Germination of seeds from herbarium specimens as a last conservation resort for resurrecting extinct or critically endangered Hawaiian plants

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Abstract

Historical herbarium collections have been proposed as a last resort for recovery of extinct plant species not represented in dedicated seed banks or other living conservation collections. For critically endangered plants at the brink of extinction, herbarium collections may also contain historical material from extinct subpopulations representing a species’ former range and lost genetic diversity of high value for conservation management. We explored the potential for germination of 81 critically endangered seed plant taxa endemic to the Hawaiian island of Kauai from herbarium specimens in herbarium PTBG of the National Tropical Botanical Garden (NTBG). Of 1250-recorded specimens of wild origin, 138 specimens representing 37 taxa contained mature seeds that could be subjected to germination testing. Seven of these taxa were not represented by any NTBG seed bank collections. Fresh embryos were observed in one seed of each of the three species Schiedea helleri, Schiedea kauaiensis, and Viola helena. While potential germination success may be low, we conclude that testing of seeds from herbarium collections should be extended from a focus on strictly extinct taxa to critically endangered taxa, which may not have sufficient representation in seed banks or other living collections of subpopulations and genetic diversity across their wild range.

KEYWORDS
extinct plant species, historical seed collections, reintroduction, resurrection ecology, Schiedea helleri, Schiedea kauaiensis: Viola helena, seed longevity

1 INTRODUCTION

A report published in 2019 by the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES, 2019) assessed that up to 1 million species of plants and animals are threatened with extinction. Other studies have shown extinction rates of seed plants to be faster than the normal turnover of species—over the past 250 years, an average of 2.3 plant species per year have gone extinct (Barnosky et al., 2011; Humphreys et al., 2019). Oceanic islands are hotspots of endemicity due to their isolation which have allowed high numbers of
plants to evolve unique adaptations to their local environment (Barnosky et al., 2011; Sakai et al., 2002). For example, the Hawaiian Islands are host to more than 1360 native plants of which 90% are endemic to the islands (Wagner et al., 1999, 2005). However, 727 taxa or about half of the native flora are considered threatened or endangered (Laukahi, 2021) and 435, or more than 30%, are listed under the U.S. Endangered Species Act (U.S. Fish and Wildlife Service, 2021). It is also estimated that 134 have already gone extinct since 1840 (Wood et al., 2019). According to the Hawai‘i Plant Extinction Prevention Program (PEPP, 2021), 237 native Hawaiian plants have less than 50 individuals left in the wild and are of high conservation priority.

The current framework for plant conservation is provided by the Global Strategy for Plant Conservation (GSPC), which is a program of the United Nations Convention on Biological Diversity (CBD) aiming specifically to halt continuing loss of plant diversity (Secretariat of the Convention on Biological Diversity, 2010). The Hawai‘i Strategy for Plant Conservation (HSPC) is the regional adaptation of the GSPC (Weisenberger & Keir, 2014). One of the targets in both the GSPC and HSPC is to ensure at least 75% of all threatened plants are conserved in ex situ collections, and seed banks are an ideal and most cost-effective and common means to store seeds for later propagation and restoration projects, including in Hawai‘i (Abeli et al., 2019; Chau et al., 2019). However, while seed banks offer optimal storage conditions for conservation collections of rare and endangered plants, seed bank collections may only represent a fraction of the wild genetic diversity (Hoban & Schlarbaum, 2014; Merritt & Dixon, 2011), when standard seed collecting protocols (e.g., Basey et al., 2015; Brown & Marshall, 1995; Hoban & Schlarbaum, 2014; Hoban & Strand, 2015) cannot be followed, as is the case with some rare species. For critically endangered plants with small population sizes (IUCN, 2021) such as *Cynanea kuhikehua* with only two individuals known to be surviving in the wild (Ronsted & Wood, 2020), mature seeds may not have been available or accessible for collection or only a few collections have been made resulting in lack of genetic diversity in the conservation collection of the most endangered plants (Hoban et al., 2021).

