


Seed freeze sensitivity and ex situ longevity of 295 species in the native Hawaiian flora

Marian M. Chau^{1,7} , Timothy Chambers², Lauren Weisenberger³, Matthew Keir⁴, Timothy I. Kroessig¹, Dustin Wolkis⁵, Roy Kam², and Alvin Y. Yoshinaga^{1,6}

Manuscript received 29 January 2019; revision accepted 24 June 2019.

¹ Lyon Arboretum, University of Hawai'i at Mānoa, Honolulu, HI 96822, USA

² U. S. Army Natural Resources Program on O'ahu, Schofield Barracks, HI 96857, USA

³ U. S. Fish and Wildlife Service, Pacific Islands Fish and Wildlife Office, Honolulu, HI 96850, USA

⁴ State of Hawai'i Department of Land and Natural Resources – Division of Forestry and Wildlife, Honolulu, HI 96813, USA

⁵ National Tropical Botanical Garden, Kalaheo, HI 96741, USA

⁶ Center for Conservation Research and Training, Pacific Biosciences Research Center, University of Hawai'i at Mānoa, Honolulu, HI 96822, USA (Retired)

⁷ Author for correspondence (e-mail: mmchau@hawaii.edu)

Citation: Chau, M. M., T. Chambers, L. Weisenberger, M. Keir, T. I. Kroessig, D. Wolkis, R. Kam, and A. Y. Yoshinaga. 2019. Seed freeze sensitivity and ex situ longevity of 295 species in the native Hawaiian flora. *American Journal of Botany* 106(9): 1–23.

doi:10.1002/ajb2.1351

PREMISE: Ex situ seed banking is critical for plant conservation globally, especially for threatened floras in tropical ecosystems like Hawai'i. Seed bank managers must maximize longevity, and species managers must plan restoration before seeds lose viability. Previous observations suggested some native Hawaiian seeds lost viability in frozen storage (−18°C). We investigated seed storage behavior in the Hawaiian flora to optimize storage conditions and recommend re-collection intervals (RCI) to maximize viability of stored seeds.

METHODS: Using 20+ years of real-time seed storage viability data, we tested freeze sensitivity for 197 species and calculated RCIs for 295 species. Using paired tests of accessions stored >2 yr at 5°C and −18°C, we developed an index of relative performance to determine freeze sensitivity. We calculated RCIs at 70% of highest germination (P70).

RESULTS: We identified four families (Campanulaceae, Cyperaceae, Rubiaceae, and Urticaceae) and four genera with seed freeze sensitivity and six additional genera with likely freeze sensitivity. Storage longevity was variable, but 195 species had viability >70% at the most recent tests (1 to 20+ yr), 123 species had RCIs >10 yr, and 45 species had RCIs <5 yr.

CONCLUSIONS: Freeze sensitive storage behavior is more widely observed in Hawai'i than any other regional flora, perhaps due to insufficient testing elsewhere. We present a new protocol to test seed freeze sensitivity, which is often not evident until 2–5 years of storage. Re-collection intervals will guide restoration practices in Hawai'i, and results inform seed conservation efforts globally, especially tropical and subtropical regions.

KEY WORDS genebank; intermediate seeds; islands; lobelioids; orthodox seeds; restoration planning; seed banking; seed conservation; seed storage behavior; temperature sensitivity.

Conservation of plant biodiversity requires in situ protection of native habitat and ex situ conservation methods to secure collections of propagules for restoration and reintroduction. Integrating both in situ and ex situ strategies is essential in Hawai'i, where over half the native flora is at risk of endangerment or extinction due to threats such as alien invasive species, habitat modification, climate change, and other human impacts (Sakai et al., 2002; Fortini et al., 2013; Weisenberger and Keir, 2014b; IUCN [International Union for the Conservation of Nature], 2018). The recent commencement of widespread in situ recovery efforts across the state has not yet stemmed rapid decline within remaining populations of many endemic species (IUCN, 2018). In fact, the number of federally listed Threatened and Endangered (T&E) plants in Hawai'i has increased

by 56% over the last decade (USFWS, 2018). Accordingly, maintaining viable propagules as an ex situ “genetic safety net” until appropriate habitat can be protected is often the only way to prevent further extinctions (Havens et al., 2004).

Restoration outplantings are often experimental, testing site suitability and mixing source material to determine which combinations are most effective (Guerrant and Kaye, 2007). One limiting factor biodiversity managers need to overcome to successfully restore habitats and populations is obtaining enough propagules to maximize genetic diversity. Thus, managers are increasingly dependent on ex situ germplasm storage to accumulate collections from small fragmented subpopulations, so that plant material collected from maternal founders over multiple seasons can be recombined

in reintroduction programs (Cochrane et al., 2007; PCA [Plant Conservation Alliance], 2015). To preserve the integrity of collections from wild individuals before they were combined and provide backup to experimental outplantings, seed collections must be preserved as long as possible, and seed bank managers must make every effort to base protocols on empirical research. When developing conservation strategies, species managers need to know how long seeds will survive in storage to schedule withdrawal for propagation before they expire (Guerrant and Fielder, 2004; PCA, 2015).

Humans have been storing seeds of agricultural crops for thousands of years, but seed storage for wild species began more recently. The U. S. Department of Agriculture (USDA) National Seed Storage Laboratory was built in 1958, primarily for agricultural species, but research led to the 1977 conversion of storage vaults from refrigeration (5°C) to freezing (−18°C), setting the stage for long-term storage (USDA, 2016). The facility is now the National Laboratory for Genetic Resources Preservation (NLGRP), the leading U. S. institution for seed physiology research on agricultural and wild plants. In 2000, the Royal Botanic Gardens, Kew Millennium Seed Bank established the first and largest global seed bank for wild species, which aims to conserve 25% of the world's plant species by 2020, and is a leading international research organization (Liu and Dickie, 2017). The Millennium Seed Bank Partnership now includes partners in over 80 countries, and seeds of 39,100 species (over 12% of wild plants) are preserved (Royal Botanic Gardens Kew, 2019a), demonstrating an increasing public recognition of seed banking as a critical tool for conservation of wild species.

The earliest recorded seed storage in Hawai'i began at the National Tropical Botanical Garden (NTBG) in 1990. Seed banking with the explicit goal of preserving seeds under conventional standards for future restoration began in 1995 at Lyon Arboretum. Conventional seed banking was also established between 2007 and 2008 at NTBG, the U.S. Army's Natural Resource Program on O'ahu (ANRP), and the Hawai'i Island Seed Bank. These four founding seed banks, along with their primary clients, funders, and/or supporters the State of Hawai'i Department of Land and Natural Resources – Division of Forestry and Wildlife and the U.S. Fish and Wildlife Service, established the Hawai'i Seed Bank Partnership (HSBP) in 2013 (Weisenberger and Keir, 2014b). By 2016, the HSBP had grown to 15 organizations with interest in or beginning to establish conventional seed storage for conservation of wild species, and now in 2019, the HSBP has over 50 members from 25 organizations. In 2013, 48% of 724 “species of conservation importance” (SCI) were represented in seed banks across the state (Weisenberger and Keir, 2014a); in 2019 over 60% of SCI are represented in seed banks (E. Grave, Laukahi: Hawai'i Plant Conservation Network personal communication). While statewide deposits of seed collections still exceed withdrawals by an order of magnitude, seeds are withdrawn on a regular basis by numerous landowners and species managers for planned restoration efforts. Seed banking is the most effective and efficient resource for ex situ plant conservation in Hawai'i (Weisenberger and Keir, 2014b).

Conventional seed banking standards include desiccation to $15 \pm 3\%$ relative humidity (RH) and freezing at $-18 \pm 3^\circ\text{C}$ (FAO, 2014). While these standard protocols are effective for most crop species, wild species present challenges such as small sample size and greater heterogeneity, requiring more species-specific knowledge about seed storage behavior (Walters et al., 2010; Walters, 2015). Early research on storage behavior of agricultural seeds established a binary designation of orthodox (i.e., storable by conventional seed

banking) or recalcitrant (i.e., desiccation-sensitive and not storable by conventional methods; Roberts, 1973). However, it soon became evident that a discrete “intermediate” category was necessary (Ellis et al., 1990; Hong and Ellis, 1995), and eventually researchers recognized that seed storage behavior is more accurately expressed as a continuum, spanning from orthodox to recalcitrant extremes (Pammenter and Berjak, 1999).

Intermediate seeds are desiccation-tolerant, but may respond to storage conditions in three ways, including (1) sensitivity to intermediate desiccation levels, (2) anomalous responses to temperatures between +10 and −30°C (hereafter, “temperature-intermediate”), and (3) loss of viability over a short time period, regardless of storage conditions (Walters, 2015). Relatively few species have been identified with intermediate seed storage behavior, comprising <2% of any compendium of over 800 species (Hong et al., 1996; Tweddle et al., 2003; Wyse and Dickie, 2018), and account for only 0.6% of the 24,242 species with designated storage behavior in the Millennium Seed Bank's Seed Information Database (Royal Botanic Gardens Kew, 2019b). However, in studies of tropical and subtropical floras, intermediate seed storage behavior may comprise up to 22% of species classified (Pritchard et al., 2004; Ellis et al., 2007; Lima et al., 2014; Salazar et al., 2018). Studies that identify multiple species with temperature-intermediate seeds are less common (but see Hong and Ellis, 1995; Lin, 1996; Ellis et al., 2007), but there are also a few studies that identify single species with temperature-intermediate seeds (e.g., Ellis et al., 1991a; Crane et al., 2003; Magistrali et al., 2013; Zhang et al., 2014). It has long been hypothesized that freeze sensitivity may be more abundant in seeds from tropical regions (Hong and Ellis, 1996; Schmidt, 2000), but more research is needed.

In seeds (and other biological materials), cooling of fluids inhibits molecular motion, often leading to the formation of a “glassy” solid composed of a matrix with pores that lacks crystalline structure (Walters et al., 2005a). In orthodox seeds, intracellular glasses exhibit low molecular mobility and dense molecular packing, with low pore volume (Buitink and Leprince, 2008). In seeds with higher pore volume and molecular mobility, “relaxation” can occur and compress the pores, and sometimes further drying or cooling has unpredictable effects on glass stability (Walters et al., 2010). In some freeze-sensitive seeds, lower-order temperature transitions (beyond the glass transition) may induce crystallization and contraction of lipid bodies, which can create additional voids, further increasing molecular mobility and destabilizing glass structure over time (Walters, 2015). Thus, seed freeze sensitivity is not likely to be apparent until after a few years in storage, which could explain why this trait has not yet been identified in other tropical regions.

There is also a high incidence of recalcitrant seeds in tropical ecosystems (Tweddle et al., 2003; Pritchard, 2004; Marques et al., 2018), though not in Hawai'i, where isolation and long-distance dispersal may have selected for desiccation tolerance (Carlquist, 1974; Yoshinaga and Walters, 2003). For non-recalcitrant seeds, it is more challenging to parse the variables influencing ex situ seed longevity, as they may relate to numerous traits or aspects such as embryo size, seed mass, cellular composition, taxonomy, genotype, and maternal environment (Pritchard and Dickie, 2003; Walters et al., 2005b; Probert et al., 2009; Long et al., 2015). There is indication that storage longevity is greater for species from hot, dry regions than for species from cool, wet regions (Probert et al., 2009) and that species from cool, dry alpine regions have short-lived seeds (Mondoni et al., 2011), but less is

known about variation of seed longevity in ex situ storage for tropical species that are from hot, wet regions.

Seed survival in ex situ storage commonly follows a sigmoidal pattern that includes a steep drop in viability, influenced by the normal distribution of viability within a seed lot and the environment to which the seeds are exposed (Ellis and Roberts, 1980). A second type of seed survival curve is also common, where the sigmoidal stage is preceded by a period of stability with little loss in viability (Bernal-Lugo and Leopold, 1998). However, a steep decline at some point is inevitable, and one challenge for seed banks is to identify when seeds should be withdrawn and used before reaching unacceptable levels in the sigmoidal stage of mortality.

The earliest published research on storage of native Hawaiian seeds was a study that included the endemic *Sophora chrysophylla* (Fabaceae), which maintained >70% viability after 3-yr storage at 15°C and 7°C, at a wide range of RH levels (Akamine, 1951). Systematic research to determine seed storage longevity of native Hawaiian plants began in 1995 at the University of Hawai'i's Lyon Arboretum. Due to the islands' tropical location, it was previously assumed that seeds of most native Hawaiian species were likely recalcitrant and that living plant collections would need to be the primary method for ex situ conservation (CPC [Center for Plant Conservation], 1994). Since 1995, hundreds of native seed collections have been made to determine storage capability using long-term research protocols developed by the Lyon Arboretum Seed Conservation Laboratory (LASCL) and NLGRP in collaboration. It became evident that many species could in fact be stored for multiple years under conventional standards without significant loss of viability, and in a study of 207 native species, Yoshinaga and Walters (2003) found a low incidence of recalcitrance. The native Hawaiian flora has a recorded total of 1061 species in 213 genera in 81 families (Wagner et al., 1999; Imada, 2012). Over the last 15 years, LASCL and its partner seed banks at ANRP and NTBG have continued to collect and bank seeds from species across the archipelago to further investigate seed storage behavior in the native Hawaiian flora.

After evidence of possible temperature-intermediate seed storage behavior accumulated for several species, we aimed to test the hypothesis that a substantial proportion of the Hawaiian flora exhibits seed freeze sensitivity at -18°C in ex situ storage. Quantifying this unique phenomenon is critical to the restoration of several endemic taxa and could be equally important in other tropical and subtropical regions. Furthermore, after more than two decades of banking native seeds, we also aimed to test the hypothesis (suggested by Yoshinaga and Walters, 2003) that the majority of the Hawaiian flora could be preserved long term (i.e., >10 yr) using conventional seed banking practices. The findings will inform conservation and restoration programs across Hawai'i and generate testable hypotheses for other tropical areas. In this study, we present the results of over 22 years of research, including data for 295 species, and provide the most comprehensive, long-term study to date on seed storage behavior and ex situ longevity for a tropical regional flora.

MATERIALS AND METHODS

Seed collections

Accessions included in this study are from collections made between 1995 and 2017 (Appendix 1). Fruits were collected from a range of plant species, families, and habitats across all of the

main Hawaiian islands (Kaua'i, O'ahu, Moloka'i, Lāna'i, Maui, and Hawai'i Island) and some of the remote northwestern Hawaiian Islands, by trained field botanists with appropriate state and/or federal permits and/or permissions from landowners. Germplasm accessions (i.e., those banked for conservation/future restoration) were usually collections from a single maternal plant, especially for rare taxa, while research accessions (i.e., those banked for storage behavior and longevity testing) were often established from pooled collections of 2–30 (rarely >50) individual plants within one population and one collection date. Replicate collections were made for many species through repeated sampling across wild populations or over time, sampling from living collections in botanical gardens, or from sampling of F1 or F2 seeds in greenhouses, living collections, or reintroduction sites. Collections of fruit were submitted to at least one of the following seed banks: Lyon Arboretum Seed Conservation Laboratory, Army Natural Resources Program on O'ahu, or National Tropical Botanical Garden.

Processing, initial testing, and storage

Each collection was assigned an accession number and details recorded in the accepting seed bank's local database. Seeds were cleaned, and numbers of seeds were counted or estimated by mass. A portion of seeds was used for initial testing to determine germination methods and/or dormancy breaking treatments and to estimate baseline seed viability (see *Germination assessments* below). Remaining seeds were designated for storage at RH levels from very dry (8%), to fairly humid (73%), and dried at 24°C using desiccation chambers or saturated salt solutions monitored with hygrometers, for 2–4 wk or until seeds reached approximate equilibrium with the target %RH (Walters, 2004). Dried seeds were transferred to storage in airtight containers such as trilaminar aluminum foil packets (Moore and Buckle; St Helens, UK) or glass vials (Thermo Fisher Scientific, Waltham, MA, USA), at temperatures from 24°C to -18°C in refrigerators or freezers (Frigidaire, Charlotte, NC, USA; or Kenmore/Sears, Hoffman Estates, IL, USA). These methods sometimes resulted in numerous treatment combinations of temperature and RH if quantities of seeds allowed, but at a minimum, most research accessions have treatments of -18°C and 5°C at the standard of 15–20% RH at storage temperature.

