds was sown. Inferred from a popular gardening handbook (Lilleeng-Rosenberger, 2005), Baskin & Baskin (2014) concluded that *S. tomentosa* exhibits physical dormancy. In such seeds, embryos are fully developed and the seed (or fruit) coat is impermeable to water (Baskin et al., 2000). Seeds were therefore scarified with a single-edged razor blade opposite the hilum to allow the seeds to imbibe. They were then placed in 60 mm Petri dishes on seed-germination paper (Anchor Paper Company, Saint Paul, USA) and placed in a sealed chamber above a saturated potassium sulphate solution (achieving an RH of c. 98% at 25 °C) overnight to avoid imbibition damage. The seeds were removed from the 98% RH sealed chamber and moved to a germination chamber between 11 and 15 November 2022. The seeds and seed-germination paper were moistened with a solution of 0.1% plant preservative mixture (Plant Cell Technology, Washington, DC, USA) in distilled water, to inhibit fungal growth without affecting germination (A. Guri, pers. comm., 2017). Dishes were then sealed with plastic paraffin film to reduce water loss. Seeds were exposed to daily alternating regimes of 12 h light (c. 41 mmol/m²/s cool white (4100 K) fluorescent light) and 12 h dark at corresponding temperatures of 25/15 °C in a germination chamber (GR36L, Percival Scientific, Perry, USA). Seeds were monitored every 7–14 days until every seed either germinated or was parasitized by fungus, or until 6 months had elapsed since sowing. Germination was defined as radicle emergence, and all resulting seedlings as well as non-germinated seeds were transferred to the NTBG Conservation Nursery for further propagation. Per cent germination was calculated by dividing the number of seeds germinated by the number of seeds sown and multiplying by 100.

Germination was observed after 7 days and had ended by day 34. Mean final germination was 88.9 ± SD 0.1 % (range 73–100%) (Table 1). The seedlings that were transferred to the NTBG Conservation Nursery appeared healthy and vigorous, and as of 6 September 2023, at least three plants per accession had survived, most of which were already flowering (R. Campbell, NTBG Nursery Manager, pers. comm., 2023).

The high germination rates indicate the seeds are of high conservation value, and therefore each seed lot was accessioned into the NTBG Seed Bank and Laboratory for conservation and continued longevity monitoring. This resulted in 100–3,101 seeds banked per accession, totalling 11,639 seeds (Table 1). An assessment of longevity in the Hawaiian flora that included data on two accessions of *S. tomentosa* stored for up to 15.5 years at a target equilibrium relative humidity (eRH) of c. 20% and −18 °C found that germinability declined to ≥ 30% of maximum (the re-collection threshold suggested by the authors of that study) in 5–10 years (Chau et al., 2019). This is in contrast with our findings of up to 100% germinability in seeds stored > 32 years, which could at least triple the re-collection threshold for the species. In other accessions of *S. tomentosa* seeds stored at −18 °C in the NTBG Seed Bank, 49 of 50 seeds germinated after 25 years (accession no. 960201), and 15 of 15 seeds germinated after 20 years (accession no. 010019) (D. Wolkis, unpubl. data). However, neither Chau et al. (2019) nor ourselves analysed survival curves (e.g. Ellis & Roberts, 1980). To understand the longevity trait fully, which is important for management of the species, seed survival curves and p_{50}, the time during storage when germinability has declined to 50%, should be investigated (Hay et al., 2022).

It has been suggested that for some species exhibiting physical dormancy characterized by a water-impermeable seed coat (hard seededness), drying induces the impermeable trait (Jaganathan et al., 2019, and references therein).

TABLE 1 Passport data and germination of each accession (seed lot), arranged from oldest to youngest accession. Accession refers to the National Tropical Botanical Garden's unique Living Collections accession number.

Accession	Collection date	Storage time (years)	Island	Germination (%)	Seeds banked
910210	28 Mar. 1990	33.1	Kauaʻi	73	1,450
900961	12 June 1990	32.9	Molokaʻi	93	100
910209	14 June 1990	32.9	Molokaʻi	100	2,550
20220293	24 June 1990	32.9	Nīhoa	80	185
20220295	25 June 1990	32.9	Nīhoa	93	115
20220294	25 June 1990	32.9	Nīhoa	93	118
910527	29 Aug. 1990	32.7	Nīhoa	93	250
920605	14 Dec. 1990	32.4	Oʻahu	93	2,001
920604	14 Jan. 1991	32.3	Hawaiʻi	80	209
920169	18 May 1991	31.9	Kauaʻi	87	533
920473	27 July 1992	30.7	Kauaʻi	87	3,101
940064	1 Jan. 1994	29.3	Hawaiʻi	93	1,027

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The unique anatomy of a water-impermeable seed coat allows the release of water from the seed (desorption), but water cannot be absorbed until the seed coat is damaged (i.e. a water gap is opened; Hay et al., 2023). Although the desiccant inside the jar with the seeds indicated ambient RH (c. 55%), it was probably much lower when the jar was sealed. As *S. tomentosa* seeds have water-impermeable seed coats (Baskin & Baskin, 2014), it is likely that the eRH of the seeds remained at this lower level, which could explain the high germinability observed after 30 years.

Many studies of seeds stored at herbarium conditions report poor germination. For example, only 1 in > 2,000 seeds harvested from herbarium specimens of rare Hawaiian species germinated (Wolkis et al., 2022); no seeds of *M. polymorpha* harvested from herbarium specimens 4–38 years old germinated (Wolkis & Deans, 2019); and only eight seeds germinated (all from a single species) in > 2,600 sown (Godefroid et al., 2011). Our finding of high germinability of *S. tomentosa* seeds suggests nevertheless that some species can be resurrected from seeds stored in overlooked repositories. This should, however, be carried out as soon as possible, before viability declines further, and if harvesting from herbarium specimens directly, the prudent use of seeds should be considered (e.g. Albani Rocchetti, 2022b). Although we found high germinability at ambient conditions after 30 years, we would expect greater longevity (i.e. p_{50} sensu Hay et al., 2022) for orthodox seeds that were dried, hermetically sealed, and stored at low temperatures (i.e. conventional storage conditions; Ellis & Roberts, 1980, FAO, 2014). Therefore, we recommend depositing seeds into seed banks where facilities and expertise can optimize longevity.

Author contributions Conceptualization: ES, DW; germination studies: ES; statistical analysis: DW; writing, revision: all authors.

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Conflicts of interest None.

Ethical standards The research was carried out under Hawaiʻi State Department of Land and Natural Resources for Natural Area Reserve, Rare Plant, and Native Invertebrate Research Permit Numbers I2986 and I5371, and otherwise abided by the *Oryx* guidelines on ethical standards.

Data availability The data supporting the findings of this study are available within the article.

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