Herbaria have, therefore, been proposed as an alternative resource for obtaining seeds of extinct or nearly extinct plants (Bowles et al., 1993; Godefroid et al., 2011) adding to the many translational uses of herbaria (Funk, 2004; Meineke et al., 2018; Renstedt et al., 2020). Herbaria generally provide poor seed storage conditions with higher temperature and humidity as well as use of chemicals and high temperature drying, compared to seed banks focused on desiccation and freezing for storage of seeds (Strang, 1999; Wolkis & Deans, 2019). However, seeds may survive even extreme conditions for long time spans as illustrated by regeneration of eight fertile plants from 30,000-year-old fruit tissue buried in Siberian permafrost (Yashina et al., 2012).

Many studies have also reported the occasional success with germination of seeds from herbarium or other archival collections up to more than 150 years old (e.g., Becquerel, 1934; Bowles et al., 1993; Daws et al., 2007; Godefroid et al., 2011; Leino & Edqvist, 2010). In one known example, propagules gained from herbarium specimens of *Crepis foetida* subsp. *foetida* resulted in the establishment of viable plant populations in the United Kingdom (Sears, 2011).

Godefroid et al. (2011) therefore proposed that routine testing of seeds from herbarium specimens of extinct plants should be performed. With the high number of plants at the brink of extinction, it could be argued that screening of seeds from herbaria should extend the focus to include Critically Endangered taxa according to the IUCN Red List criteria (IUCN, 2021) with no or limited representation in ex situ living collections.

The aim of the present study is to test the potential for using seeds from the PTBG herbarium collections of the National Tropical Botanical Garden on Kaua‘i as a backup for resurrecting already extinct Hawaiian plant species or restoring species at the brink of extinction.

## 2 METHODS

### 2.1 Sampling strategy

As a case study, we focused on the accessioned herbarium collections in herbarium PTBG of the National Tropical Botanical Garden (formerly Pacific Tropical Botanical Garden) on Kaua‘i, Hawai‘i. Herbarium PTBG includes nearly 90,000 herbarium specimens focused primarily on the plants of the Hawaiian Islands and areas of the tropical Pacific (primarily Polynesia, Micronesia and Melanesia) with ca. 86% of the collections from these areas. The oldest collections are from 1837 and the majority of the collections are from the last 50–60 years following the establishment of the herbarium as part of the National Tropical Botanical Garden in 1964. The PTBG herbarium is well curated and all specimens have updated annotations based on the taxonomic concepts defined by the online Flora of the Hawaiian Islands (Wagner et al., 2005).