Germination assessments at LASCL and ANRP

LASCL and ANRP began as the same seed bank at Lyon Arboretum in 1995. Seeds and associated data of rare and endemic species found on lands managed by the U. S. Army on O'ahu were moved to a separate facility in 2007. The two seed banks continue the same practices initially developed; thus, they have essentially the same methods for the purposes of this study. To assess relative viability after storage, seeds were withdrawn at pre-determined intervals for germination trials. Scheduled intervals typically included 6 mo, 1 yr, 2 yr, 5 yr, and every 5 yr thereafter, but exact times between collection and testing varied; therefore, we report the number of years elapsed since collection for germination test results. Number of seeds sown varied by accession, but when possible, at least two replicates of 25 to 100 re-humidified seeds were sown for each treatment combination. Dormancy breaking treatments, when needed, varied by species but most often included mechanical scarification, soaking in water, or treatment with gibberellic acid (GA₃) or potassium nitrate. Seeds were sown on 1% agar solution or blotter

paper (Anchor Paper, St. Paul, MN, USA) in Petri dishes placed in growth chambers (Hoffman Manufacturing, Corvallis, OR, USA; or Percival Scientific, Perry, IA, USA), or in a perlite-vermiculite medium in nursery pots for some of the larger seeds. Growth chambers were maintained at 90–100% RH and diurnally programmed for each corresponding month for high (from 22 to 26°C) and low (from 15 to 19°C) temperatures and light on/off settings (day length from 10.83 to 13.25 h) equivalent to monthly averages for 500–600 m a.s.l. on the island of O'ahu. Test seeds were watered and checked for germination approximately every 7–14 days, and the number of germinated seeds in each treatment was recorded. For seeds <1 mm long, cotyledon emergence was used as an indicator of germination, while radicle emergence was used for seeds >1 mm. Seedlings were removed from Petri dishes or pots during counting (and usually potted up for propagation, especially for species of conservation importance). Tests were concluded when all seeds had germinated or died, or after species-specific intervals ranging from 3 mo to several years. Percentage germination was calculated as the number of seeds germinated divided by the number of seeds sown. Resulting seedlings from rare taxa were transplanted for propagation in Lyon Arboretum Hawaiian Rare Plant Program or ANRP greenhouse facilities.

Germination assessments at NTBG

Seed storage at NTBG began in 1990 with a target RH of 15–25% at ambient temperatures in open containers. In 2008, these and all following seed accessions were hermetically sealed in trilaminate foil packets and transferred to storage at 5 and/or –18°C. Longevity monitoring started in 2016 with testing intervals of 1, 2, 5, 10, and every 10 yr thereafter. For assessing germination, in most cases, three replicates of 10–50 seeds were sown per germination assay in Petri dishes on blotter paper moistened with a 0.1% solution of a plant preservative mixture (PPM; Plant Cell Technology, Washington, D. C., USA) in distilled water to inhibit fungal growth without affecting germination (A. Guri, Plant Cell Technology, personal communication), and sealed with plastic paraffin film. Each day seeds were exposed to 12 h light (~41 $\mu\text{mol m}^{-2} \text{s}^{-1}$ cool white (4100 K) fluorescent light)/12 h dark with daily alternating temperature regimes of 25°/15°C or 30°/20°C in a germination chamber (GR36L; Percival Scientific). Germination tests were monitored every 14 days and concluded after all seeds had either germinated or died, or after 1 yr had elapsed since sow date. Percentage germination, defined by radicle emergence, is equal to the number of seeds that germinated divided by the number of seeds sown. Resulting seedlings from rare taxa were transplanted in the NTBG Conservation and Horticulture Center Nursery.

Database query design and data export

LASCL and ANRP have Microsoft Access (2013; Redmond, WA, USA) databases built on the same original design, and divergence from initial design has been minimal. The similarity of the two databases made it possible for the database manager at ANRP to develop a series of queries that could be applied to both seed banks. Two unique series were developed: (1) for export of data to support analysis of freeze sensitivity in storage behavior and (2) to determine re-collection intervals for Hawaiian species. Freeze-sensitivity queries returned all seed accessions with an initial germination test and all subsequent storage viability tests from each

accession with a sample size of 15 or more seeds. Filters allowed further selection of data to include all accessions with paired viability tests conducted on seeds stored at 5°C and –18°C, and from these accessions, the most recent paired tests (the greatest amount of time between collection date and viability test start date) with the highest germination percentage for both storage temperatures (5°C and –18°C; seeds were stored at various RH levels at both temperatures). For each test, time between collection date and viability test start date was calculated in years. Because freeze sensitivity was often observed after 2 yr of storage, only tests where seeds had been stored for at least 2 yr were included. Re-collection interval queries returned all accessions with an initial germination test (<1 yr elapsed since collection) and subsequent storage viability tests from each accession with a sample size of 15 or more seeds, regardless of storage temperature or storage RH. For re-collection intervals, percentage germination equal to 70% of the highest germination within each accession (P70) was calculated along with number of years between the time of collection and the viability test start date.

Exceptions—Two species, *Bobea sandwicensis* (Rubiaceae) and *Euphorbia haelealeana* (Euphorbiaceae), were included with 10+ seeds for initial tests, since initial germination was $\geq 40\%$ and subsequent tests had >15 seeds. *Leptecophylla tameiameia* (Ericaceae) met criteria but was excluded from both exports, because the test units sowed were bony endocarps, containing up to 5–8 seeds each (Wagner et al., 1999); thus, interpretation of data was uncertain.

Determining freeze sensitivity in seed storage behavior

To determine which species had freeze-sensitive seeds, we used the data set described above to evaluate whether or not seed viability was greater under refrigerated (5°C) or frozen (–18°C) storage. We created and calculated a relative performance value (RP) for each accession to produce a value on a scale of –1 to 1, using the following equations, where C = percentage viability after 5°C storage, and D = percentage viability after –18°C storage:

if $C > D$, then $(D/C) - 1$, RP is negative;

if $D > C$, then $1 - (C/D)$, RP is positive.

Freeze sensitivity was determined at family and genus levels, where there were three or more accessions per family and/or genus. If $RP > 0$, frozen seeds age slower than refrigerated seeds, and the species likely has orthodox storage behavior. If $RP < 0$, seeds age slower refrigerated than frozen, and the species has intermediate, freeze-sensitive storage behavior. A one-sample Wilcoxon signed-rank test was used to determine whether RP values were significantly different from (greater than or less than) zero.

Recommending seed re-collection intervals

We used the P70 threshold to recommend seed re-collection intervals (RCI) by species. Although international regeneration standards are set at P85 (FAO, 2014), we chose P70 because cryptic nonviable seeds (i.e., those lacking an embryo or otherwise internally compromised) were not excluded from our seed bank accessions, since we lack x-ray technology and often cannot cut-test seeds of rare or endemic species with limited quantities before

germination studies. Anecdotally, we have also seen a general trend of low viability among many Hawaiian species. Ultimately, re-collection intervals are meant to provide practical guidance to land and species managers making decisions about how to use the services provided by seed banks.

We calculated P70 values relative to the highest germination, rather than the initial germination, due to dormancy issues, improvements in germination testing methods over 22+ years, and/or possible discovery of better dormancy breaking treatments over time; and because for 67% of the species included in this study, the highest germination was not the initial test. From the reports generated by the P70 queries, we excluded tests currently running for less than 4 mo and those conducted under (now) known suboptimal conditions. In Appendix 1, we report percentage germination and storage data for the longest running accession within each species, and the number of supporting accessions and number of conflicting accessions (see definitions below). Accessions for subspecific taxa were combined to report longest running, supporting, and conflicting accessions at the species level. For the number of supporting/conflicting accessions, we only included tests of seeds stored >5 yr, unless all tests were <5 yr, in which case we included tests >1 yr. Supporting accessions may support the P70 interval if it has been reached, but more often support the minimum value of the interval; e.g., a species with a 5–10 yr re-collection interval may have an accession that supports >5 yr P70 values but has not declined below P70 yet. Supporting accessions also may not have reached the minimum value of the P70 interval, but do not conflict: e.g., a species with a 15–20 yr re-collection interval may have an accession that supports >10 yr P70 values. For species that have not yet reached P70, the re-collection interval is notated as such, indicating ongoing research to determine P70 in real time.

RESULTS

Freeze sensitivity in seed storage behavior

There were 326 accessions that fit the criteria to be included in the determination of freeze sensitivity (stored for 2.00 to 20.44 yr; see data query methods above); 81 accessions were included from ANRP and 245 from LASCL, pertaining to 47 plant families and 95 genera. To determine freeze sensitivity at the family level, 32 families were analyzed (15 families only had one or two accessions with RP values). To determine freeze sensitivity at the genus level, 40 genera were analyzed (55 genera only had one or two accessions with RP values).

Campanulaceae, Cyperaceae, Rubiaceae, and Urticaceae show freeze sensitivity when analyzed at the family level (Table 1, Fig. 1), though not all genera tested within these families have enough replicates to confirm freeze sensitivity (Fig. 2), whereas *Clermontia*, *Cyanea*, and *Lobelia* in Campanulaceae, and *Eragrostis* in Poaceae show freeze sensitivity when analyzed at the genus level (Table 1, Fig. 2), with RP values significantly <0. There are six additional genera, representing three additional families, which have at least three replicate accessions and mean RP values between –0.630 and –0.967 and are likely freeze sensitive (Table 2).

Only one family, Ericaceae, represented only by the genus *Vaccinium*, had a positive RP value indicating orthodox storage behavior ($n = 5$, median RP = 0.500, $P = 0.043$). All remaining families and genera had RP values not significantly different from zero.

Seed longevity and re-collection intervals

There were 877 accessions that fit the criteria to be included in the determination of storage longevity and RCIs (stored for 0.43 to 20.44 yr; see data query methods above); 369 from ANRP, 501 from LASCL, and seven from NTBG, including 64 plant families and 131 genera. In total, we report recommended re-collection intervals at P70 for a total of 295 species, or >25% of native Hawaiian angiosperms.

Of the 295 species tested, 195 had viability >P70 at the time of their most recent test (Table 3). Of these, over half (102 species) have RCIs >10 yr, and 57 of those 102 species have RCIs >15 yr (Fig. 3; Appendix 1). Seven species have been stored for over 20 yr, including three that have been identified as having freeze-sensitive seeds, and four of the seven species have RCIs >20 yr (Appendix S1).

Of the 295 species tested, 100 have viability <P70 and had reached RCIs at the time of their most recent test (Table 3). Of these species, over half (50 species) have RCIs >5 yr, and 21 of those 50 species have RCIs between 10–20 yr (Fig. 3). However, there are 14 species that have RCIs <1 year and may have very short-lived or recalcitrant seeds, although nine of these species are represented by only one accession (Appendix S2). Similarly, 32 species have identified RCIs of <5 yr, although 23 of these species are represented by only one accession (Appendix S3).

The 295 species assessed for re-collection intervals represent 64 of the 80 flowering plant families in the native Hawaiian flora (Wagner et al., 1999; Imada, 2012). Of the top 10 families assessed, minimum RCIs were <5 yr for six families, but maximum RCIs were >10 yr for all 10, showing wide variation within most families (Table 4). For all but one of the top 10 families, the majority of species have P70 >5 yr, except for Gesneriaceae (45% have P70 >5 yr). Five families

TABLE 1. Native Hawaiian plant families and genera with freeze-sensitive seed storage behavior.

Family	Genus	<i>n</i>	Mean RP	SE	Min	Max	Median	Wilcoxon signed-rank test	<i>P</i>
Campanulaceae	all species	55	–0.582	0.059	–1.000	0.739	–0.746	–5.893	0.000
Campanulaceae	<i>Clermontia</i>	11	–0.485	0.129	–1.000	0.044	–0.338	–2.708	0.007
Campanulaceae	<i>Cyanea</i>	21	–0.640	0.077	–1.000	0.143	–0.792	–3.913	0.000
Campanulaceae	<i>Lobelia</i>	14	–0.667	0.129	–1.000	0.739	–0.811	–2.922	0.003
Cyperaceae	all species	14	–0.383	0.104	–1.000	0.090	–0.268	–2.794	0.005
Poaceae	<i>Eragrostis</i>	5	–0.332	0.129	–0.786	–0.064	–0.257	–2.023	0.043
Rubiaceae	all species	23	–0.253	0.117	–1.000	1.000	–0.130	–1.929	0.054*
Urticaceae	all species	10	–0.737	0.129	–1.000	0.000	–0.966	–2.689	0.007

Notes: *n* = number samples; RP = relative performance; *marginally significant.

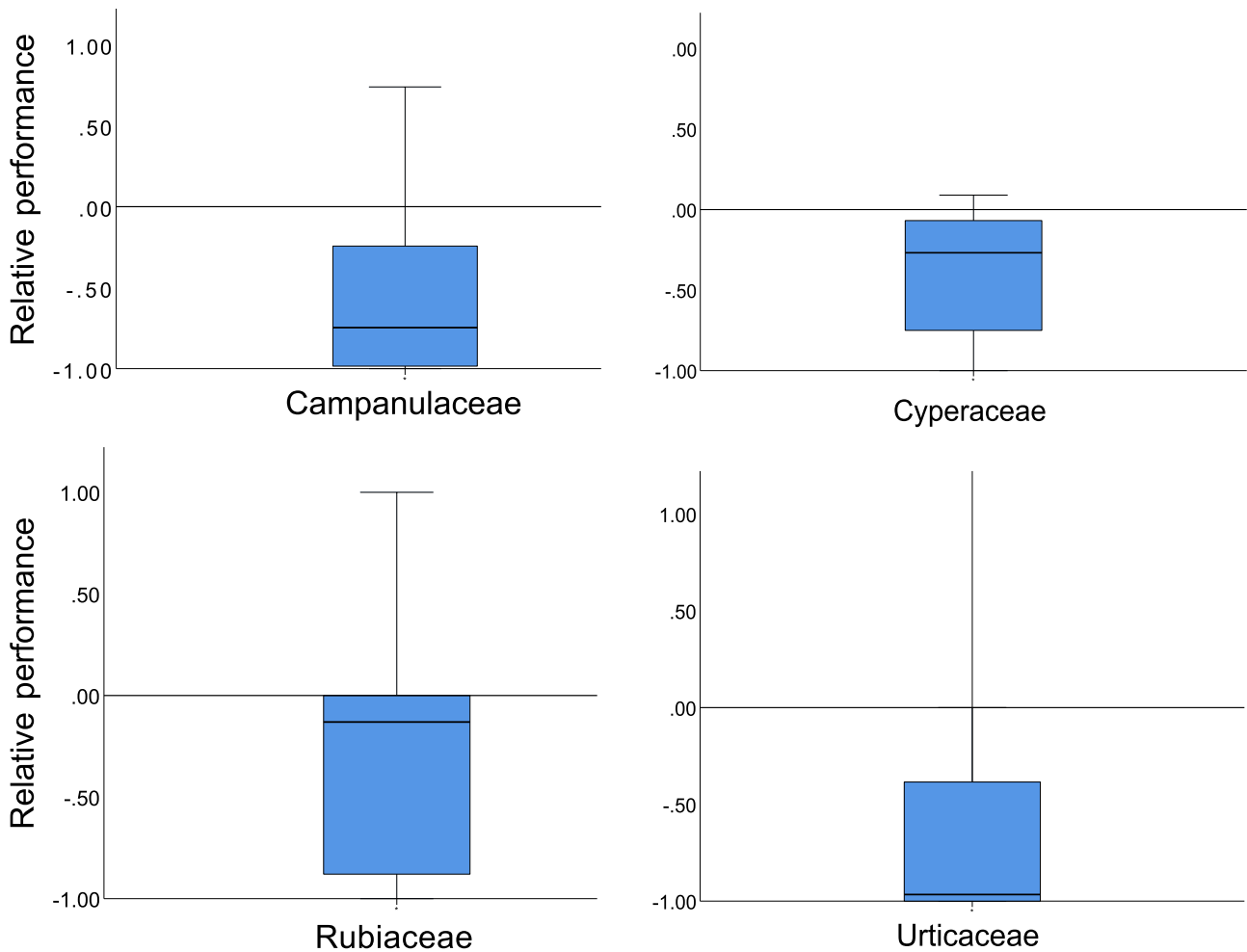


FIGURE 1. Relative performance (RP) of four families in the Hawaiian flora found to have freeze-sensitive seed storage behavior. RP values <1 indicate freeze-sensitive behavior.

had >50% of species with data indicating P70 >10 yr, including Asteraceae, Caryophyllaceae, Malvaceae, Cyperaceae, and Fabaceae.

The best-represented family in this study is Campanulaceae, for which we assessed RCIs for 56 species (representing all six genera), or 41% of the family, and found that 86% of species have RCIs >5 yr, while 45% have data indicating RCIs >10 yr (Table 4). Additionally, despite having freeze-sensitive seeds (with the exception of *Trematolobelia* species) and being stored at 5°C, 12 species have RCIs >15 yr, and *Clermotia kakeana* has an RCI >20 yr (Fig. 4). However, three species (*Clermontia montis-loa*, *Cyanea koolauensis*, and *Lobelia koolauensis*) have RCIs <5 yr, and *L. koolauensis* is represented by four accessions (Appendix 1).

