Comparative analysis of the effects of ultra-dry seed storage on long-term viability

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Abstract

This research investigates the long-term viability of seeds stored under ultra-dry conditions, which involves reducing seed moisture content to levels below conventional seed bank standards. The study is conducted within the context of ex-situ plant conservation of native Hawaiian species. The germination rates of ultra-dry and conventionally dried seed samples were analyzed by extracting more than 20 years of data from routine viability tests. A comparative analysis was performed to evaluate if ultra-dry seed storage has an effect on viability over time. Mixed-effects models were employed in the statistical analysis and applied separately according to seeds' storage behaviors. Relative humidity was measured inside glass vials and laminated aluminum foil packets used for long-term seed storage. The results suggested no significant differences in germination rates between the two desiccation methods. Hygrometer measurements revealed that seeds' relative humidity in storage is not reaching the expected value and seeds are, in fact, usually stored more humid than intended. The differences in recorded relative humidity may be attributed to the desiccation protocol, storage methods (containers and temperatures), and seeds' chemical properties. This research shows that seeds could maintain long-term viability (longevity) in storage, regardless of RH treatment. It also suggests that drying seeds below generally accepted levels may not be deleterious to longevity.

Keywords: ultra-dry seed storage, seed viability, longevity, ex-situ plant conservation, seed banking

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Abbreviations

CPC	Center for Plant Conservation
eMC	Equilibrium Moisture Content
eRH	Equilibrium Relative Humidity
ISTA	International Seed Testing Association
K2CO3	Potassium Carbonate
LME	Mixed-Effects Model
MC	Moisture Content
MgCl2	Magnesium Chloride
RH	Relative Humidity (expressed as a percentage)
SCL	Seed Conservation Laboratory at UH Lyon Arboretum
SD	Standard Deviation

1. Introduction

Seeds represent the most critical phase of a plant's life cycle and are responsible for the evolutionary continuum of plant species (Desai, 2004). Drying of fruits is part of the natural dispersal of many species. This drying process prepares the seeds for dormancy until the next season, when they may germinate (Thomsen & Stubsgaard, 1998). The ability of the seed to withstand drying as part of its natural process is utilized in seed conservation. Properly managed off-site collections of seeds and plant material can make the critical difference between extinction and survival (Maunder et al., 2004). *Ex-situ* plant conservation safeguards the biodiversity of species facing threats and habitat loss for posterior reintroduction efforts. With more than 500 years of plant conservation in botanical gardens, *ex-situ* plant conservation via living germplasm held in seed banks has recently been getting more attention, acting as a cost-effective solution to the ongoing environmental changes and biodiversity decline.

The universal rule of seed banking is keeping seeds cool and dry to prolong seed longevity in storage over time. A global consensus on what is considered too dry has yet to be reached, and significant differences in desiccation response are observable among seeds of different species and even within seeds of the same provenance. A "better safe than sorry" approach has been adopted in Hawai'i, avoiding the risk of killing seeds by overcooling or over-drying.

Nonetheless, recent research has shown that some species may withstand desiccation far below the standard levels while retaining acceptable levels (≥ 50% germination rate) of viability over extended periods. This approach to seed drying has been qualified as "ultra-dry" and supports the hypothesis that under-drying seeds may be more deleterious than over-drying seeds. The debate originates from the various experimental designs of seed desiccation research with viability equations extrapolated from seed aging experiments and the less commonly observed seed viability from long-term germination studies. It is now understood that even tropical species produce seeds with a certain tolerance to desiccation. This characteristic is utilized in the long-term conservation of germplasm of endemic plant populations.

The seed conservation laboratory (SCL) at the University of Hawai'i's Lyon Arboretum is the largest repository for Threatened and Endangered tropical plant species in Hawai'i, USA. Seed sampling for ultra-dry storage began 27 years ago,

and routine viability assessment over regular intervals of seed germination testing was performed. A comparison of the effects of ultra-dry and conventional desiccation treatments at cold and sub-zero temperatures can now be achieved with comprehensive longevity data after more than two decades of long-term storage.

2. Study objectives and hypotheses

Extraneous viability studies assessing ultra-dry seed storage have shown divergent results, and controversy exists around desiccation protocols (Smith et al., 2003; FAO, 2014; CPC, 2019). This research aims at addressing the general effects of ultra-dry storage conditions on seed longevity and investigates if low RH desiccation treatments can optimize viability for longer periods of cold storage compared to conventional seed drying methods.