We used the PEPP species list (PEPP, 2019) as a basis for taxon sampling. The PEPP species list includes taxa that have less than 50 individuals left in the wild. Since completion of the sampling for this study, the PEPP list was updated in 2021 excluding *Euphorbia eleanoriae* as a PEPP species due to recent findings of new subpopulations by drone survey increasing the wild population of
| Family       | Taxon                                         | Collection date range | Number of sheets | Specimens with seeds present | Date range of specimens with seeds | Seed harvested | Seeds sown | Number of seed bank accessions |
|-------------|-----------------------------------------------|-----------------------|------------------|-------------------------------|-----------------------------------|----------------|------------|-------------------------------|
| Asteliaceae | *Astelia waialealae* Wawra                    | 1989–2006             | 9                | 0                             | NA                                | NA             | 0          | 0                             |
| Campanulaceae | *Cyanea kolekoleensis* (H. St. John) Lammers | 1993–1998             | 2                | 0                             | NA                                | NA             | 0          | 0                             |
| Campanulaceae | *Cyanea kuhíhewa* Lammers                     | 1991–2017             | 6                | 0                             | NA                                | NA             | 0          | 6                             |
| Campanulaceae | *Cyanea remyi* Rock                            | 1972–2017             | 37               | 1                             | 1991                              | Yes            | 21         | 6                             |
| Campanulaceae | *Cyanea rivularis* Rock                        | 1988–2016             | 26               | 4                             | 1993–1994                         | Yes            | 50         | 6                             |
| Campanulaceae | *Cyanea undulata* C. N. Forbes                 | 1989–2019             | 21               | 1                             | 2012                              | Yes            | 13         | 0                             |
| Gesneriaceae | *Cyrtandra paliku* W. L. Wagner, K. R. Wood & Lorence | 1993–2019             | 7                | 0                             | NA                                | NA             | 0          | 0                             |
| Campanulaceae | *Delissea kauaiensis* (Lammers) Lammers       | 1972–2016             | 18               | 6                             | 1990–2204                         | Yes            | 207        | 86                            |
| Campanulaceae | *Delissea rhytidosperma* H. Mann              | 1984–2017             | 11               | 2                             | 1984–1992                         | Yes            | 60         | 43                            |
| Asteraceae   | *Dubautia kalalauensis* B.G. Balwin & G.D. Carr | 1985–2004             | 4                | 1                             | 1985                              | Yes            | 3          | 13                            |
| Asteraceae   | *Dubautia kenwoodii* G. D. Carr                | 1991                 | 1                | 0                             | NA                                | NA             | 0          | 0                             |
| Asteraceae   | *Dubautia pauciflorula* H. St. John & G. D. Carr | 1979–2012             | 21               | 12                            | 1987–2012                         | Yes            | 307        | 1                             |
| Euphorbiaceae | *Euphorbia eleanoriae* (Lorence & W. L. Wagner) Govaerts | 1990–2016             | 22               | 0                             | NA                                | NA             | 0          | 0                             |
| Euphorbiaceae | *Euphorbia remyi* var. *hanaleiensis* Scherff | 1972–1993             | 2                | 0                             | NA                                | NA             | 0          | 0                             |
| Phyllanthaceae | *Flueggea neoawawae* W. J. Hayden              | 1987–2018             | 38               | 6                             | 1990–2007                         | Yes            | 60         | 9                             |
| Rhamnaceae   | *Gouania meyenii* Steud.                      | 1991–1994             | 8                | 2                             | 1992–1994                         | Yes            | 13         | 3                             |
| Malvaceae    | *Hibiscadelphus distans* L.E.Bishop & D.R.Herbst | 1982–2005             | 13               | 1                             | 1992                              | Yes            | 1          | 28                            |
| Malvaceae    | *Hibiscadelphus woodii* Lorence & W. L. Wagner | 1991–2005             | 5                | 0                             | NA                                | NA             | 0          | 0                             |
| Rubiaceae    | *Kadua haupuensis* Lorence & W. L. Wagner     | 1998                 | 3                | 0                             | NA                                | NA             | 0          | 10                            |
| Rubiaceae    | *Kadua st.-johnii* B. C. Stone & Lane W. L. Wagner & Lorence | 1993–2013             | 8                | 0                             | NA                                | NA             | 0          | 32                            |