Rubiaceae is well represented in this study, was also found to be freeze-sensitive, and is variable in longevity (Fig. 1, Table 4; Appendix S4). The longest-lived species is *Kadua affinis* with a RCI >20 yr, despite being stored at 5°C. All other species of *Kadua* remain >P70 at the last interval tested except *K. acuminata*, which has three accessions supporting a RCI <1 year (Appendix 1). The genus *Coprosma* is represented by multiple species >P70 at 2–5 yr, except *C. rhyncocarpa* with a RCI <2 yr. The genus *Bobea* is represented by two species; *B. elatior* with a RCI of 10–15 yr and *B. sandwicensis* with a RCI <2 yr. Similarly, *Psychotria* is represented by two species; *P. hobdyi* with a RCI >5 yr and *P. mariniana* with a RCI <1 year. Other genera that

have been tested for >8 yr have reached their RCIs at 8 or 10 yr, such as species of *Gardenia*, *Nertera*, and *Psydrax*.

Gesneriaceae, represented only by the genus *Cyrtandra* in Hawai'i, has the widest variation in longevity within a genus (Table 4). Of 11 species tested, five have RCIs <5 yr, each with one or two supporting accessions, while *C. cordifolia* and *C. grandiflora* have RCIs of 10–15 yr, the former with two supporting accessions, the latter with one supporting and one conflicting accession. Three species remain >P70 at the time of the latest test, including *C. dentata*, a federally listed T&E species, which remains >P70 at 10 yr, with 10 supporting accessions and two conflicting accessions (Appendix 1).

DISCUSSION

We identified four families and 10 genera in the native Hawaiian flora, potentially representing hundreds of species, with freeze-sensitive or likely freeze-sensitive seed storage behavior. In these taxa, viability after storage at 5°C was greater than viability after storage at –18°C, indicating that their seeds do not tolerate frozen storage and, therefore, are not storable by conventional seed banking standards. These results are unique, as this phenomenon is not reported in a substantial proportion

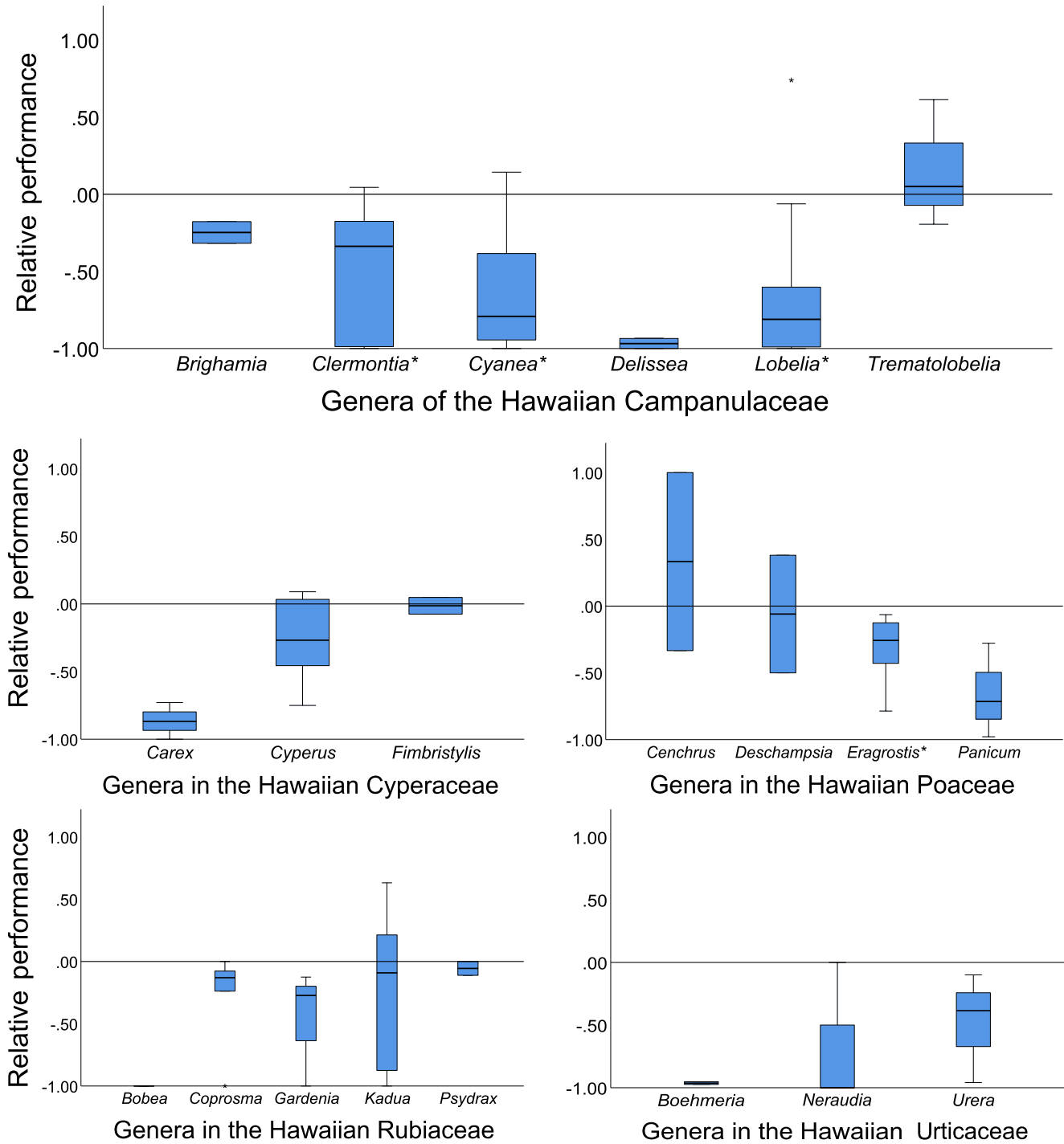


FIGURE 2. Relative performance of genera in the Hawaiian flora found to have freeze-sensitive seed storage behavior or contributing to families with this behavior. RP values <1 indicate freeze-sensitive behavior.

of any other regional flora. We provide a new index of relative performance and a new protocol (Fig. 5; see discussion below) for testing seed freeze sensitivity, which can directly inform seed conservation globally.

Contrary to previous understanding, our real time data show that seeds of many Hawaiian species can be stored using conventional seed bank protocols. Some can be stored for relatively long-term periods, alleviating immediate threats for many rare taxa.

However, there is also variation in seed longevity that warrants further research. Our results have implications for seed conservation in Hawai'i and in other tropical, subtropical, and island floras.

Freeze sensitivity in seed storage behavior

A subcategory within temperature-intermediate seed storage behavior is emerging in Hawai'i, where seeds age quickly and become

TABLE 2. Native Hawaiian plant genera with low replication but likely freeze-sensitive seed storage behavior.

Family	Genus	<i>n</i>	Mean RP	SE	Min	Max	Median	Wilcoxon signed-rank test	<i>P</i>
Asteliaceae	<i>Astelia</i>	3	−0.630	0.171	−0.956	−0.375	−0.558	−1.604	0.109
Campanulaceae	<i>Delissea</i>	4	−0.967	0.019	−1.000	−0.933	−0.968	−1.841	0.066
Cyperaceae	<i>Carex</i>	4	−0.866	0.055	−1.000	−0.729	−0.868	−1.826	0.068
Poaceae	<i>Panicum</i>	3	−0.657	0.204	−0.978	−0.278	−0.714	−1.604	0.109
Santalaceae	<i>Santalum</i>	4	−0.723	0.096	−1.000	−0.560	−0.666	−1.826	0.068
Urticaceae	<i>Neraudia</i>	4	−0.750	0.250	−1.000	0.000	−1.000	−1.732	0.083

Notes: *n* = number samples; RP = relative performance.

nonviable, usually within 5 yr, in conventional storage (15–20% RH, −18°C), but remain viable or have a higher viability for longer under dry, refrigerated conditions (15–20% RH, 5°C). By storing seeds from the same accessions at both 5°C and −18°C in paired tests for several years, we were able to compare viability in storage by developing an index of relative performance (RP). These values indicate if frozen seeds outperform refrigerated seeds, or vice versa. As defined here, the latter indicates seed freeze sensitivity. Freeze sensitivity falls within the broader defined category of temperature-intermediate seeds (Walters, 2015), but our study explicitly tested species for sensitivity to −18°C, or freezing to the temperature standard for conventional seed banking (FAO, 2014).

Seed freeze sensitivity in native Hawaiian plants—Here we identify four plant families in Hawai'i that have freeze-sensitive seed storage behavior: Campanulaceae, Cyperaceae, Rubiaceae, and Urticaceae, which potentially represent 255 native species in total (Appendix 1; Wagner et al., 1999; Imada, 2012). In addition to the four genera identified with freeze sensitive seeds, six additional genera exhibit “likely freeze sensitive” seed storage behavior. These genera introduce two additional families, Asteliaceae and Santalaceae, which may show seed freeze sensitivity in at least some of their Hawaiian representatives. All 10 genera with freeze-sensitive or likely freeze-sensitive seeds potentially represent 162 native species (21 not in the four families above). In total, the 276 unique species found in freeze-sensitive or likely freeze-sensitive families or genera make up 26% of the native Hawaiian flora (1061 species; Wagner et al., 1999; Imada, 2012), although there are a few likely exceptions (such as *Trematolobelia* spp.; see below).

The genera in the “likely freeze sensitive” category highlight the importance of sample size and replicated research accessions. For example, *Delissea* had a mean RP of −0.967, but was not significant because of the limited sample size of four accessions. More collections should be made to further investigate species that are hypothesized to be freeze sensitive, but for some rare genera, it can be difficult to acquire enough collections to statistically assign a

storage behavior category. Therefore, from a practical standpoint it would be irresponsible to recommend conventional storage, so we have added the category of ‘likely freeze sensitive’ to guide users of seed banks in Hawai'i. Other genera such as *Pittosporum* and *Vitex* have RP values less than −0.9, but only a single sample (data not shown), and should be prioritized for further research.

One of the most exceptional examples of seed freeze sensitivity in the Hawaiian flora is the Campanulaceae. There are six native genera (all endemic to Hawai'i except *Lobelia*), comprising 142 species that evolved from one colonization event ca. 13 Mya, representing the largest plant family in the native Hawaiian flora (Givnish et al., 2008). Seed freeze sensitivity is observed at the family level, as well as at the genus level for *Clermontia*, *Cyanea*, and *Lobelia*, and is likely for *Delissea*. *Brighamia* could not be analyzed due to low sample size. *Trematolobelia* was the only genus in the family with RP = 0, indicating possible orthodox behavior for its four species. More investigations are needed into the morphological and physiological differences among these genera that may be associated with seed freeze sensitivity. Species in this family also occupy different habitats throughout Hawai'i, from mesic valleys to wind-swept wet forests to rocky cliffs (Wagner et al., 1999), so further research is also warranted to determine whether habitat might influence freeze sensitivity.

The identification of the Hawaiian Rubiaceae as freeze sensitive, in addition to *Coffea* spp. and *Genipa americana* in other tropical areas (Ellis et al., 1990; Hong and Ellis, 1995; Eira et al., 2006; Magistrali et al., 2013), suggests a need for further research on the family globally, especially in the tropics. Although numerous Rubiaceae species are identified as orthodox in the Seed Information Database (SID; Royal Botanic Gardens Kew, 2019b), these determinations are usually made on timescales of <2 yr, perhaps insufficient to identify freeze sensitive storage behavior. Likewise, numerous species in Campanulaceae, Cyperaceae, Urticaceae, Poaceae, Asteliaceae, and Santalaceae are identified as orthodox, but often after <2 yr. Thus more research is needed globally to compare storage of genera in these families at 5°C and −18°C.

TABLE 3. Numbers of species tested and categorized into different re-collection intervals (RCI) within testing periods.

Test period (yr)	Number of species							
	Tested	Not reached P70	RCI 15-20	RCI 10-15	RCI 5-10	RCI <5	RCI <1	RCI uncertain
>20	7	4	2	1	0	0	0	0
15-20	81	50	1	14	7	6	2	1
10-15	71	43		3	18	3	1	3
5-10	79	57			4	17	1	0
<5	57	41				6	10	0
Totals	295	195	3	18	29	32	13	4

Note: P70 = no. years to decline below 70% of highest germination.

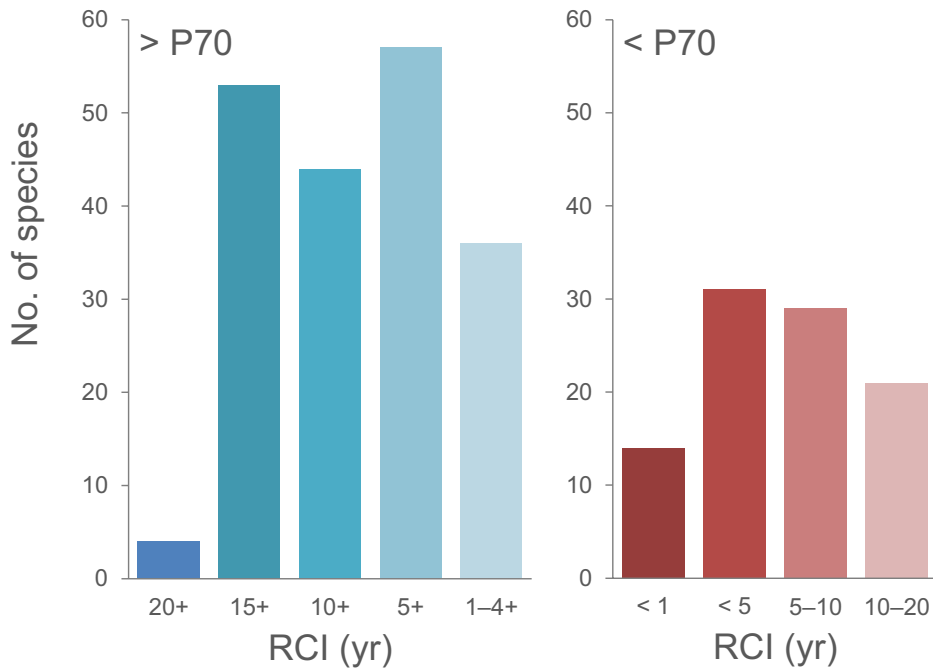


FIGURE 3. Numbers of Hawaiian species, stored in an ex situ seed bank for up to 22 years, with > or <70% of highest germination (P70) at the time of most recent tests and their recommended re-collection intervals (RCI).

Freeze-sensitive seed storage behavior worldwide—The SID identifies the seed storage behavior of 73 species as “intermediate” and 77 species as “intermediate?” (= likely intermediate). Most frequently, this category is assigned based on desiccation-intermediate behavior rather than temperature-intermediate behavior, which is rarely studied comparatively. Regardless, the total 150 species that are designated intermediate or likely intermediate only make up 0.6% of the 24,242 species with designated seed storage behavior data (not including the “uncertain” category) in SID. Our results show that the Hawaiian flora has a disproportionately higher occurrence of intermediate storage behavior (driven by the occurrence

TABLE 4. Ten families with the highest number of species tested, with re-collection interval (RCI) ranges, compared to numbers of species within the native Hawaiian flora (Wagner et al., 1999; Imada, 2012; USFWS, 2018).

Family	No. species tested			RCI		No. HI native species	
	Total	P70 >5	P70 >10	Min	Max	Total	T&E
Campanulaceae	56	48	25	<5	20+	136	47
Asteraceae	21	17	12	<5	20+	98	13
Rubiaceae	20	11	4	<1	20+	59	6
Caryophyllaceae	14	14	8	5+	15+	41	16
Malvaceae	13	11	8	1+	15+	27	8
Cyperaceae	12	10	6	3+	15+	45	2
Poaceae	12	7	5	<5	15+	50	3
Gesneriaceae	11	5	3	<5	10-15	58	12
Fabaceae	10	10	7	5-10	15+	25	4
Lamiaceae	9	6	2	<5	15+	62	10

Notes: P70 = no. years to decline below 70% of highest germination; T&E = federally listed as threatened and endangered.

of freeze sensitivity, a subcategory of temperature-intermediate behavior) compared to the global estimates in SID.