Two alternative hypotheses were evaluated:

- H1: If seeds are subjected to ultra-dry desiccation at ambient temperatures, then longevity will be negatively impacted over time in cold storage.
- H2: If seeds are subjected to ultra-dry desiccation at ambient temperatures, then longevity will be optimized over time in cold storage.

Additionally, the null hyphothesis (H0) ascertaining a non-significance of the effects of ultra-dry seed desiccation on germination rates compared to conventional desiccation was theorized.

3. Seed banking

3.1 Seed storage behaviors

Seed dormancy is often linked to seed longevity; it is believed that seeds with a hard seed coat often store longer as they limit the movement of air and water. The link between embryo dormancy and longevity is complex to verify, as seed dormancy confounds viability measurements for longevity tests (Walters, 2013). Seed dormancy slows down metabolic processes while seed longevity expresses the retention of seed viability and vigor throughout storage time. Some seeds are genetically and chemically well-equipped for longer storability than others under similar conditions (Desai, 2004). This observation of seed storage behaviors led to their initial division into two categories according to their dehydration response: orthodox seeds are desiccation-tolerant and long-lived, produced by most annual and biennial species and most arable and horticultural crop species (Pritchard, 2004). Recalcitrant seeds are desiccation-sensitive, they quickly lose viability once moisture (<40%) has been removed and are primarily found in tropical regions and among timber species of more temperate latitudes. Recently, a third category has been proposed for seeds with an intermediate behavior as they can tolerate desiccation but may be short-lived (<5 years) or temperature-sensitive at conventional freezing temperatures of -18°C (Pritchard & Dickie, 2003). Seed aging is a function of time, temperature, and moisture content (MC). Therefore, it is possible to influence the survival period of seeds, primarily those exhibiting orthodox and intermediate behaviors, by controlling the seed storage environment (Cromarty et al., 1982). The protocols for seed storage depend predominantly on the storage behavior of the seed (Walters, 2004) and the storage longevity intended by the ex-situ facility for conservation or restoration use.

3.1.1 Desiccation protocols

Upon reception in a seed bank facility, fresh seeds have a MC associated with a high decline in vigor and viability and, therefore, need to be reduced to a level where the declines are acceptable (Hay et al., 2023). Since the beginning of ex-situ

plant conservation, drying strategies have been a topic of significant interest, leading to variations in processes and subsequent seed longevity. Saturated solutions of electrolytes or color-indicated silica gel are generally used for seed drying. Silica gel is advantageous, particularly because it is easily regenerated and moisture can be observed directly as the methyl violet indicator of the gel gradually turns from orange (reactivated) to green (saturated) starting around 20-25% relative humidity (RH) (Sutcliffe & Adams, 2014b). When drying, fresh seeds are sealed with a desiccant that removes moisture from the air and consequently dries the seeds. Electronic drying chambers with forced air circulation, controlled humidity and temperature are generally preferred and a more robust option for seed desiccation. Fresh seeds are placed in thin layers on open trays to maximize air recirculation and obtain an even desiccation. The time for the seeds to reach equilibrium relative humidity (eRH) within the chamber is a function of the temperature and the seed's internal moisture diffusion ability. Consequently, larger seeds may take longer to reach equilibrium simply due to the increased distance through which moisture must diffuse before it reaches the seed surface. An average of 30 days at ambient temperatures with good air recirculation will give them enough time to reach eRH regardless of their internal structures (Cromarty et al., 1982). Seeds can then be stored in hermetic containers made of glass or aluminum. Laminated aluminum foil packets are a space efficient alternative for long-term germplasm storage in seed banks.