(Continues)
| Family     | Taxon                                                                 | Collection date range | Number of sheets | Specimens with seeds present | Date range of specimens with seeds | Seed harvested | Seeds sown | Number of seed bank accessions |
|------------|----------------------------------------------------------------------|-----------------------|------------------|-------------------------------|-----------------------------------|----------------|------------|--------------------------------|
| Malvaceae  | *Kokia kauaiensis* (Rock) O. Deg. & Duvel                           | 1971–2017             | 27               | 0                             | NA                                | NA             | 0          | 20                             |
| Loganiaceae| *Geniostoma lorenciana* (K. R. Wood, W. L. Wagner & T. Motley) Byng & Christenh. | 1998–2206             | 20               | 1                             | 2001                              | Yes            | 10         | 1                              |
| Loganiaceae| *Geniostoma lydgatei* (C. N. Forbes) Byng & Christenh.               | 1987–2018             | 85               | 7                             | 1993–2012                         | Yes            | 253        | 19                             |
| Loganiaceae| *Geniostoma tinifolia* var. *wahiawaense* (H. St. John) Byng & Christenh. | 1979–2013             | 18               | 3                             | 1991–1996                         | Yes            | 53         | 3                              |
| Brassicaceae| *Lepidium orbiculare* St. John                                      | 1992–2015             | 11               | 8                             | 1992–2015                         | Yes            | 111        | 13                             |
| Primulaceae| *Lysimachia iniki* K. L. Marr                                        | 1992–2017             | 12               | 0                             | NA                                | NA             | 0          | 5                              |
| Primulaceae| *Lysimachia ovoidea* H. St. John                                     | 1987–2016             | 9                | 0                             | NA                                | NA             | 0          | 1                              |
| Primulaceae| *Lysimachia pendens* K. L. Marr                                       | 1987–2010             | 7                | 0                             | NA                                | NA             | 0          | 0                              |
| Primulaceae| *Lysimachia scopulensis* Marr                                        | 1991–2017             | 18               | 8                             | 1991–2016                         | Yes            | 66         | 5                              |
| Primulaceae| *Lysimachia venosa* (Wawra) H. St. John                             | 2012–2015             | 7                | 0                             | NA                                | NA             | 0          | 1                              |
| Asteraceae | *Melanthera micrantha* subsp. *micrantha* (Nutt.) W. L. Wagner & H. Rob. | 1990–2016             | 10               | 0                             | NA                                | NA             | 0          | 2                              |
| Asteraceae | *Melanthera waimeaensis* (H. St. John) W. L. Wagner & H. Rob.        | 1991–2018             | 6                | 0                             | NA                                | NA             | 0          | 11                             |
| Rutaceae   | *Melicope degeneri* (B. C. Stone) T. G. Hartley & B. C. Stone         | 1995–2016             | 45               | 4                             | 2004–2016                         | Yes            | 14         | 0                              |
| Rutaceae   | *Melicope haupuenis* (H. St. John) T. G. Hartley & B. C. Stone        | 1990–2018             | 41               | 1                             | 2011                              | Yes            | 2          | 0                              |
| Rutaceae   | *Melicope knudsenii* (Hillebr.) T. G. Hartley & B. C. Stone           | 1990–2016             | 28               | 0                             | NA                                | NA             | 0          | 1                              |
| Rutaceae   | *Melicope quadrangularis* (H. St. John & E. P. Hume) T. G. Hartley & B. C. Stone | 1991–2018             | 13               | 0                             | NA                                | NA             | 0          | 0                              |
| Rutaceae   | *Melicope stonei* K. R. Wood, Appelhans & W. L. Wagner               | 1994–2018             | 6                | 0                             | NA                                | NA             | 0          | 0                              |
| Family | Taxon | Collection date range | Number of sheets | Specimens with seeds present | Date range of specimens with seeds | Seed harvested | Seeds sown | Number of seed bank accessions |
|--------|-------|-----------------------|------------------|-------------------------------|-----------------------------------|----------------|------------|--------------------------------|
| Fabaceae | *Mezoneuron kavaiense* (H. Mann) Hillebr. | 1987–2011 | 4 | 0 | NA | NA | 0 | 36 |
| Primulaceae | *Myrsine knudsenii* (Rock) Hosaka | 1986–2018 | 59 | 10 | 1996–2014 | Yes | 58 | 8 |
| Primulaceae | *Myrsine mezii* Hosaka | 1993–2018 | 14 | 0 | NA | NA | 0 | 0 |
| Solanaceae | *Nothocestrum peltatum* Skottsb. | 1985–2018 | 47 | 3 | 1991–2003 | Yes | 36 | 3 |
| Poaceae | *Panicum niuaiense* H. St. John | 1985–2014 | 8 | 2 | 1985–1993 | Yes | 75 | 66 |
| Lamiaceae | *Phyllostegia electra* C. N. Forbes | 1976–2019 | 86 | 17 | 1988–2017 | Yes | 179 | 18 |
| Lamiaceae | *Phyllostegia helleri* Sherff | 1993–2015 | 6 | 1 | 2015 | Yes | 5 | 1 |
| Lamiaceae | *Phyllostegia knudsenii* Hillebr. | 1994–2001 | 2 | 0 | NA | NA | 0 | 0 |
| Lamiaceae | *Phyllostegia waimeae* Wawra | 2000–2010 | 6 | 1 | 2010 | Yes | 15 | 1 |
| Lamiaceae | *Phyllostegia wawrana* Sherff | 1993–2017 | 18 | 7 | 1993–2004 | Yes | 71 | 1 |
| Plantaginaceae | *Plantago princeps var. anomala* Rock | 1986–2014 | 17 | 2 | 1993–2014 | Yes | 9 | 15 |
| Orchidaceae | *Platanthera holochila* (Hillebr.) Kranzsl. | 1991–2005 | 4 | 0 | NA | NA | 0 | 3 |
| Araliaceae | *Polyscias bisattenuata* (Sherff) Lowry & G. M. Plunkett | 1988–2018 | 54 | 0 | NA | NA | 0 | 7 |
| Araliaceae | *Polyscias fynnii* (Lowry & K. R. Wood) Lowry & G. M. Plunkett | 1986–2019 | 45 | 0 | NA | NA | 0 | 1 |
| Arecales | *Pritchardia viscosa* Rock | 1989–2012 | 3 | 1 | 1989 | Yes | 3 | 1 |
| Rubiaceae | *Psychotria grandiflora* H. Mann | 1993–2016 | 16 | 0 | NA | NA | 0 | 1 |
| Asteraceae | *Remya montgomeryi* W.L. Wagner & D.R. Herbst | 1986–2000 | 14 | 4 | 1986–1994 | Yes | 92 | 10 |
| Caryophyllaceae | *Schiedea attenuata* W. L. Wagner, Weller & A. K. Sakai | 1991–1994 | 3 | 2 | 1991–1994 | Yes | 79 | 17 |
| Caryophyllaceae | *Schiedea helleri* Sherff | 1993–2018 | 20 | 3 | 1993–2001 | Yes | 25 | 25 |
| Caryophyllaceae | *Schiedea kauaiensis* H. St. John | 1992–2017 | 15 | 1 | 2017 | Yes | 13 | 95 |
| Caryophyllaceae | *Schiedea membranacea* H. St. John | 1974–2019 | 29 | 2 | 1974–1981 | Yes | 7 | 27 |
| Caryophyllaceae | *Schiedea perlmanni* W. L. Wagner & Weller | 1992–2011 | 11 | 0 | NA | NA | 0 | 1 |
We restricted sampling to only taxa endemic to Kaua‘i Island as those are the main focus of the herbarium PTBG and constitute nearly 40% of Hawaii single-island endemicity (Sakai et al., 2002). We also excluded non-seed plants (seven fern and one clubmoss species) from the PEPP list. This resulted in a curated list of 81 plant taxa endemic to Kaua‘i and considered Critically Endangered according to the IUCN Red List criteria (IUCN, 2021). We followed the taxonomic concepts of the online Flora of the Hawaiian Islands (Wagner et al., 2005).