Seed freeze sensitivity has been documented in the literature for 29 species, in 14 genera, in 9 families (each represented by 1–3 genera): Arecaeae, Caricaceae, Flacourtiaceae, Lauraceae, Lythraceae, Orchidaceae, Rubiaceae, Rutaceae, and Sapotaceae (Seaton and Hailes, 1989; Ellis et al., 1990, 1991a, b, 2007; Hong and Ellis, 1995; Lin, 1996; Crane et al., 2003; Magistrali et al., 2013; Zhang et al., 2014). All of these studies compared seeds stored at temperatures above 0°C to seeds stored at –18 or –20°C for periods from 3 mo to 10 yr. Several of these examples are economically important species, and none of these studies represent a substantial proportion of a regional flora.

The limited record of seed freeze sensitivity outside Hawai'i may be due to a general lack of long-term seed storage behavior research in many tropical regions, as intermediate behavior is thought to be more abundant in the tropics, especially for temperature-intermediate seeds (Hong and Ellis, 1996; Schmidt, 2000; Pritchard, 2013). The gradual structural deterioration in seeds caused by temperature transitions and glass destabilization (Walters, 2015) may explain why freeze sensitivity is often detected only after 2–5 yr of storage, and seed storage experiments are often too short to detect this behavior. Additionally, when seed storage behavior is analyzed for large data sets, intermediate behavior is often dismissed or not addressed experimentally (Tweddle et al., 2003; Wyse and Dickie, 2018).

Freeze-sensitive seed storage behavior in the tropics—Previous studies of tropical seed storage behavior did not identify any species with seed freeze sensitivity (Pritchard et al., 2004; Daws et al., 2005) or only identified one species (Lima et al., 2014). Seven of 53 species from South Florida, United States, were classified as freeze sensitive, but based on predictive models using single accessions, desiccated and frozen only 3 and 7 days, respectively (Salazar et al., 2018). Six of 60 tree species in Vietnam showed possible freeze sensitivity (Ellis et al., 2007) but lost all viability after 3 mo, so may be better classified as having short-lived seeds (Walters, 2015). Further investigation is needed to determine whether freeze-sensitive seed storage behavior is common in tropical/subtropical regions.

Next steps for seed freeze sensitivity research—The mechanisms for freeze sensitivity in seed storage behavior are largely unknown. There is some evidence that lipid composition and abundance within seeds may impact seed aging at –18°C, especially where a melting point close to –18°C may allow lipids to slowly freeze and melt repeatedly, causing cellular damage through the constant movement of lipids in and out of a crystalline structure (Crane et al., 2003, 2006). Investigations into lipid composition and concentration in seeds of endemic Hawaiian species at NLGRP are ongoing; however, to date no correlation has been detected between lipid composition and

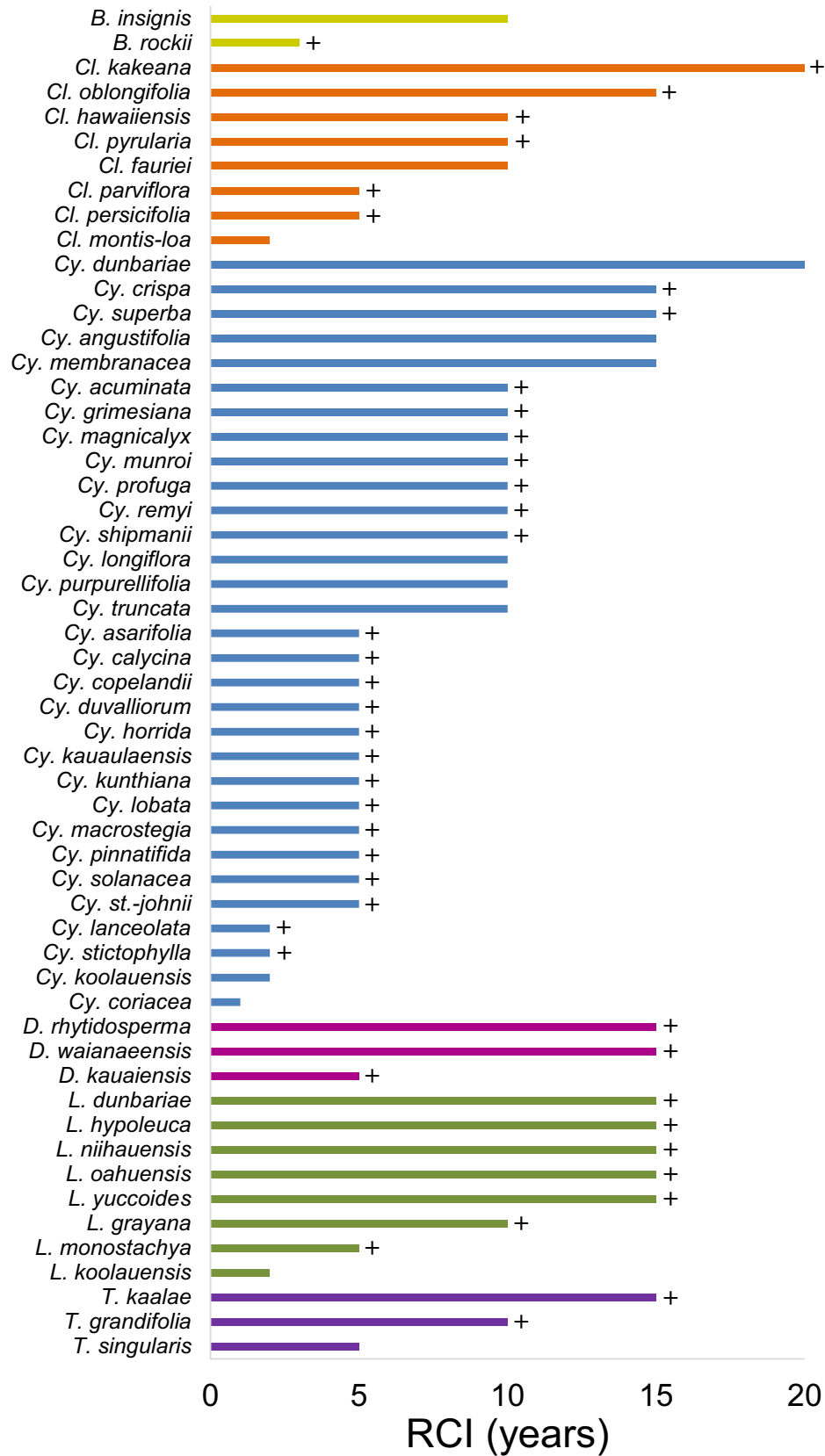


FIGURE 4. Recommended seed re-collection intervals (RCI) to maintain 70% of highest germination for all species tested in the Hawaiian Campanulaceae, in the genera *Brighamia*, *Clermontia*, *Cyanea*, *Delissea*, *Lobelia*, and *Trematolobelia*. +: RCI has not yet been reached.

freeze sensitivity in Hawaiian species (L. Hill and C. Walters, NLGRP, unpublished data). Further investigations into this relationship, and other potential interactions between biochemistry and structure at temperatures near -18°C , are warranted in Hawai'i, pantropically, and globally. Additional research is also needed to investigate whether cooling seeds to lower temperatures, such as through ultra-low freezers (-80°C) or cryopreservation (less than -150°C), might bypass the temperature range that damages freeze-sensitive seeds, potentially allowing for longer term seed storage.

To facilitate research on seed freeze sensitivity in tropical/subtropical floras and in the cosmopolitan plant families identified in our research (Campanulaceae, Cyperaceae, Rubiaceae, and Urticaceae) beyond Hawai'i, we propose a simplified protocol adapted from Hong and Ellis (1996) to determine this category of intermediate seed storage behavior (Fig. 5). There is a third type of intermediate storage behavior – sensitivity to intermediate levels of desiccation – not included in this figure because we did not test this aspect, and other protocols exist (e.g., Hong and Ellis, 1996). Our protocol requires monitoring viability of seeds stored in equilibrium with 15–20% RH at different temperatures (5°C and -18°C) and time intervals. It is critical that tests be carried out for 1–5 yr because freeze sensitivity may not be identifiable earlier. The determination of short-lived seed storage behavior

is essentially a byproduct of carrying out the protocol for the required time period and provides important information for seed bank managers and users. For undertaking seed storage behavior studies, especially in the tropics, our research demonstrates the importance of including comparative, long-term assessments of temperature sensitivity, and this study provides a model and hypotheses for testing seed storage behavior of other tropical floras.

Using the protocol above (Fig. 5), we have initiated storage at subfreezing temperatures (-80°C) to investigate long-term storage solutions for species with freeze-sensitive and likely freeze-sensitive seeds, as a more economical alternative to cryopreservation. Our paired experiments at -80°C and 5°C test the efficacy and safety of storing seeds at temperatures potentially outside the range at which lipid crystallization reactions take place (Crane et al., 2006). We have data for accessions stored for 0.98 to 11.16 yr, representing nine species in Campanulaceae and one each in Rubiaceae and Loganiaceae. High variation in our results reveals that our research to date on subfreezing temperature storage is inconclusive and often conflicting. Additional research is needed to develop protocols for the safe handling of seeds at subfreezing temperatures and to confirm or invalidate -80°C storage for the long-term conservation of freeze-sensitive seeds.

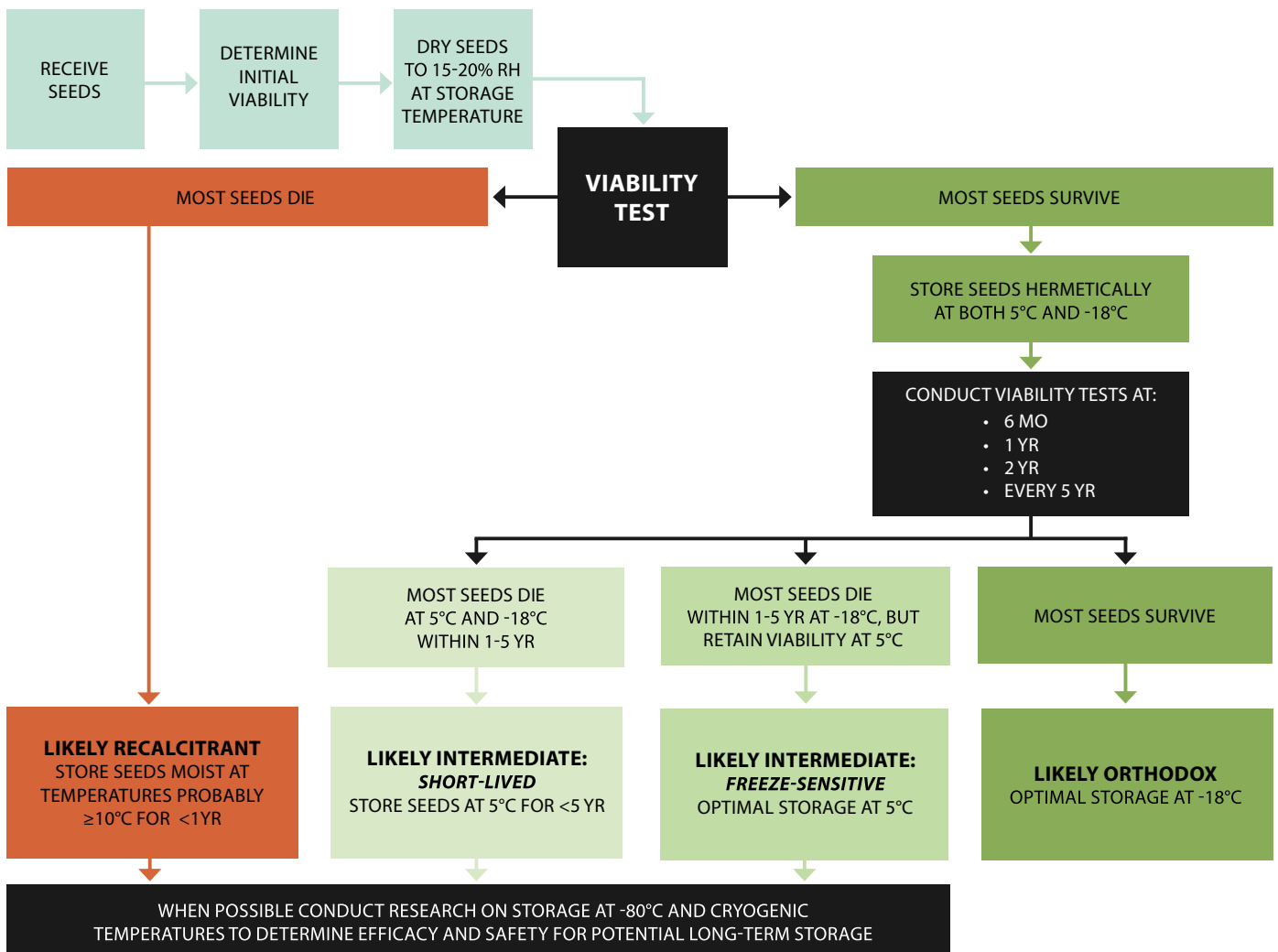


FIGURE 5. A protocol to determine freeze-sensitive seed storage behavior, adapted from Hong and Ellis (1996).

Positive relative performance—We identified one genus with a positive RP value: *Vaccinium* (Ericaceae). Positive RP values indicate that seeds stored at -18°C have a higher viability than seeds stored at 5°C . If a species is long-lived in conventional storage, it is likely that aging, or a decline in viability, has not been detected at either 5°C or -18°C , and the resulting RP value would therefore be zero. It is worth noting that the large majority of species examined here, 88% (36 genera), have RP values at or around zero, many of which likely have orthodox storage behavior, and aging has not been detected. However, *Vaccinium* species have orthodox seeds, but since aging has been detected at 5°C , they may not be very long-lived by conventional seed banking standards (FAO, 2014).

Seed longevity and re-collection intervals

Seed banking is an effective tool for ex situ conservation of the Hawaiian flora, as RCIs of 10 yr or greater have been estimated for 123 (77%) of 159 species tested for >10 yr. Efficacy of conventional storage is confirmed, especially on the scale of emergency seed banking to prevent extinction, and/or for the medium-term purposes of “buying time” until preparations can be made for restoration efforts for land management, threat mitigation, species monitoring, etc. (Cochrane et al., 2007; FAO, 2014; PCA, 2015). Even for species that have been stored and tested for <10 yr, 98 (72%) of 136 species tested maintain viability $>P70$, which supports the use of seed banking for short- to medium-term restoration purposes. The determination of three species (representing three genera and two families) with RCIs >20 yr, despite having freeze-sensitive seeds and being stored at 5°C (Appendices 1, S1), supports the use of seed banking even for some species that cannot be conventionally stored.

Although it was previously thought that most Hawaiian species would have recalcitrant seeds (CPC, 1994), our study confirms the hypothesis of Yoshinaga and Walters (2003) that many Hawaiian species could be stored long term using conventional seed banking standards. The Hawai'i Strategy for Plant Conservation identified seed banking as one of the most useful, effective, and cost-efficient methods to preserve “species of conservation importance”, and the majority of ex situ collections of native Hawaiian plants are now held in seed banks (Weisenberger and Keir, 2014b). Our data support conventional seed banking as the recommended ex situ germplasm conservation method for most of the Hawaiian flora. However, it is critical that we understand seed storage behavior and base our seed bank storage and usage protocols on empirical evidence (Guerrant and Fielder, 2004). With this need in mind, it is important to also examine the potential shortcomings of seed banking for some species, so that alternative methods can be utilized when appropriate.

Short-lived or recalcitrant species with RCI <1 yr—We have identified 13 species with RCI <1 yr, which indicates they may have recalcitrant or very short-lived seeds, or seeds not yet identified as intermediate and stored under suboptimal conditions (Walters, 2015). Another possibility is that some species, especially those only represented by one accession, may have been collected under suboptimal conditions. Seed longevity is dependent on postharvest moisture and temperature conditions, but also on “seed quality”, which includes physiological traits influenced by collection timing (Walters, 1998; Walters et al., 2010). Acquisition of desiccation tolerance relies on accumulation of sugars and late embryogenesis abundant (LEA) proteins, which facilitate glass formation, and occurs in later stages of seed development (Leprince and Buitink,

2010; Long et al., 2015; Teixeira et al., 2018). When fruit collections are made before the point of natural abscission, seeds may not have developed the desiccation tolerance of mature seeds, which in turn decreases storage longevity (Leprince et al., 1993, 2017; Hay and Probert, 1995; Angelovici et al., 2010). On the other end of the spectrum, seeds collected after the point of natural abscission may lose viability rapidly as they are exposed to desiccation and heat, which can also compromise longevity (Ellis and Roberts, 1981; Hay and Probert, 1995). It is critical to understand species characteristics for peak fruit maturation and to collect fruits and seeds as close to this point as possible. It is also important to transport propagules from the field to the seed bank under conditions that do not age or deteriorate seeds, including heat, anaerobic conditions, or substantial time between collection and seed processing.

