MARIAN CHAU COLLECTING SEEDS FROM *METROSIDEROS POLYMORPHA* ON O'AHU.
PHOTO BY TIM KROESSIG

keep cool, stay dry, and you may live long

Major seed storage findings published

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In the summer of 1989 NTBG's future Breadfruit Institute Director Dr. Diane Ragone collected seeds from the critically endangered papala *(Charpentiera densiflora)*, at Kahanu Garden on Maui, marking this as the oldest known collection in Hawai'i.

At that time, the Kew Millennium Seed Bank was still seven years from establishment, and the Svalbard Global Seed Vault wouldn't be constructed until 2008, the same year NTBG's Juliet Rice Wichman Botanical Research Center and future site of the NTBG Seed Bank and Laboratory was completed. NTBG has banked seeds every year since, and conserving these early collections for future use showed incredible forethought by NTBG staff.

Seed banking, especially at botanical institutions, would not hit the mainstream for at least another decade. But what would the shelf life of these collections be, and at what intervals should they be re-collected? The term "seed storage behavior" describes the physiological response to storage methods. If seeds tolerate desiccation and freezing, they are considered to have "orthodox behavior."

Until the early 2000s, it was assumed that because of Hawai'i's tropical location, the conventional storage methods of desiccating and freezing seeds would prove lethal to the majority of the Hawaiian flora, and therefore seeds from most species were not orthodox and could not be banked.

FREEZE SENSITIVE SEEDS OF ENDANGERED, ENDEMIC HAWAIIAN SPECIES IN THE LOBELIA FAMILY; CLOCKWISE FROM TOP LEFT: *BRIGHAMIA ROCKII, CLERMONTIA LINDSEYANA, DELISSEA KAUAIENSIS,* AND *LOBELIA OAHUENSIS*. PHOTOS BY MARIAN CHAU

Fortunately, in 1995 Alvin Yoshinaga, then Seed Conservation Laboratory Manager at the Harold L. Lyon Arboretum at the University of Hawai'i at Mānoa, had the tremendous foresight to initiate real time seed longevity experiments that would eventually transform the way we view seed storage behavior in Hawai'i.

The experimental design was simple, but would take great fortitude to carry out. Yoshinaga's experiment determined initial viability, then preserved the same seed collection in different ways (varying temperature and relative humidity), and continuously tested seeds stored at those different environmental conditions over the life the collection. These methods were eventually adopted by seed labs at the U.S. Army Natural Resources Program on O'ahu and at NTBG.

It is easy to see how these experiments would transcend the duration of staff employment and even lifetimes. Even so, some patterns began to emerge, including how some species seemed to exhibit the curious seed storage behavior of tolerating desiccation (like that of an orthodox species) yet with viability declining faster at subfreezing temperatures (i.e. -18°C) compared to refrigeration temperatures (i.e. +5°C).

These differences often take several years to detect, and are described by the seed storage behavior category "freeze sensitive." It became clear that the time had come to make sense of these data and help inform seed conservation in Hawai'i and globally.

With efforts led by Yoshinaga's successor Dr. Marian Chau (currently owner of Kalehua Seed Conservation Consulting and Hawai'i Seed Bank Partnership Coordinator for Laukahi Plant Conservation Network) and collaborators from around the state of Hawai'i including NTBG and others , results from these experiments were published open access in September in the American Journal of Botany2.

The study utilizes over 20 years of real-time germination data pooled from the partner seed labs, including tests for freeze sensitivity and re-collection intervals for 197 and 295 native Hawaiian plant species, respectively. We found that species in Campanulaceae (lobelia, bellflower), Cyperaceae (sedge), Rubiaceae (coffee), and Urticaceae (nettle) families, and other genera exhibit freeze sensitive storage behavior.

We also presented a new metric and a simple protocol to determine freeze sensitivity, which can often take two to five years to detect. Interestingly, Hawai'i has a greater proportion of its flora exhibiting freeze sensitive seed storage behavior than any regional flora assessed. Longevity varied from less than one to twenty plus years.

To give managers an idea of how long each species' seeds might last in storage, we established a threshold of 70 percent of the maximum germination for each collection. After seeds drop below 70 percent, their viability often continues to decline quickly. The number of years it takes seeds to reach that threshold is called the re-collection interval, which is the recommended period when managers should consider withdrawing seeds, before they lose significant viability.

At the time of the most recent test, 195 species had not yet reached their re-collection interval. Only 45 species had a re-collection interval of less than 5 years. It is of note that by their nature these experiments are ongoing, and thus new results are continuously being generated.

These results provide seed bank, land, and natural resource managers with critical information on ex situ (off site) plant conservation in Hawai'i, and also offer an excellent starting point for future research. With this study being the largest to test real time seed longevity of native species, one of the only studies to test storage behavior in a large proportion of a tropical flora, and the first to find a large proportion of species to have freeze sensitive seeds, the Hawaiian flora can serve as a model for other tropical, subtropical, and/or island ecosystems looking to use seed banking as a means of plant conservation.

Read the full article open access at https://doi.org/10.1002/ajb2.1351 or search for "Seed freeze sensitivity and ex situ longevity of 295 species in the native Hawaiian flora."

[1] Timothy Chambers and Roy Kam (US Army Natural Resources Program on O'ahu); Lauren Weisenberger (US Fish and Wildlife Service, Pacific Islands Fish and Wildlife Office); Matthew Keir (State of Hawai'i Department of Land and Natural Resources – Division of Forestry and Wildlife); Timothy I. Kroessig and Alvin Y. Yoshinaga (Lyon Arboretum, University of Hawai'i at Mānoa); and Dustin Wolkis (NTBG Seed Bank and Laboratory Manager)

[2] Chau MM, Chambers T, Weisenberger L, Keir M, Kroessig TI, Wolkis D, Kam R, Yoshinaga AY (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.

DUSTIN WOLKIS IN THE FIELD. PHOTO BY RUTH BONE