For each taxon, we counted the total number of herbarium sheets held at PTBG excluding specimens of horticultural origin or outside the taxon’s endemic range. For each taxon, this was then considered the number of available specimens (Table 1). Next, we recorded if each sheet included fruit or potentially mature seeds that could be tested for germination. As removal of seeds can reduce the diagnostic value of a specimen, we then set a threshold of only sampling seeds if there were more than five seeds present on a specimen and to a maximum of 20 percent of those seeds. Even when abundant seeds were present, no more than 50 seeds were sampled from any specimen to avoid unnecessary oversampling.

### 2.2 Seed viability

Seed viability was estimated through germination testing. We used standard protocols for rare Hawaiian plants already in use by the NTBG Seed Bank and Lab (www.ntbg.org/collections/seedbank) and the Hawai‘i Seed Bank Partnership (Laukahi, 2021). A maximum of 50 seeds were tested per accession. To prevent imbibition damage, all seeds were rehydrated overnight by placing seeds in a sealed chamber over a saturated solution of potassium sulfate at ambient lab temperature (~22°C) achieving a relative humidity of ~91%. To inhibit fungal growth without affecting germination (Guri & Patel, 1998) seeds were soaked for 7 h in a 5% solution of plant preservative mixture (PPM™; Plant Cell Technologies, Washington, DC) in distilled water.