In practice, optimum collection timing can be difficult to achieve, especially for rare plants, so some variation in collection quality is to be expected. Thus, for several species with RCIs <1 year and based on only one accession (Appendix S2), results are inconclusive, and further sampling is needed to determine seed storage behavior and longevity. However, of these 13 species we identified as short-lived or recalcitrant, other studies support this conclusion for *Chrysodracon* (Asparagaceae), *Planchonella* and *Sideroxylon* (Sapotaceae), and *Pritchardia* (Arecaceae; Pérez et al., 2012; Royal Botanic Gardens Kew, 2019b; C. Walters and L. Hill, NLGRP, unpublished data).

Short-lived species with RCI <5 yr—There are 32 species with RCIs between 1–5 yr, and these species are considered suitable for short-term seed storage (Appendix S3). This determination is subject to similar caveats as described for species with a RCI <1 year. For species in this group with higher sample sizes, we would strongly recommend following these RCI guidelines. *Lobelia koolauensis* ($n = 4$) is an interesting case study, as other *Lobelia* spp. studied have longer-lived seeds, even at 5°C (Fig. 4). This species is endangered, and its range is restricted to wet, windswept summits in the central Kōolau Mountains of O'ahu (Wagner et al., 1999, 2012). It is possible that habitat and rarity may affect seed longevity in storage, but more research is needed. For some short-lived species, we hypothesize that they have freeze-sensitive seeds (Appendix S3) because accessions of other species in these genera tested at 5°C had longer RCIs (Appendix 1), and further research is needed. For other species with low sample size, an RCI between 1–5 yr can be taken as evidence that at least some collections of that species could be short-lived. Extra care might be warranted to maintain the highest collection quality possible, and more frequent viability testing of ex situ collections is recommended.

Other factors that may decrease seed longevity in some of the species above could include low seed set or low viability, possibly due to the consequences of small and declining population sizes (e.g., inbreeding depression, genetic drift) and loss of pollinators (Barrett et al., 1996; Inman-Narahari et al., 2013; Jennings et al., 2016). Some taxa also have consistently low seed fill (e.g., species of *Metrosideros* [Myrtaceae] and Asteraceae; Appendix 1) possibly due to factors above, which can also complicate results of germination tests, so care should be taken in interpreting these results.

Additionally, seed dormancy is sometimes complex in the Hawaiian flora (Baskin and Baskin, 2014) and could confound the determination of RCIs if best protocols for germination have not been fully ascertained. Protocols for a species' required germination conditions, including moisture, temperature, and photoperiod,

must be determined to test seed longevity (and for species to have had data included in this study). Additionally, any dormancy must either be broken through germination pretreatment, or tests must be allowed to run for extended periods, even up to several years. In the majority of species, both protocols have been established—often there is no dormancy, requiring no pretreatment, or pretreatments have been identified. However, we do occasionally refine or test new germination and/or dormancy protocols when we receive replicate or larger collections. In some cases, ex situ storage appears to break dormancy after a number of years (e.g., species in Cyperaceae and Poaceae; Appendix 1), so earlier tests could have misleading results. Further analyses on the relationship between dormancy and seed longevity in the Hawaiian flora are planned for future studies.

Seed longevity in Hawaiian plant families—In the 10 families best represented in our longevity study, we observed a wide range of RCIs. These 10 families include four of the five largest plant families in the world (Asteraceae, Fabaceae, Rubiaceae, and Poaceae; Willis, 2017), as well as other families represented by >20 species in the native Hawaiian flora, and five families with >10 species federally listed as T&E (Wagner et al., 1999; Imada, 2012; USFWS, 2018; Table 4). The top 10 families assessed also represent eight of the largest families in the Hawaiian flora, while Malvaceae and Fabaceae rank in the 15 largest families.

The largest family in the Hawaiian flora and the best represented in this study is Campanulaceae, with 142 species, including 47 species federally listed T&E (Wagner et al., 1999; Imada, 2012; USFWS, 2018). Of the 56 species we tested, 12 species in five genera had viability >P70 at 15 or 20 yr (Fig. 4), 11 of which were stored at 5°C, suggesting that this family can have fairly long-lived seeds and may be best categorized as a family with a wide range of seed longevities. This result contrasts that of a comparative study of 195 species in 71 families held at the Millennium Seed Bank, where 32 collections of four species in Campanulaceae were tested after 20 yr of storage, and 59% had a significant drop in germination (Probert et al., 2009). However, because the Millennium Seed Bank stores all collections at 15% RH and –18°C, their real-time results could be confounded by freeze sensitivity of seeds, if this characteristic is common for the family outside of Hawai'i. Seeds of five species in 21 populations of Australian *Wahlenbergia* (Campanulaceae) had a wide range of P50 values, due to variation among populations within species; variation in seed lifespan was also possibly correlated with breeding system (Kochanek et al., 2009). Further research is needed on biological and ecological factors, at species and population levels, which may affect seed longevity of often rare Campanulaceae in Hawai'i.

Rubiaceae is the fifth largest family in Hawai'i and third best represented in this study. Because we identified the family as having freeze-sensitive seeds, along with other Rubiaceae species in the literature (Eira et al., 2006; Hong and Ellis, 1995; Magistrali et al., 2013), this trait could confound estimates of longevity in ex situ storage. Our results suggest that Rubiaceae is another family with wide variation in seed longevity, even excluding accessions stored at –18°C. More research is needed on both storage behavior and longevity of seeds in Rubiaceae globally.

Gesneriaceae is the fourth largest family in the Hawaiian flora, represented by only the genus *Cyrtandra*, but with 59 species it is one of the largest Hawaiian radiations, likely from a single colonizing ancestor (Wagner et al., 1999; Clark et al., 2008; Imada, 2012;). While the species in this study vary considerably in longevity, the majority had reached their RCI before 10 yr, and even the two

longest-lived species (tested so far) have RCIs of 10–15 yr, so this family might be best suited for medium-term storage in Hawai'i. However, the variation of RCIs found within this one genus suggests that collection quality may be affecting estimates of longevity (Walters et al., 2010). For example, *C. dentata* and *C. grandiflora* have RCIs >10 yr, but with conflicting accessions. While both have the ability to remain viable in storage for 10 yr, species in this genus may be sensitive to collection timing, due to the limited phenological changes in the fruit during the ripening process, as both immature and mature fruit typically appear white in color (Wagner et al., 1999). It is also possible for species to vary in seed longevity at the population level (Hereford and Moriuchi, 2005).

Longevity and relative performance—The genus *Vaccinium* (Ericaceae) may show signs of aging in storage due to its significantly positive RP value. Ericaceae also had short-lived seeds in artificial aging tests (Probert et al., 2009). However, *Vaccinium* seed longevity in an experimental soil seed bank was highly variable, and multiple variables related to habitat were correlated with seed longevity (Hill and Kloet, 2005). Our study found variable RCIs for *Vaccinium* spp. (Appendix 1), paralleling the soil seed bank study and warranting further research.

Factors affecting variation in seed longevity—As evidenced here, there is wide variation in seed longevity throughout the Hawaiian flora. Variation throughout other floras and taxa has been documented, even under identical storage conditions (Probert et al., 2009), and there can be intraspecific variation in seed storage behavior (Argyris et al., 2005; Niedzielski et al., 2009; Walters et al., 2010). Seed longevity may also be influenced by aspects of the maternal plant environment, such as flower type, photoperiod, temperature, and moisture levels (Gutterman, 2000; Hereford and Moriuchi, 2005; Kochanek et al., 2010; Long et al., 2015). Shared habitat characteristics can sometimes determine longevity of seeds across varying taxa; e.g., alpine plants have short-lived seeds (Mondoni et al., 2011), while in a larger and more heterogeneous region like Western Australia, conventional seed banking is deemed suitable for at least short- to medium-term storage across the flora (Crawford et al., 2007). However, classification of storage behavior and longevity could certainly change based on results from longer studies.

Even in our study, caveats apply, such as those discussed above, and the fact that while we controlled for storage treatment when optimal treatments were known, for some species, different accessions were tested under different storage conditions over time. Storage environment is known to influence either the shape or the duration of the survival curve of a seed lot (Ellis and Roberts, 1980). It should be noted that the P70 threshold may fall within the sigmoidal phase of seed mortality (Bernal-Lugo and Leopold, 1998), such that a steep decline in viability may occur during or soon after a species' recommended RCI. It is also possible that some species currently classified as short-lived could have desiccation-intermediate storage behavior, because we can rarely quantify the percent moisture content of seeds, since many species are rare and sample sizes are often small. Accordingly, re-collection intervals presented here are only a guideline, a starting place for biodiversity managers to make conservation planning decisions. Thus, it is important that long-term studies like this one be conducted across many taxa and floras and continued in perpetuity to inform adaptive management, especially for a flora as threatened as Hawai'i's.

CONCLUSIONS

Seed storage is the most efficient way to preserve most of the Hawaiian flora (Weisenberger and Keir, 2014b), and our study supports these claims, suggesting conventional seed storage is applicable for most species for which recalcitrant and short-lived seed storage behaviors have not been detected. Conservation programs in Hawai'i are working to achieve Target 8 of the Hawai'i Strategy for Plant Conservation and the Global Strategy for Plant Conservation, and 75% of the Species of Conservation Importance rely on being able to be secured as seed collections in ex situ storage (Convention on Biological Diversity, 2011; Weisenberger and Keir, 2014b). Freeze sensitivity in seeds has obvious implications for seed banking because this behavior may reduce longevity in storage, currently limited to 5°C, until research can confirm the efficacy and safety of other potential storage methods, such as -80°C or cryopreservation. However, the confirmed longevity of several species stored at 5°C for 15–20 years or more suggests that “conventional seed banking” may not be the only form of low-cost seed banking that can provide for relatively long-term storage. Wyse et al. (2018) cautioned that Target 8 of the Global Strategy for Plant Conservation is likely unattainable for threatened species globally, because more than 25% of threatened species have recalcitrant seeds. Based on our study, it is probable that species with freeze-sensitive seeds account for more than 25% of rare species in Hawai'i, but with the knowledge that many of them can be stored medium to long term at 5°C, Target 8 of the Hawai'i Strategy for Plant Conservation for rare species is likely attainable with regard to ex situ capacity, as long as re-collection intervals are applied to maintain the viability of these collections.

Across all taxa, it is critical for conservation practitioners to have estimates of seed longevity in storage, so that collections can be used for restoration and replenished before significant loss of viability occurs (Guerrant and Fielder, 2004; Cochrane et al., 2007; PCA, 2015). This study will empower conservation programs across the state of Hawai'i to better utilize ex situ seed banking as a tool for species preservation and ecosystem restoration. This study also represents the largest number of species within a regional flora to be assessed for real-time seed longevity, with over 20 years of data. It is one of few studies to assess seed storage behavior for a large sampling of a native tropical flora and the first to find a large proportion of species with freeze-sensitive seed storage behavior. Thus, the Hawaiian flora can serve as a model for other tropical, subtropical, and island regions that might utilize seed banking to preserve species of conservation importance, now or in the future.

ACKNOWLEDGMENTS

We gratefully acknowledge the mentorship of Christina Walters and Lisa Hill, who were instrumental in identifying the freeze-sensitive seed storage behavior trend in Hawai'i. We thank Vickie Caraway and Nellie Sugii, whose support has made possible the longevity of this study and seed banking of T&E species in Hawai'i; and Margaret Clark, Loyal Mehrhoff, Steve Perlman, Jill Wagner, and Ken Wood for their intellectual contributions to the understanding of Hawaiian seed storage behavior. We gratefully acknowledge the dozens of dedicated field botanists who submitted collections to the seed banks over the last two decades, including from the authors' affiliated organizations and the Plant Extinction Prevention Program

of Hawai'i. We thank Celeste Hanley for assistance with graphical design, and Vickie Caraway, Rachael Davies, Nellie Sugii, Leland Werden, Rakan Zahawi, Kay Havens, and anonymous reviewers for providing feedback that improved the manuscript. We are grateful to the authors' affiliated organizations for providing funding and support for this research, including U.S. Fish and Wildlife Service (USFWS) Grant F13AC00918/F13AC00987 and Hawai'i Department of Land and Natural Resources—Division of Forestry and Wildlife (DLNR-DOFAW) Grant 15.165 to Lyon Arboretum. All collections of T&E species and/or from public lands were made or received under permits, including DLNR-DOFAW permits P-120 and ODF-011718AC (Lyon Arboretum Hawaiian Rare Plant Program); DLNR-DOFAW permit P-155 and USFWS permit TE043638-11 (ANRP); and DLNR-DOFAW permits P-233 and K2018-3018cc (NTBG); and we thank these and other landowners for granting permissions for seed collections.

AUTHOR CONTRIBUTIONS

A.Y.Y. designed the study; M.M.C., T.C., L.W., M.K., T.K., D.W., and A.Y.Y. carried out the experiments in the seed banks; R.K. designed databases and queries; M.M.C., T.C., and L.W. analyzed the data; M.M.C., T.C., L.W., M.K., T.K., D.W., and A.Y.Y. wrote and revised the manuscript.

DATA AVAILABILITY STATEMENT

Additional Supporting Information may be found online in the supporting information tab for this article. Collection locality information is withheld, because many species are rare, threatened, and/or endangered.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. Species tested for over 20 years and their re-collection intervals and storage behavior.

APPENDIX S2. Species with re-collection intervals <1 year and notes about storage behavior.

APPENDIX S3. Species with re-collection intervals <5 years.

APPENDIX S4. Recommended seed re-collection intervals to maintain 70% of highest germination, for all species tested in the Hawaiian Rubiaceae.

APPENDIX S5. Full data set for seed freeze sensitivity.

APPENDIX S6. Full data set for seed ex situ longevity.

APPENDIX S7. Summary information about the meaning of each column in each data set.

LITERATURE CITED

Akamine, E. K. 1951. Viability of Hawaiian forest tree seeds in storage at various temperatures and relative humidities. *Pacific Science* 5: 37–46.

- Angelovici, R., G. Galili, A. R. Fernie, and A. Fait. 2010. Seed desiccation: a bridge between maturation and germination. *Trends in Plant Science* 15: 211–218.
- Argyris, J., M. J. Truco, O. Ochoa, S. J. Knapp, D. W. Still, G. M. Lenssen, J. W. Schut, et al. 2005. Quantitative trait loci associated with seed and seedling traits in *Lactuca*. *Theoretical and Applied Genetics* 111: 1365–1376.
- Barrett, S. C. H., B. Emerson, and J. Mallet. 1996. The reproductive biology and genetics of island plants. *Philosophical Transactions of the Royal Society, B, Biological Sciences* 351: 725–733.
- Baskin, C. C., and J. M. Baskin. 2014. Seeds: ecology, biogeography, and evolution of dormancy and germination. Academic Press, San Diego, CA, USA.
- Bernal-Lugo, I., and A. C. Leopold. 1998. The dynamics of seed mortality. *Journal of Experimental Botany* 49: 1455–1461.
- Buitink, J., and O. Leprince. 2008. Intracellular glasses and seed survival in the dry state. *Comptes Rendus Biologies* 331: 788–795.
- Carlquist, S. J. 1974. Island biology. Columbia University Press, NY, NY, USA.
- Clark, J. R., R. H. Ree, M. E. Alfaro, M. G. King, W. L. Wagner, and E. H. Roalson. 2008. A comparative study in ancestral range reconstruction methods: re-tracing the uncertain histories of insular lineages. *Systematic Biology* 57: 693–707.
- Cochrane, J. A., A. D. Crawford, and L. T. Monks. 2007. The significance of ex situ seed conservation to reintroduction of threatened plants. *Australian Journal of Botany* 55: 356–361.
- Convention on Biological Diversity. 2011. Global strategy for plant conservation. Available at <https://www.cbd.int/gspc/strategy.shtml> [accessed 31 December 2018].
- CPC [Center for Plant Conservation]. 1994. An action plan for conserving hawaiian plant diversity. Center for Plant Conservation, St. Louis, MO, USA.
- Crane, J., A. L. Miller, J. W. Van Roekel, and C. Walters. 2003. Triacylglycerols determine the unusual storage physiology of *Cuphea* seed. *Planta* 217: 699–708.
- Crane, J., D. Kovach, C. Gardner, and C. Walters. 2006. Triacylglycerol phase and ‘intermediate’ seed storage physiology: a study of *Cuphea carthagenensis*. *Planta* 223: 1081–1089.
- Crawford, A. D., K. J. Steadman, J. A. Plummer, A. Cochrane, and R. J. Probert. 2007. Analysis of seed-bank data confirms suitability of international seed-storage standards for the Australian flora. *Australian Journal of Botany* 55: 18–29.
- Daws, M. I., N. C. Garwood, and H. W. Pritchard. 2005. Traits of recalcitrant seeds in a semi-deciduous tropical forest in Panama: some ecological implications. *Functional Ecology* 19: 874–885.
- Eira, M. T. S., E. A. A. da Silva, R. D. De Castro, S. Dussert, C. Walters, J. D. Bewley, and H. W. M. Hilhorst. 2006. Coffee seed physiology. *Brazilian Journal of Plant Physiology* 18: 149–163.
- Ellis, R. H., and E. H. Roberts. 1980. Improved equations for the prediction of seed longevity. *Annals of Botany* 45: 13–30.
- Ellis, R. H., and E. H. Roberts. 1981. The quantification of aging and survival in orthodox seeds. *Seed Science and Technology* 9: 373–409.
- Ellis, R. H., T. D. Hong, and E. H. Roberts. 1990. An intermediate category of seed storage behaviour? I. Coffee. *Journal of Experimental Botany* 41: 1167–1174.
- Ellis, R. H., T. D. Hong, and E. H. Roberts. 1991a. Effect of storage temperature and moisture on the germination of papaya seeds. *Seed Science Research* 1: 69–72.
- Ellis, R. H., T. D. Hong, E. H. Roberts, and U. Soetisna. 1991b. Seed storage behaviour in *Elaeis guineensis*. *Seed Science Research* 1: 99–104.
- Ellis, R. H., T. Mai-Hong, T. D. Hong, T. T. Tan, N. D. Xuan-Chuong, L. Q. Hung, B. Ngoc-Tam, and V. T. Le-Tam. 2007. Comparative analysis by protocol and key of seed storage behaviour of sixty Vietnamese tree species. *Seed Science and Technology* 35: 460–476.
- FAO. 2014. Genebank standards for plant genetic resources for food and agriculture. Food and Agriculture Organization of the United Nations, Rome, Italy.
- Fortini, L. B., J. Price, J. Jacobi, A. Vorsino, J. Burgett, K. W. Brinck, F. Amidon, et al. 2013. A landscape-based assessment of climate change vulnerability for all native Hawaiian plants. University of Hawai‘i, Hilo, HI, USA.
- Givnish, T. J., K. C. Millam, A. R. Mast, T. B. Paterson, T. J. Theim, A. L. Hipp, J. M. Henss, et al. 2008. Origin, adaptive radiation and diversification of the Hawaiian lobeliads (Asterales: Campanulaceae). *Proceedings of the Royal Society, B, Biological Sciences* 276: 407–416.
- Guerrant, E. O., and P. Fielder. 2004. Accounting for sample decline during ex situ storage and reintroduction. In E. O. Guerrant, K. Havens, and M. Maunder [eds.], *Ex situ plant conservation: supporting species survival in the wild*, 454–473. Island Press, Washington, D.C., USA.
- Guerrant, E. O., and T. N. Kaye. 2007. Reintroduction of rare and endangered plants: common factors, questions and approaches. *Australian Journal of Botany* 55: 362–370.
- Gutterman, Y. 2000. Maternal effects on seeds during development. In M. Fenner [ed.], *Seeds: the ecology of regeneration in plant communities*, 59–84. CABI Publishing, New York, New York, USA.
- Havens, K., E. O. Guerrant, M. Maunder, and P. Vitt. 2004. Guidelines for ex situ conservation collection management: minimizing risks. In E. O. Guerrant, K. Havens, and M. Maunder [eds.], *Ex situ plant conservation: supporting species survival in the wild*, 454–473. Island Press, Washington, D.C., USA.
- Hay, F. R., and R. J. Probert. 1995. Seed maturity and the effects of different drying conditions on desiccation tolerance and seed longevity in foxglove (*Digitalis purpurea* L.). *Annals of Botany* 76: 639–647.
- Hereford, J., and K. S. Moriuchi. 2005. Variation among populations of *Diodia teres* (Rubiaceae) in environmental maternal effects. *Journal of Evolutionary Biology* 18: 124–131.
- Hill, N. M., and S. P. V. Kloet. 2005. Longevity of experimentally buried seed in *Vaccinium*: relationship to climate, reproductive factors and natural seed banks. *Journal of Ecology* 93: 1167–1176.
- Hong, T., and R. Ellis. 1995. Interspecific variation in seed storage behaviour within 2 genera – *Coffea* and *Citrus*. *Seed Science and Technology* 23: 165–181.
- Hong, T. D., and R. H. Ellis. 1996. A protocol to determine seed storage behaviour. IPGRI Technical Bulletin no. 1. International Plant Genetic Resources Institute, Rome, Italy.
- Hong, T. D., S. Linington, and R. H. Ellis. 1996. seed storage behavior: a compendium. IPGRI Handbook for Genebanks no. 4. International Plant Genetic Resources Institute, Rome, Italy.
- Imada, C. 2012. Hawaiian native and naturalized vascular plants checklist. Bishop Museum, Honolulu, HI, USA.
- Inman-Narahari, F., R. Ostertag, S. Cordell, C. P. Giardina, K. Nelson-Kaula, and L. Sack. 2013. Seedling recruitment factors in low-diversity Hawaiian wet forest: towards global comparisons among tropical forests. *Ecosphere* 4: UNSP 24.
- IUCN [International Union for the Conservation of Nature]. 2018. The IUCN red list of threatened species, version 2018-2. Available at <http://www.iucn.org> [accessed 15 November 2018].
- Jennings, H., K. Wallin, J. Brennan, A. D. Valle, A. Guzman, D. Hein, S. Hunter, et al. 2016. Inbreeding, low genetic diversity, and spatial genetic structure in the endemic Hawaiian lobeliads *Clermontia fauriei* and *Cyanea pilosa* ssp. *longipedunculata*. *Conservation Genetics* 17: 497–502.
- Kochanek, J., K. J. Steadman, R. J. Probert, and S. W. Adkins. 2009. Variation in seed longevity among different populations, species and genera found in collections from wild Australian plants. *Australian Journal of Botany* 57: 123–131.
- Kochanek, J., Y. M. Buckley, R. J. Probert, S. W. Adkins, and K. J. Steadman. 2010. Pre-zygotic parental environment modulates seed longevity. *Austral Ecology* 35: 837–848.
- Leprince, O., and J. Buitink. 2010. Desiccation tolerance: from genomics to the field. *Plant Science* 179: 554–564.
- Leprince, O., G. A. F. Hendry, and B. D. McKersie. 1993. The mechanisms of desiccation tolerance in developing seeds. *Seed Science Research* 3: 231–246.
- Leprince, O., A. Pellizzaro, S. Berriri, and J. Buitink. 2017. Late seed maturation: drying without dying. *Journal of Experimental Botany* 68: 827–841.
- Lima, M. de Jr, T. D. Hong, Y. M. B. C. Arruda, A. M. S. Mendes, and R. H. Ellis. 2014. Classification of seed storage behaviour of 67 Amazonian tree species. *Seed Science and Technology* 42: 363–392.

- Lin, T.-P. 1996. Seed storage behaviour deviating from the orthodox and recalcitrant type. *Seed Science and Technology* 24: 523–532.
- Liu, U., and J. Dickie. 2017. Managing ex situ collections of wild species' seeds: use of biodiversity informatics in the Millennium Seed Bank to address challenges. *Proceedings of TDWG* 1: e20197. <https://doi.org/10.3897/twgdproceedings.1.20197>.
- Long, R. L., M. J. Gorecki, M. Renton, J. K. Scott, L. Colville, D. E. Goggin, L. E. Commander, et al. 2015. The ecophysiology of seed persistence: a mechanistic view of the journey to germination or demise. *Biological Reviews* 90: 31–59.
- Magistrali, P. R., A. C. José, J. M. R. Faria, and E. Gasparin. 2013. Physiological behavior of *Genipa americana* L. seeds regarding the capacity for desiccation and storage tolerance. *Journal of Seed Science* 35: 495–500.
- Marques, A., G. Buijs, W. Ligterink, and H. Hilhorst. 2018. Evolutionary ecophysiology of seed desiccation sensitivity. *Functional Plant Biology* 45: 1083–1095.
- Mondoni, A., R. J. Probert, G. Rossi, E. Vegini, and F. R. Hay. 2011. Seeds of alpine plants are short lived: implications for long-term conservation. *Annals of Botany* 107: 171–179.
- Niedzielski, M., C. Walters, W. Luczak, L. M. Hill, L. J. Wheeler, and J. Puchalski. 2009. Assessment of variation in seed longevity within rye, wheat and the intergeneric hybrid triticale. *Seed Science Research* 19: 213–224.
- Pammenter, N. W., and P. Berjak. 1999. A review of recalcitrant seed physiology in relation to desiccation-tolerance mechanisms. *Seed Science Research* 9: 13–37.
- PCA [Plant Conservation Alliance]. 2015. National Seed Strategy for Rehabilitation and Restoration. Plant Conservation Alliance, Washington, D.C., USA. Available at <https://www.blm.gov/programs/natural-resources/native-plant-communities/national-seed-strategy> [accessed 29 November 2018].
- PEPP [Plant Extinction Prevention Program of Hawai'i]. 2018. PEP species list. Website <http://www.peppi.org/pep-species-list.html> [accessed 29 November 2018].
- Pérez, H. E., L. M. Hill, and C. Walters. 2012. An analysis of embryo development in palm: interactions between dry matter accumulation and water relations in *Pritchardia remota* (Arecaceae). *Seed Science Research* 22: 97–111.
- Pritchard, H. W. 2004. Classification of seed storage types for ex situ conservation in relation to temperature and moisture. In E. O. Guerrant, K. Havens, and M. Maunder [eds.], *Ex situ plant conservation: supporting species survival in the wild*, 139–161. Island Press, Washington, D.C., USA.
- Pritchard, H. W. 2013. Classification of seed storage types for ex situ conservation in relation to temperature and moisture. In E. O. Guerrant, K. Havens, and M. Maunder [eds.], *Ex Situ Plant Conservation: Supporting Species Survival in the Wild*, 139–161. Island Press, Washington, DC.
- Pritchard, H. W., and J. B. Dickie. 2003. Predicting seed longevity: the use and abuse of seed viability equations. In R. D. Smith, J. B. Dickie, S. H. Linington, H. W. Pritchard, and R. J. Probert [eds.], *Seed conservation: turning science into practice*, 653–722. Royal Botanic Gardens, Kew, UK.
- Pritchard, H. W., M. I. Daws, B. J. Fletcher, C. S. Gaméné, H. P. Msanga, and W. Omondi. 2004. Ecological correlates of seed desiccation tolerance in tropical African dryland trees. *American Journal of Botany* 91: 863–870.
- Probert, R. J., M. I. Daws, and F. R. Hay. 2009. Ecological correlates of ex situ seed longevity: a comparative study on 195 species. *Annals of Botany* 104: 57–69.
- Roberts, E. H. 1973. Predicting the storage life of seeds. *Seed Science and Technology* 1: 499–514.
- Royal Botanic Gardens Kew. 2019a. Seed collection [online]. Website <https://www.kew.org/science/collections/seed-collection/about-millennium-seed-bank> [accessed 5 May 2019].
- Royal Botanic Gardens Kew. 2019b. Seed Information Database—SID, release 7.1. Available at <http://data.kew.org/sid/> [accessed 4 January 2019].
- Sakai, A. K., W. L. Wagner, and L. A. Mehrhoff. 2002. Patterns of endangerment in the Hawaiian flora. *Systematic Biology* 51: 276–302.
- Salazar, A., J. Maschinski, J. Possley, and K. Heineman. 2018. Seed germination of 53 species from the globally critically imperiled pine rockland ecosystem of South Florida, USA: effects of storage, phylogeny and life-history traits. *Seed Science Research* 28: 82–92.
- Schmidt, L. H. 2000. Guide to handling of tropical and subtropical forest seed. Danida Forest Seed Centre, Copenhagen, Denmark.
- Seaton, P. T., and N. S. J. Hailes. 1989. Effect of temperature and moisture content on the viability of *Cattleya aurantiaca* seed. In H. W. Pritchard [ed.], *Modern methods in orchid conservation*, 17–30. Cambridge University Press, Cambridge, UK.
- Teixeira, F. P., J. M. Rocha Faria, W. V. Souza Pereira, and A. C. Jose. 2018. Maturation and desiccation tolerance in seeds of *Sesbania virgata* (Cav.) Pers. *Floresta E Ambiente* 25: e20160419.
- Tweddle, J. C., J. B. Dickie, C. C. Baskin, and J. M. Baskin. 2003. Ecological aspects of seed desiccation sensitivity. *Journal of Ecology* 91: 294–304.
- USDA [U.S. Department of Agriculture] 2016. History of NCGRP: USDA ARS [online]. Available at <https://web.archive.org/web/20171223201718/https://www.ars.usda.gov/plains-area/fort-collins-co/center-for-agricultural-resources-research/plant-germplasm-preservation-research/docs/history-of-n-cgrp/> [accessed 23 November 2018].
- USFWS [U.S. Fish & Wildlife Service]. 2018. Listed species believed to or known to occur in Hawaii. ECOS Environmental Conservation Online System, Species occurrence by state. Available at https://ecos.fws.gov/tess_public/reports/species-listed-by-state-report?state=HI&status=list/. U.S. Fish & Wildlife Service, Washington, D.C., USA. [accessed 23 November 2018].
- Wagner, W. L., D. R. Herbst, and S. H. Sohmer. 1999. Manual of the flowering plants of Hawai'i, vols. 1 and 2. University of Hawai'i Press, Honolulu, HI, USA.
- Wagner, W. L., D. R. Herbst, N. Khan, and T. Flynn. 2012. Hawaiian vascular plant updates: a supplement to the manual of the flowering plants of Hawai'i and Hawai'i's ferns and fern allies, version 1.3. Smithsonian National Museum of Natural History, Washington, D.C., USA.
- Walters, C. 1998. Understanding the mechanisms and kinetics of seed aging. *Seed Science Research* 8: 223–244.
- Walters, C. 2004. Guidelines for seed storage. In E. O. Guerrant, K. Havens, and M. Maunder [eds.], *Ex situ plant conservation: supporting species survival in the wild*, 442–453. Island Press, Washington, D.C., USA.
- Walters, C. 2015. Orthodoxy, recalcitrance and in-between: describing variation in seed storage characteristics using threshold responses to water loss. *Planta* 242: 397–406.
- Walters, C., L. M. Hill, and L. J. Wheeler. 2005a. Dying while dry: kinetics and mechanisms of deterioration in desiccated organisms. *Integrative and Comparative Biology* 45: 751–758.
- Walters, C., L. M. Wheeler, and J. M. Grotenhuis. 2005b. Longevity of seeds stored in a genebank: species characteristics. *Seed Science Research* 15: 1–20.
- Walters, C., D. Ballesteros, and V. A. Vertucci. 2010. Structural mechanics of seed deterioration: standing the test of time. *Plant Science* 179: 565–573.
- Weisenberger, L., and M. J. Keir. 2014a. Assessing status, capacity, and needs for the ex situ conservation of the Hawaiian flora. *Pacific Science* 68: 525–536.
- Weisenberger, L., and M. Keir. 2014b. Hawai'i strategy for plant conservation. Hawai'i Plant Conservation Network, Honolulu, HI, USA.
- Willis, K. J. [ed.]. 2017. State of the world's plants 2017. Report. Royal Botanic Gardens, Kew, UK.
- Wyse, S. V., and J. B. Dickie. 2018. Taxonomic affinity, habitat and seed mass strongly predict seed desiccation response: a boosted regression trees analysis based on 17 539 species. *Annals of Botany* 121: 71–83.
- Wyse, S. V., J. B. Dickie, and K. J. Willis. 2018. Seed banking not an option for many threatened plants. *Nature Plants* 4: 848–850.
- Yoshinaga, A. Y., and C. Walters. 2003. Conservation of tropical island seeds - an example from Hawai'i. In R. D. Smith, J. B. Dickie, S. H. Linington, H. W. Pritchard, and R. J. Probert [eds.], *Seed conservation: turning science into practice*, 955–963. Royal Botanic Gardens, Kew, UK.
- Zhang, N., B. Wen, M. Ji, and Q. Yan. 2014. Low-temperature storage and cryopreservation of grapefruit (*Citrus paradisi* Macfad.) seeds. *Cryoletters* 35: 418–426.