For some accessions of Labordia, Myrsine, Phillostegia, and Stenogyne, the seeds could not be separated from the surrounding fruit without damaging the seeds and they were therefore sown in their surrounding fruit material. In some cases, seeds could be separated from the fruit material after soaking in distilled water (e.g., Solanum sandwicense), or with 5% PPM and in other cases whole fruits or seeds with attached fruit material were sown. Seeds (or seeds with fruit material) were then sown in 60-mm-diameter Petri dishes on blotter paper moistened with a
0.05% solution of PPM in distilled water, and sealed with plastic paraffin film, and exposed to a daily 12-h light (41 mmol/m²/s cool white [4100 K] fluorescent light)/12-h dark with simultaneous, alternating temperature regimens of 25/15°C. To elicit germination in taxa that may require cold stratification, after 97–176 days, all non-germinated seeds were transferred to a 15/5°C thermoperiod (and 12/12-h photoperiod) for 28–43 days, then transferred back to the original 25/15°C conditions for 27–41 days.

Seeds were assayed once every 2 weeks and rewatered with a 0.1% solution of PPM in distilled water as needed. Germination was defined as radical emergence. Seeds that germinated at any point during the experiment were transferred to the NTBG Conservation Nursery for propagation. The germination experiments were ended after a total of 152–260 days elapsed since sowing, after which all non-germinated seeds were transferred to the nursery for transplantation and propagation attempt. Embryos and seedlings were included in the conservation collections and mature plants can potentially be used to harvest F1 seeds, make cuttings, or conduct pollination experiments to further extend the conservation collection of that specific taxon.

3 | RESULTS

3.1 | Availability and taxonomic diversity of specimens and seeds

We recorded 1250 specimens collected between 1971 and 2019 representing 70 taxa from 26 families (86% of 81 Kaua’i endemic PEPP taxa sensu 2019) of wild origin within the taxon’s native range (Table 1; Appendix S1). Of these, 1112 specimens contained no seeds or fruits and were excluded from further experiments. In a few cases of Myrsine knudsenii, no seeds were present inside the fruits and these specimens were also excluded from further studies. Two accessions of Melicope degeneri (PTBG 20140308 & PTBG 20140193) contained seeds that did not look like Melicope seeds but were sown anyway.

![Figure 1](image-url)
A total of 2103 seeds from 138 specimens representing 38 taxa from 17 families (47% of Kaua‘i endemic PEPP taxa sensu 2019) collected between 1974 and 2017 were harvested for germination studies (Table 1, Appendix S1).

3.2 Germination success of historical seeds

Germination was observed in only a single seed of Schiedea kauaiensis from 2017 (PTBG sheet number 076512), after 232 days of incubation (after returning to 25/15 °C from 15/5 °C). Embryos were observed outside of their coats in Schiedea helleri from 1996 (PTBG sheet number 054716) and Viola helena from 1991 (PTBG sheet number 012540) after 237 (after returning to 25/15°C from 15/5°C), and 90 days of incubation, respectively (Figure 1).

4 DISCUSSION

The herbarium collections of PTBG contained a large number of sheets representing 70 of 81 critically endangered PEPP listed species with less than 50 individuals left in the wild. This confirms that herbarium collections may contain a relatively broad representation of even very rare species which may include collections from species or subpopulations that have been already lost. For example, Nakahama et al. (2015) identified a unique allele no longer detected in wild populations of the threatened Japanese Vincetoxicum pycnostelma from herbarium specimens up to 18 years old.

A large proportion of the sheets did not contain any seeds and it has been suggested (Godefroid et al., 2011) that general collecting efforts focus on flowering specimens rather than fruiting or sterile specimens. Even specimens collected with fruits may in fact only contain immature seeds and the longevity of immature seeds is reduced compared to fully matured ones (Hay & Smith, 2003), which could also account for the low viability observed.