APPENDIX 1. (Continued)

Family	Taxon	Auth.	Rare	Freeze-sensitivity in genus or family	Germination (%)		Storage		No. Accessions		Seed bank(s)	Storage notes
					Highest	Most Recent	Years stored	RCI (Yr)	Supp.	Con.		
Campanulaceae	<i>Brighamia insignis</i>	A.Gray	Y	F	96	26	10.0	5-10	1	0	L	
	<i>Brighamia rockii</i>	St.John	Y	F	27	22	3.6	3+	1	4	L	
	<i>Clermontia fauriei</i>	H.Lév.	N	G/F	96	35	10.0	5-10	1	0	L	
	<i>Clermontia hawaiiensis</i>	Rock	N	G/F	100	98	10.0	10+	2	0	L	
	<i>Clermontia kakeana</i>	Meyen	N	G/F	98	93	20.3	20+	5	1	A, L	
	<i>Clermontia montis-loa</i>	Rock	N	G/F	24	7	17.5	<5	1	0	L	
	<i>Clermontia oblongifolia</i>	Gaudich.	Y	G/F	100	93	17.4	15+	4	1	L	
	<i>Clermontia parviflora</i>	Gaudich. ex A.Gray	N	G/F	98	95	5.1	5+	1	0	L	
	<i>Clermontia persicifolia</i>	Gaudich.	N	G/F	94	84	5.3	5+	1	0	L	
	<i>Clermontia pyricularia</i>	Hillebr.	Y	G/F	97	77	12.6	10+	1	0	L	
	<i>Cyanea acuminata</i>	Hillebr.	Y	G/F	97	81	10.1	10+	1	2	A	
	<i>Cyanea angustifolia</i>	Hillebr.	N	G/F	98	39	20.3	10-15	5	0	L	
	<i>Cyanea asarifolia</i>	H.St.John	Y	G/F	72	72	5.5	5+	1	1	L	
	<i>Cyanea calycina</i>	(Cham.) Lammers	Y	G/F	100	92	5.1	5+	1	0	A	
	<i>Cyanea copelandii</i>	Rock	Y	G/F	84	62	9.1	5+	1	0	L	
	<i>Cyanea coriacea</i>	Hillebr.	N	G/F	48	48	1.0	1+	1	0	N	
	<i>Cyanea crispa</i>	(Gaudich.) Lammers, Givnish & Sytsma	Y	G/F	83	70	15.0	15+	5	0	A, L	
	<i>Cyanea dunbariae</i>	Rock	Y	G/F	50	42	17.4	15-20	1	0	L	
	<i>Cyanea duvalliorum</i>	Lammers & H.Oppenh.	Y	G/F	100	96	5.0	5+	1	0	L	
	<i>Cyanea grimesiana</i>	Gaudich.	Y	G/F	92	90	14.2	10+	8	9	A	
	<i>Cyanea horrida</i>	(Rock) O.Deg. & Hosaka	Y	G/F	72	72	5.3	5+	6	0	L	
	<i>Cyanea kauaualaensis</i>	H.Oppenh. & Lorence	Y	G/F	88	72	8.3	5+	3	0	L	
	<i>Cyanea koolauensis</i>	Lammers, Givnish & Sytsma	Y	G/F	64	8	6.2	<5	1	0	A	
	<i>Cyanea kunthiana</i>	Hillebr.	Y	G/F	96	90	5.0	5+	1	0	L	
	<i>Cyanea lanceolata</i>	(Gaudich.) Lammers, Givnish & Sytsma	Y	G/F	100	76	2.0	2+	1	0	L	
	<i>Cyanea lobata</i>	H.Mann	Y	G/F	79	69	5.4	5+	1	0	L	
	<i>Cyanea longiflora</i>	(Wawra) Lammers, Givnish & Sytsma	Y	G/F	86	49	14.4	5-10	30	3	A	
	<i>Cyanea macrostegia</i>	Hillebr.	N	G/F	94	94	5.5	5+	1	0	L	
	<i>Cyanea magnicalyx</i>	Lammers	Y	G/F	100	86	10.4	10+	3	0	L	
	<i>Cyanea membranacea</i>	Rock	Y	G/F	90	36	18.5	10-15	4	0	L	
	<i>Cyanea munitoi</i>	(Hosaka) Lammers	Y	G/F	48	48	13.2	10+	1	0	L	
	<i>Cyanea pinnatifida</i>	(Cham.) E.Wimm.	Y	G/F	96	89	5.3	5+	2	0	L	
	<i>Cyanea profuga</i>	C.N.Forbes	Y	G/F	92	92	9.8	10+	1	0	L	
	<i>Cyanea purpurellifolia</i>	(Rock) Lammers, Givnish & Sytsma	Y	G/F	84	50	10.5	5-10	2	2	L	
	<i>Cyanea remyi</i>	Rock	Y	G/F	60	60	10.7	10+	1	0	L	
	<i>Cyanea rivularis</i>	Rock	Y	G/F	96	34	10.2	<10	1	0	L	
	<i>Cyanea shipmanii</i>	Rock	Y	G/F	100	98	10.5	10+	2	0	L	
	<i>Cyanea solanacea</i>	Hillebr.	Y	G/F	100	96	8.6	5+	2	0	L	
	<i>Cyanea st-johnii</i>	(Hosaka) Lammers, Givnish & Sytsma	Y	G/F	100	84	5.1	5+	5	2	A, L	
<i>Cyanea stictophylla</i>	Rock	Y	G/F	95	94	2.6	2+	1	0	L		
<i>Cyanea superba</i>	A.Gray	Y	G/F	91	91	15.2	15+	7	2	A, L		
<i>Cyanea truncata</i>	Rock	Y	G/F	70	16	11.5	5-10	2	1	L		
<i>Delissea kauaiensis</i>	(Lammers) Lammers	Y	G*/F	100	88	5.3	5+	3	0	L		
<i>Delissea rhytidosperma</i>	H.Mann	Y	G*/F	92	76	18.5	15+	4	2	L		
<i>Delissea waianaensis</i>	Lammers	Y	G*/F	100	84	15.1	15+	13	9	A, L		
<i>Labelia dunbariae</i>	Rock	Y	G/F	98	80	15.5	15+	2	0	L		
<i>Labelia grayana</i>	E.Wimm.	N	G/F	80	71	10.4	10+	1	0	L		

(Continued)

APPENDIX 1. (Continued)

Family	Taxon	Auth.	Rare	Freeze-sensitivity in genus or family	Germination (%)		Storage		No. Accessions		Seed bank(s)	Storage notes	
					Highest	Most Recent	Years stored	RCI (Yr)	Supp.	Con.			
Capparidaceae Caryophyllaceae	<i>Labelia hypoleuca</i>	Hillebr.	N	G/F	100	78	15.0	15+	1	1	A, L		
	<i>Labelia koolauensis</i>	(Hosaka & Fosberg) Lammer	Y	G/F	80	0	15.0	<5	4	0	A		
	<i>Labelia monostachya</i>	(Rock) Lammer	Y	G/F	66	64	5.5	5+	6	1	L		
	<i>Labelia niihauensis</i>	St.John	Y	G/F	89	88	17.2	15+	3	0	L		
	<i>Labelia oahuensis</i>	Rock & Hosaka	Y	G/F	97	92	16.0	15+	3	0	A, L		
	<i>Labelia yuccoides</i>	Hillebr.	N	G/F	92	80	18.7	15+	1	0	L		
	<i>Trematolobelia grandifolia</i>	(Rock) O.Deg.	N	F	88	44	12.4	5-10	1	0	L		
	<i>Trematolobelia kaale</i>	(O.Deg.) Lammer	N	F	92	72	15.1	15+	2	1	L		
	<i>Trematolobelia singularis</i>	H.St.John	Y	F	100	100	5.1	5+	1	0	L		
	<i>Capparis sandwichiensis</i>	DC.	N		100	68	3.3	<5	4	0	L		
	<i>Schiedea adamantis</i>	H.St.John	Y		90	88	16.8	15+	21	1	L		
	<i>Schiedea diffusa</i>	A.Gray	Y		97	97	5.0	5+	1	0	L		
	<i>Schiedea globosa</i>	H.Mann	N		100	82	5.5	5+	2	0	L		
	<i>Schiedea helleri</i>	Sherff	Y		21	21	5.5	5+	1	0	L		
	<i>Schiedea kaale</i>	Wawra	Y		80	76	16.0	15+	3	1	A		
	<i>Schiedea lynchoides</i>	Hillebr.	Y		88	81	5.5	5+	1	0	L		
	<i>Schiedea nuttallii</i>	Hook.	Y		87	87	15.6	15+	2	0	A		
	<i>Schiedea obovata</i>	(Sherff) W.L.Wagner & Weller	Y		100	96	16.4	15+	12	0	A		
	<i>Schiedea trinervis</i>	(H.Mann) Pax & K.Hoffm.	Y		92	100	15.8	15+	7	2	A, L		
	Convolvulaceae	<i>Schiedea viscosa</i>	H.Mann	Y		96	96	5.6	5+	1	0	L	
<i>Silene alexandri</i>		Hillebr.	Y		94	94	5.5	5+	3	0	L		
<i>Silene hawaiiensis</i>		Sherff	Y		100	97	10.1	10+	1	0	A		
<i>Silene lanceolata</i>		A.Gray	Y		68	62	15.1	15+	4	0	A, L		
<i>Silene perlimanii</i>		W.L.Wagner, D.R.Herbst & Sohmer	Y		86	85	9.1	10+	9	3	A, L		
<i>Bonania menziesii</i>		A.Gray	Y		60	28	8.4	5-10	1	0	L		
<i>Cuscuta sandwichiensis</i>		Choisy	N		58	58	2.4	2+	1	0	L		
<i>Jacquemontia sandwicensis</i>		A.Gray	N		52	90	10.0	10+	1	0	L	D	
<i>Sicyos pachycarpus</i>		Hook. & Arn.	N		50	8	5.6	<5	1	0	L		
<i>Sicyos waimanaloensis</i>		H.St.John	N		32	11	2.5	<5	1	0	L		
Cucurbitaceae		<i>Carex alligata</i>	Boott	N	G*/F	76	55	5.4	5+	1	0	L	D
		<i>Carex meyenii</i>	Nees	N	G*/F	36	7	17.5	10-15	1	0	L	D
	<i>Carex wahuensis</i>	C.A.Mey.	N		28	23	15.1	15+	1	1	L		
	<i>Cyperus hillebrandii</i>	Boeckler	N		41	41	15.2	15+	3	0	L	D	
	<i>Cyperus javanicus</i>	Houtt.	N		96	96	17.3	15+	3	0	L	D	
	<i>Cyperus pennatifolius</i>	Kük.	Y		42	42	4.1	4+	1	0	L	D	
	<i>Cyperus trachysanthos</i>	Hook. & Arn.	Y		88	88	17.5	15+	7	0	L	D	
	<i>Fimbristylis cymosa</i>	R.Br.	N		96	83	5.1	5+	2	0	L		
	<i>Gahnia beecheyi</i>	H.Mann	N		20	14	3.0	3+	1	0	L		
	<i>Machaena angustifolia</i>	(Gaudich.) T.Koyama	N		80	74	10.0	10+	1	0	L	D	
Dipentodontaceae	<i>Morelia gahniiformis</i>	(Gaudich.) Kern	N		88	88	5.0	5+	1	0	A		
	<i>Schoenoplectella juncooides</i>	(Roxb.) Lye	N		94	57	11.1	5-10	1	0	L		
	<i>Perrottetia sandwicensis</i>	A.Gray	N		60	52	2.0	2+	1	0	L		
	<i>Drosera anglica</i>	Huds.	N		83	83	2.3	2+	2	0	L		
	<i>Vaccinium calycinum</i>	Sm.	N		76	75	18.4	15+	3	0	L		
	<i>Vaccinium dentatum</i>	Sm.	N		100	16	5.0	<5	1	0	L		
	<i>Vaccinium reticulatum</i>	Sm.	N		90	66	12.5	10+	1	1	L		
	<i>Euphorbia celastroides</i>	Boiss.	Y		68	66	10.0	10+	1	0	A		
	<i>Euphorbia haebeleana</i>	DR.Herbst	Y		82	67	10.1	10+	1	0	A		
	<i>Euphorbia herbstii</i>	(W.L.Wagner) Oudejans	Y		83	63	10.0	10+	1	0	A		
Euphorbiaceae	<i>Euphorbia skottsbergii</i>	Sherff	Y		35	5	12.5	10-15	4	0	L		

(Continued)

APPENDIX 1. (Continued)

Family	Taxon	Auth.	Rare	Freeze-sensitivity in genus or family	Germination (%)		Storage		No. Accessions		Seed bank(s)	Storage notes	
					Highest	Most Recent	Years stored	RCI (Yr)	Supp.	Con.			
Fabaceae	<i>Acacia koa</i>	A.Gray	N		100	94	15.2	15+	2	0	L		
	<i>Canavalia galeata</i>	Gaudich.	N		96	88	10.1	10+	2	0	A, L		
	<i>Canavalia hawaiiensis</i>	O.Deg. & Sauer	N		87	87	15.5	15+	1	0	L		
	<i>Erythrina sandwicensis</i>	O.Deg.	N		92	68	10.2	10+	2	0	L		
	<i>Mezoneuron kavaiense</i>	Hillebr.	Y		80	80	10.3	10+	3	0	L		
	<i>Senna gaudichaudii</i>	(Hook. & Arn.) H.S.Irwin & Barneby	N		76	27	10.0	5-10	2	0	L		
	<i>Sesbania tomentosa</i>	Hook. & Arn.	Y		100	36	15.8	5-10	2	0	L		
	<i>Sophora chrysophylla</i>	Seem.	N		88	4	15.5	10-15	1	1	L		
	<i>Vigna marina</i>	Merr.	N		100	100	10.1	10+	2	0	L		
	<i>Vigna o-wahuensis</i>	Vogel	Y		100	55	10.1	5-10	1	0	L		
	Gentianaceae	<i>Schenkia sebaeoides</i>	Griseb.	Y		88	33	10.2	5-10	3	1	L	
		<i>Cyrtandra cordifolia</i>	Gaudich.	N		92	59	12.5	10-15	2	0	L	
	Gesneriaceae	<i>Cyrtandra dentata</i>	St.John & Storey	Y		98	82	13.9	10+	10	2	A, L	
		<i>Cyrtandra filipes</i>	Hillebr.	Y		98	54	5.5	<5	1	0	L	
		<i>Cyrtandra gracilis</i>	Hillebr.	Y		74	50	10.4	<10	3	0	L	
		<i>Cyrtandra grandiflora</i>	Gaudich.	N		97	42	15.4	10-15	1	1	L	
		<i>Cyrtandra hawaiiensis</i>	C.B.Clark	N		97	82	5.4	5+	1	0	L	
<i>Cyrtandra lessoniana</i>		Gaudich.	N		86	78	5.1	5+	1	0	L		
<i>Cyrtandra rivularis</i>		St.John & Storey	N		94	58	5.5	<5	1	0	L		
<i>Cyrtandra sessilis</i>		St.John & Storey	Y		100	10	10.2	<5	2	0	L		
<i>Cyrtandra subumbellata</i>		(Hillebr.) St.John & Storey	Y		88	42	8.0	<5	1	0	A		
<i>Cyrtandra viridiflora</i>		St.John & Storey	Y		96	36	5.0	<5	2	0	L		
Goodeniaceae	<i>Scaevola chamissoniana</i>	Gaudich.	N		70	68	5.4	5+	1	0	L		
	<i>Scaevola gaudichaudiana</i>	Cham.	N		64	64	5.0	5+	2	0	L		
	<i>Scaevola gaudichaudii</i>	Hook. & Arn.	N		86	86	5.4	5+	1	0	L		
	<i>Scaevola procera</i>	Hillebr.	N		76	60	10.2	10+	1	0	L		
	<i>Scaevola taccada</i>	(Gaertn.) Roxb.	N		88	88	15.4	15+	1	0	L		
	<i>Gunnera petaloides</i>	Gaudich.	N		88	0	10.4	<1	2	0	L	SL	
	<i>Hydrangea arguta</i>	(Gaudich.) Y.De Smet & C.Granados	N		86	9	15.0	<5	4	0	A, L		
	<i>Sisyrinchium acre</i>	H.Mann	N		94	94	5.0	5+	1	0	L	D	
	<i>Joinvillea ascendens</i>	Gaudich. ex Brongn. & Gris	Y		47	47	5.1	5+	1	4	A, L		
	<i>Luzula hawaiiensis</i>	(Hillebr.) O.Deg. & I.Deg.	N		87	80	5.0	5+	1	0	L		
Juncaceae	<i>Phyllostegia electra</i>	C.N.Forbes	Y		70	70	1.0	1+	1	0	N		
	<i>Phyllostegia floribunda</i>	Benth.	Y		95	95	5.0	5+	1	0	L		
	<i>Phyllostegia grandiflora</i>	Benth.	N		64	32	5.4	<5	1	0	L		
	<i>Phyllostegia kaalaensis</i>	H.St.John	Y		84	24	10.1	5-10	1	0	A		
	<i>Phyllostegia mollis</i>	Benth.	Y		86	56	10.0	5-10	4	0	A, L		
	<i>Phyllostegia stachyoides</i>	A.Gray	Y		88	18	15.5	<15	1	0	L		
	<i>Phyllostegia velutina</i>	(Sherff) H.St.John	Y		40	0	15.0	5-10	1	1	L		
	<i>Plectranthus parviflorus</i>	R.Br.	N		96	84	15.1	15+	2	0	L		
	<i>Vitex rotundifolia</i>	Lf.	N		88	78	10.5	10+	1	0	L		
	Loganiaceae	<i>Labordia cyrtandrae</i>	(Baill.) Skottsb.	Y		88	80	7.7	5+	3	2	A	
<i>Labordia kaalae</i>		C.N.Forbes	Y		85	74	10.0	10+	1	0	A		
<i>Labordia lorenciana</i>		K.R.Wood, W.L.Wagner & T.J.Motley	Y		64	32	5.4	<5	1	0	L		
<i>Labordia tinifolia</i>		A.Gray	Y		84	82	15.3	15+	2	0	L		

(Continued)

APPENDIX 1. (Continued)

Family	Taxon	Auth.	Rare	Freeze-sensitivity in genus or family	Germination (%)			Storage			No. Accessions		Seed bank(s)	Storage notes
					Highest	Most Recent	Years stored	RCI (Yr)	Supp.	Con.				
Malvaceae	<i>Abutilon menziesii</i>	Seem.	Y		66	66	15.3	15+	1	1	1	L	D	
	<i>Abutilon sandwicense</i>	(O.Deg.) Christoph.	Y		80	50	11.7	5-10	1	0	0	A	D	
Myrtaceae	<i>Gossypium tomentosum</i>	Nutt. ex Seem.	N		95	76	10.0	10+	2	0	0	L		
	<i>Hibiscadelphus distans</i>	L.E.Bishop & D.R.Herbst	Y		45	39	5.0	5+	1	0	0	L		
	<i>Hibiscadelphus hualalaiensis</i>	Rock	Y		72	72	3.0	3+	1	0	0	L		
	<i>Hibiscus arnottianus</i>	A.Gray	Y		82	82	1.3	1+	1	0	0	L		
	<i>Hibiscus brackenridgei</i>	A.Gray	Y		55	42	13.8	10+	2	2	2	A, L		
	<i>Hibiscus furcellatus</i>	Lam.	N		76	76	17.4	15+	2	0	0	L		
	<i>Hibiscus waiameae</i>	A.Heller	Y		60	60	6.4	5+	1	0	0	L		
	<i>Kokia drynarioides</i>	Lewton	Y		90	90	10.3	10+	1	0	0	L		
	<i>Kokia kauaiensis</i>	(Rock) O.Deg. & Duvel	Y		48	45	10.6	10+	1	0	0	L		
	<i>Sida fallax</i>	Walp.	N		40	31	20.4	20+	1	1	1	L		
	<i>Thespesia populnea</i>	(L.) Correa	N		100	80	17.8	15+	1	0	0	L		
	<i>Metrosideros macropus</i>	Hook. & Arn.	N		38	15	15.0	10-15	2	0	0	L		
	<i>Metrosideros polymorpha</i>	Gaudich.	N		84	28	20.0	15-20	7	2	2	A, L		
	<i>Metrosideros rugosa</i>	A.Gray	N		9	3	5.0	<5	1	0	0	L	LSF	
	<i>Metrosideros tremuloides</i>	Knuth	N		52	52	7.6	5+	1	1	1	L		
<i>Pisonia brunoniana</i>	Endl.	N		68	0	6.7	<1	2	0	0	L	SL		
<i>Liparis hawaiiensis</i>	H.Mann	N		90	85	2.5	2+	3	0	0	L			
<i>Freycinetia arborea</i>	Gaudich.	N		66	0	5.0	<5	1	0	0	L			
<i>Argemone glauca</i>	Pope	N		60	13	15.4	5-10	4	3	3	L	D		
<i>Antidesma platyphyllum</i>	H.Mann	N		70	8	4.0	<1	5	0	0	A, L	SL		
<i>Flueggea neowawraea</i>	W.J.Hayden	Y		100	48	15.1	5-10	1	0	0	A			
<i>Phytolacca sandwicensis</i>	Endl.	N		98	48	5.2	<5	2	0	0	L			
<i>Peperomia latifolia</i>	Miq.	N		96	80	15.5	15+	1	2	2	L			
<i>Peperomia leptostachya</i>	Hook. & Arn.	N		96	89	15.1	15+	1	1	1	L			
<i>Peperomia membranacea</i>	Hook. & Arn.	N		94	46	15.1	10-15	1	0	0	L			
<i>Peperomia tetraphylla</i>	Hook. & Arn.	N		96	78	15.5	15+	2	1	1	L			
<i>Pittosporum flocculosum</i>	(Hillebr.) Sherff	N		76	52	10.1	<10	1	0	0	A			
<i>Pittosporum glabrum</i>	Hook. & Arn.	N		85	72	1.0	1+	1	0	0	A			
<i>Pittosporum hosmeri</i>	Rock	N		61	61	2.1	2+	1	0	0	L			
<i>Plantago princeps</i>	Cham. & Schitdl.	Y		100	100	13.6	10+	5	1	1	A, L			
<i>Cenchrus agrimonoides</i>	Trin.	Y		69	69	10.1	10+	1	1	1	A			
<i>Deschampsia nubigena</i>	Hillebr.	N		42	21	10.0	<5	2	0	0	L			
<i>Dissochordrus biflorus</i>	Kuntze	N		20	12	5.2	<5	1	0	0	L			
<i>Eragrostis atropioides</i>	Hillebr.	N		92	49	15.4	10-15	1	0	0	L			
<i>Eragrostis deflexa</i>	Hitchc.	N		100	80	17.4	15+	3	0	0	L			
<i>Eragrostis grandis</i>	Hillebr.	N		89	89	1.1	1+	1	1	1	L	D		
<i>Eragrostis variabilis</i>	Gaudich.	N		78	78	5.1	5+	1	0	0	L	D		
<i>Isachne distichophylla</i>	Munro ex Hillebr.	N		95	53	5.3	<5	1	0	0	L	D		
<i>Ischaemum byrone</i>	Hitchc.	Y		10	10	8.5	5+	1	0	0	L			
<i>Panicum niuhauense</i>	St.John	Y		94	94	14.0	10+	3	0	0	L			
<i>Panicum tenuifolium</i>	Hook. & Arn.	N		66	46	15.1	10-15	1	0	0	L			
<i>Panicum torridum</i>	Gaudich.	N		72	64	2.0	2+	1	0	0	L			
<i>Rumex albenscens</i>	Hillebr.	N		88	76	15.4	15+	2	0	0	L			
<i>Portulaca lutea</i>	Sol. ex G.Forst.	N		100	100	5.2	5+	1	0	0	L			
<i>Portulaca sclerocarpa</i>	A.Gray	Y		100	89	10.0	10+	1	0	0	A			

(Continued)

APPENDIX 1. (Continued)

Family	Taxon	Auth.	Rare	Freeze-sensitivity in genus or family	Germination (%)		Storage		No. Accessions		Seed bank(s)	Storage notes
					Highest	Most Recent	Years stored	RCI (Yr)	Supp.	Con.		
Primulaceae	<i>Lysimachia hillebrandii</i>	Hook.f. ex A.Gray	N		54	54	5.0	5+	2	0	A, L	
	<i>Lysimachia mauritiana</i>	Lam.	N		90	90	15.5	15+	1	0	L	
	<i>Lysimachia remyi</i>	Hillebr.	N		23	23	1.1	1+	1	1	A, L	
Ranunculaceae	<i>Myrsine lessertiana</i>	A.D.C.	N		92	79	3.5	3+	1	0	L	
	<i>Ranunculus mauianus</i>	A.Gray	Y		94	94	1.4	1+	1	0	L	
	<i>Alphitonia ponderosa</i>	Hillebr.	N		16	16	1.4	1+	1	0	L	
	<i>Colubrina oppositifolia</i>	Brongn. ex H.Mann	Y		52	52	15.0	15+	2	0	L	
	<i>Gouania vitifolia</i>	A.Gray	Y		68	61	5.2	5+	1	0	A	
	<i>Osteomeles anthyllifolia</i>	Lindl.	N		80	74	17.4	15+	2	1	L	
Rosaceae	<i>Rubus hawaiiensis</i>	A.Gray	N		54	54	0.9	1+	1	0	L	
	<i>Bobea elatior</i>	Gaudich.	N	F	28	3	16.3	10-15	2	0	L	
Rubiaceae	<i>Bobea sandwicensis</i>	Hillebr.	N	F	40	5	10.01	<5	1	0	A	
	<i>Coprosma ernodeoides</i>	A.Gray	N	F	100	92	1.8	2+	1	0	L	
	<i>Coprosma foliosa</i>	A.Gray	N	F	100	100	5.2	5+	1	1	L	
	<i>Coprosma kauensis</i>	A.Heller	Y	F	52	42	2.4	2+	1	0	L	
	<i>Coprosma longifolia</i>	Hook.f.	N	F	84	84	5.0	5+	1	0	A	
	<i>Coprosma rhynochocarpa</i>	A.Gray	N	F	92	52	5.2	<5	1	0	L	
	<i>Gardenia brighamii</i>	H.Mann	Y	F	72	47	13.4	5-10	9	2	L	
	<i>Gardenia manni</i>	St.John & Kuykendall	Y	F	80	80	1.0	1+	1	0	A	
	<i>Kadua acuminata</i>	Cham. & Schitdl.	N	F	49	5	15.1	<5	3	0	L	
	<i>Kadua affinis</i>	Cham. & Schitdl.	N	F	99	88	20.4	20+	3	1	A, L	
	<i>Kadua centranthoides</i>	Hook. & Arn.	N	F	77	61	2.1	2+	2	0	A, L	
	<i>Kadua degeneri</i>	W.L.Wagner & Lorence	Y	F	84	84	14.2	10+	4	0	A	
Rutaceae	<i>Kadua littoralis</i>	Hillebr.	N	F	100	88	5.0	5+	1	0	L	
	<i>Kadua parvula</i>	A.Gray	Y	F	76	70	17.3	15+	5	0	A, L	
	<i>Kadua tryblilum</i>	(D.R.Herbst & W.L.Wagner) W.L.Wagner & Lorence	N	F	70	70	2.0	2+	1	0	N	
	<i>Nertera granadensis</i>	Druce	N	F	78	36	8.4	5-10	1	0	L	
	<i>Psychotria hobyi</i>	Sohmer	Y	F	72	72	7.5	5+	1	0	L	
Santalaceae	<i>Psychotria mariniana</i>	(Cham. & Schitdl.) Fosberg	N	F	70	0	15.4	<1	1	0	L	SL
	<i>Psychotria odorata</i>	(G.Forst.) A.C.Sm. & S.P.Darwin	N	F	52	36	15.4	5-10	1	0	L	
	<i>Melicope clusiifolia</i>	(A.Gray) T.G.Hartley & B.C.Stone	N	F	80	9	1.3	<1	1	0	L	FS?
	<i>Melicope cornuta</i>	(Hillebr.) Appelhans, K.R.Wood & W.L.Wagner	Y	F	24	12	2.4	<5	1	0	A	
Sapindaceae	<i>Melicope hawaiiensis</i>	(Wawra) T.G.Hartley & B.C.Stone	N	F	76	76	9.3	10+	2	0	L	
	<i>Santalum ellipticum</i>	Gaudich.	N	G*	100	100	5.0	5+	3	0	L	
	<i>Santalum freycinetianum</i>	Gaudich.	E	G*	100	67	10.3	5-10	1	0	L	
	<i>Santalum paniculatum</i>	Hook. & Arn.	N	G*	100	100	2.0	2+	1	0	L	
Sapotaceae	<i>Dodonaea viscosa</i>	Jacq.	N		88	70	15.0	15+	3	0	A, L	
	<i>Sapindus oahuensis</i>	Hillebr.	N		24	10	2.6	<1	1	0	L	SL
	<i>Sapindus saponaria</i>	L.	N		86	24	15.0	10-15	1	1	L	
Scrophulariaceae	<i>Planchonella sandwicensis</i>	Pierre	N		10	0	0.7	<1	1	0	L	SL/R
	<i>Sideroxylon polynesicum</i>	(Hillebr.) Smedmark & Anderb.	N		8	5	0.9	<1	1	0	L	SL/R
Scrophulariaceae	<i>Myoporium sandwicense</i>	A.Gray	N		72	32	7.5	5-10	2	1	L	
	<i>Myoporium stellatum</i>	(G.L.Webster) O.Deg. & I.Deg.	N		66	20	2.3	<5	1	0	L	

(Continued)

APPENDIX 1. (Continued)

Family	Taxon	Auth.	Rare	Freeze-sensitivity in genus or family	Germination (%)		Storage		No. Accessions		Seed bank(s)	Storage notes
					Highest	Most Recent	Years stored	RCI (Yr)	Supp.	Con.		
Solanaceae	<i>Lycium sandwicense</i>	A.Gray	N		86	78	2.6	2+	3	0	L, N	
	<i>Solanum americanum</i>	Mill.	N		100	94	15.5	15+	2	0	L	
	<i>Solanum incompletum</i>	Dunal	Y		94	90	15.4	15+	1	2	A, L	
	<i>Solanum nelsonii</i>	Dunal	Y		76	76	2.1	2+	1	0	L, N	
	<i>Solanum sandwicense</i>	Hook. & Arn.	Y		90	90	18.4	15+	4	0	L	
Thymeleaceae	<i>Wikstroemia oahuensis</i>	Rock	N		80	24	0.5	<1	1	0	A	FS?
	<i>Wikstroemia uva-ursi</i>	A.Gray	N		88	25	15.2	5-10	2	0	L	FS?
Urticaceae	<i>Boehmeria grandis</i>	A.Heller	N	F	54	38	15.0	15+	3	2	L	
	<i>Neraudia angulata</i>	R.S.Cowan	Y	G*/F	64	12	10.2	5-10	3	3	A	
	<i>Neraudia ovata</i>	Gaudich.	Y	G*/F	20	20	6.1	5+	2	0	L	
	<i>Pipturus albidus</i>	A.Gray ex H.Mann	N	F	45	22	20.4	15-20	1	1	L	
	<i>Touchardia latifolia</i>	Gaudich.	N	F	64	32	17.4	10-15	2	1	A, L	
	<i>Urera glabra</i>	Wedd.	N	F	65	48	5.3	5+	1	0	L	
	<i>Urera kaalae</i>	Wawra	Y	F	68	16	10.5	5-10	1	2	A	
Violaceae	<i>Isodendron laurifolium</i>	A.Gray	Y		96	65	3.5	3+	1	0	L	
	<i>Viola chamissoniana</i>	Ging.	Y		72	68	15.0	15+	6	0	A, L	
Xanthorrhoeaceae	<i>Dianella sandwicense</i>	Hook. & Arn.	N		100	94	10.0	10+	1	0	L	
Zygophyllaceae	<i>Tribulus cistoides</i>	L.	N		40	28	2.1	2+	1	0	L	

Notes: Rare: Y = taxon is either federally listed as Threatened or Endangered (USFWS, 2018) or known to have ≤50 plants remaining in the wild (PEPP, 2018); N = taxon is not rare. Freeze sensitivity in genus or family: seed freeze sensitivity is detected at F = the family level, or G = the genus level; F* or G* = likely freeze sensitive. Individual species could be exceptions to freeze-sensitive storage behavior, especially at the family level (e.g. *Trematolobelia* spp.). Germination % is shown for the longest running accession of each species. Highest is italicized when it was the initial test. Years stored = number of years in storage at most recent test. RCI = re-collection interval, to avoid decline below 70% of highest germination (P70), and a plus (+) indicates when most recent test has not yet reached P70. Supp. = number accessions supporting the RCI; Con. = number accessions conflicting with the RCI (see Methods). Seed bank(s): accessions held at A = ANRP, L = LASCL, and/or N = NTBG. Storage notes: SL = short lived; SL/R = short lived or recalcitrant; or results may be complicated by LSF = low seed fill, D = dormancy, or FS? = hypothesized freeze-sensitive seeds tested at -18°C