Specimens deposited as vouchers in connection with seed collecting for seed banking and restoration purposes may also deliberately have had all seeds removed from the voucher specimens before depositing. However, we did not observe a trend of more specimens without fruit originating after the establishment of a NTBG seed collecting program in 1989 and the time range for specimens with and without seeds present were roughly the same. While seeds deposited with herbarium voucher specimens may not be viable, they may still contribute to seed morphology, genetic, and other studies (Walters et al., 2006).

To avoid oversampling of the specimens, seeds were only sampled from specimens with five or more seeds present, but no specimens with less than five seeds were observed in the present study. In extreme cases where the seeds of a herbarium specimen would for example be the absolutely last opportunity for resurrecting an already extinct species, any such sheets could have been considered too.

Thirty-one (82%) of the 38 taxa sampled for germination testing were also represented by seed accessions stored in the NTBG Seed Bank and Lab. In addition to the seven taxa (18%) not currently secured in the seed bank (Cyanea kolekoleensis, Cyanea undulata, M. degeneri, Melicope haupuensis, V. helena, Viola kauaensis var. wahiawaensis, and Xylosma crenatum), another seven (18%) of the sampled taxa (Dubautia pauciflora, Geniostoma lorecianica, Phylostegia helleri, Phyllostegia waiameae, Phyllostegia wawrana, Pritchardia viscosa, and Stenogyne campanulata) were only represented by one accession in the seed bank (see Table S1 for details) suggesting the seeds obtained from the herbarium collections could represent additional geographic or genetic diversity, including from subpopulations that could subsequently have gone extinct and could therefore no longer be sampled in the wild.

The three species for which germination was observed date back to 1991 or nearly 30 years. V. helena is not currently stored in the seed bank, and successful propagation from the herbarium seed collection would therefore represent a new conservation collection. S. helleri and S. kauaiensis were already backed by 25 and 96 accessions, respectively, in the seed bank of which 8 and 13 are from the same accessions represented by the herbarium specimen, respectively.

While herbarium samples may represent lost or additional diversity, the germination success is very limited. Germination or embryo emergence were only observed for three of 2103 sown seeds (0.14%) and in two of the cases it was not clear if the seeds had germinated or if the embryos were ejected from the seed coat by some mechanical process. Pericarp decomposition has been observed for Erechtites hieracifolia seeds buried in soil for 8 years (Baskin & Baskin, 1996). This was not the case for our study as the seed coats were intact when the embryos presented themselves. This is comparable to the findings of Godefroid et al. (2011), who observed germination of 8 seeds out of 2672 seeds (0.30%) from herbarium vouchers of extinct Belgium plants at herbarium BR of Meise Botanic Garden.

None of the two embryos and one seedling, that were transferred to the nursery survived. Low survival was also found in the study by Godefroid et al. (2011).

Optimized germination protocols were used in the present study, but it is possible that higher germination
rates could be obtained with more experimentation. In extreme cases, where filled, apparently healthy seeds fail to germinate and grow, embryo rescue may be also be a possibility (Sarasan et al., 2016).

It is also well known that rare wild species may exhibit individual variation in seed behavior both under storage and in germination processes (Chau et al., 2019; Godefroid et al., 2010). However, we suspect that the low germination success could be due to the generally poor drying and storage conditions for seeds in herbarium collections compared to modern seed banks (Bowles et al., 1993; Strang, 1999; Wolkis & Deans, 2019), although seeds of a common Hawaiian endemic species, Metrosideros polymorpha, was able to withstand the PTBG herbarium entry protocol of drying then freezing (Wolkis & Deans, 2019). It is also possible that postharvest specimen handling was not optimal, which could have resulted in loss of viability before transfer from the herbarium (Wolkis & Deans, 2019).

Despite the low germination success overall, any germination of an otherwise extinct species is an encouraging result significantly increasing the potential of resurrection and conservation of that species. Our results thus support the recommendation of Godefroid et al. (2011) to conduct routine germination testing on seeds from extinct species held in herbaria in the hope of finding even a few needles in the haystack. We further recommend extending the focus from strictly extinct taxa to also critically endangered taxa, which may not have sufficient representation in seed banks or other living collections of subpopulations and genetic diversity across their wild range (Hoban & Schlarbaum, 2014; Merritt & Dixon, 2011).

ACKNOWLEDGMENTS
The authors thank the many collectors of PTBG herbarium specimens and seed accessions for contributing to the collections over the years and the volunteers in the PTBG herbarium and the NTBG Seed Bank and Laboratory for help maintaining the collections. Rhian Campbell of the NTBG Conservation Nursery is thanked for help with transplantation and propagation attempt of seeds and germinants and Neil Brosnahan for scanning the herbarium sheets for the figure.

CONFLICT OF INTEREST
The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS
Nina Ronsted and Dustin Wolkis designed the study. Tim Flynn curated the herbarium specimens and provided recommendations for collecting materials. K.J. conducted the sampling and the germination tests. Mike DeMotta led the transplantation and propagation of seeds in the conservation nursery. Nina Ronsted drafted the manuscript with Dustin Wolkis and Kelli Jones. All authors revised and approved the final manuscript.

DATA AVAILABILITY STATEMENT
The datasets analyzed during the current study are available by request from National Tropical Botanical Garden.

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Baskin, C. C., & Baskin, J. M. (1996). Role of temperature and individual variation in seed behavior both under storage and in germination processes (Chau et al., 2019; Godefroid et al., 2010). However, we suspect that the low germination success could be due to the generally poor drying and storage conditions for seeds in herbarium collections compared to modern seed banks (Bowles et al., 1993; Strang, 1999; Wolkis & Deans, 2019), although seeds of a common Hawaiian endemic species, Metrosideros polymorpha, was able to withstand the PTBG herbarium entry protocol of drying then freezing (Wolkis & Deans, 2019). It is also possible that postharvest specimen handling was not optimal, which could have resulted in loss of viability before transfer from the herbarium (Wolkis & Deans, 2019).

Despite the low germination success overall, any germination of an otherwise extinct species is an encouraging result significantly increasing the potential of resurrection and conservation of that species. Our results thus support the recommendation of Godefroid et al. (2011) to conduct routine germination testing on seeds from extinct species held in herbaria in the hope of finding even a few needles in the haystack. We further recommend extending the focus from strictly extinct taxa to also critically endangered taxa, which may not have sufficient representation in seed banks or other living collections of subpopulations and genetic diversity across their wild range (Hoban & Schlarbaum, 2014; Merritt & Dixon, 2011).

ACKNOWLEDGMENTS
The authors thank the many collectors of PTBG herbarium specimens and seed accessions for contributing to the collections over the years and the volunteers in the PTBG herbarium and the NTBG Seed Bank and Laboratory for help maintaining the collections. Rhian Campbell of the NTBG Conservation Nursery is thanked for help with transplantation and propagation attempt of seeds and germinants and Neil Brosnahan for scanning the herbarium sheets for the figure.

CONFLICT OF INTEREST
The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS
Nina Ronsted and Dustin Wolkis designed the study. Tim Flynn curated the herbarium specimens and provided recommendations for collecting materials. K.J. conducted the sampling and the germination tests. Mike DeMotta led the transplantation and propagation of seeds in the conservation nursery. Nina Ronsted drafted the manuscript with Dustin Wolkis and Kelli Jones. All authors revised and approved the final manuscript.

DATA AVAILABILITY STATEMENT
The datasets analyzed during the current study are available by request from National Tropical Botanical Garden.

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**How to cite this article:** Wolkis, D., Jones, K., Flynn, T., DeMotta, M., & Rønsted, N. (2022). Germination of seeds from herbarium specimens as a last conservation resort for resurrecting extinct or critically endangered Hawaiian plants. *Conservation Science and Practice, 4*(1), e576. https://doi.org/10.1111/csp2.576