3.2 Relationship between seed moisture content and relative humidity

The term “dry” is not precise; seeds can feel dry and look dry under different MCs, so it is necessary to understand and control the drying processes (Thomsen & Stubsgaard, 1998). MC determination is one of the most important assessments made on seeds for research and conservation interested in maintaining seed viability (Hay et al., 2023). Seed MC expresses the weight of water present in the seed as a percentage of the total weight of the seed. Traditionally, seed MC is determined following the standards defined by the International Seed Testing Association (ISTA) with the oven drying method, measuring sample’s weight change after drying at constant temperature (Nijënstein et al., 2005). Seed MC measurement is a straightforward method for assessing the amount of water in a seed, however, accurate measurements require destructive sampling. Measuring the RH of the air in equilibrium with seed samples is a reliable, non-destructive alternative to gravimetric MC determination where the eRH of the seeds is directly related to the seed water activity and water potential (Probert, 2003). Sorption isotherms predict seeds' response to desiccation and represent the relationship between MC and RH

at a given temperature (Gold & Hay, 2014). Seed composition can create differences in the MC-RH relationship. Starchy seeds will have a higher MC than oily seeds at the same RH due to the proportion of dry matter in the seed available for water exchange. It has been found that the variance between MC and RH tends to decrease at low seed moisture as the relationship becomes similar for contrasting species (Ellis et al., 1990). This is due to the different categories of bound water occurring in seed tissues with only the strongly bound fraction present at low MC and less variable in quantities than the weakly bound fraction and multimolecular water at intermediate and high MCs.

3.3 Conventional and ultra-dry seed desiccation

The chemical composition of a seed significantly influences water activity and subsequent longevity in storage. Water activity describes the water concentration in thermodynamics, i.e., its availability for reactions and present only in carbohydrates and proteins (Walters & Engels, 1998). Knowledge of seed moisture is important to maintain seed longevity in storage (Sutcliffe & Adams, 2014a). The term “ultra-dry” seed storage was introduced by the International Board for Plant Genetic Resourcesin 1998 following the investigation of the effects of very low MC on seed longevity. This research was based on the general theory that seed longevity doubles for every 1% reduction in MC (10% reduction in RH) or for every 5°C reduction in temperature, dependent on a species-specific variation and on the prospect of developing uniform methods for medium to long-term storage of seed germplasm (Black et al., 1998). This method involves reducing the seed MC to levels below seed bank standards without compromising the integrity of cellular membrane structure and seed function (Malagatti, 2023). Conventional drying addresses a combination of temperature and RH that can be widely applicable to most gene banks and their seed collections. These values differ across scientific literature and geographical locations, most likely due to the differences in the range of taxa held in respective seed banks and their collection's end use. The Center for Plant Conservation (CPC) recommends drying seeds between 21-40% RH at 15-25°C to obtain a storage eRH of ~20% respective to storage temperatures (5°C or -20°C)(CPC, 2019). The Royal Botanic Gardens at Kew recommend desiccation to ~15% RH at ~15°C (Sutcliffe & Adams, 2014a). Drying below this threshold is generally considered ultra-dry. Below this level of hydration, longevity may increase, remain the same, or decrease depending on the seed’s chemical composition and the seed lot quality (Pritchard & Dickie, 2003). The critical (or optimum) MC, the value below which seed longevity is not improved, is one of the most important points in the debate, determining the best conditions for seed storage and predicting seed longevity. While empirical data has shown that dry and cold conditions will increase the shelf life of biological material, the optimum MC

and temperature are not well understood and vary among species and as a factor of the lipid content of the seed (Vertucci & Roos, 1990). The investigation of a MC threshold has led to the elaboration of complex seed viability equations aiming at predicting the loss of seed viability as a function of MC and temperature by calculating longevity for ambient and cold-stored seeds (Pritchard & Dickie, 2003). The validity of such equations on the concrete viability of seeds has since then been argued mainly because some longevity studies require extrapolation of results from short-term experiments or artificial aging experiments where seeds are exposed to suboptimal conditions of elevated temperatures (Ellis et al., 1993; Solberg et al., 2020). These differences in experimental designs may explain the strong variation of germination response compared to long-term studies with routine viability testing.

4. Materials and methods

The seed conservation laboratory at UH Lyon Arboretum is the largest repository for tropical plant species in Hawai'i. Preserving a large collection of seeds permitted the research potential on the effects of ultra-dry storage of seeds of native and endemic species more than 20 years ago. Hypotheses can now be tested by retrieving long-term germination data of cold-stored seeds under different desiccation treatments and performing comparative analyses.

4.1 Initial seed sampling

The species selection in the ultra-dry study followed the availability of seeds at the SCL and lasted between 1997 to 2015. Since the SCL deals almost exclusively with plants protected under the United States Fish and Wildlife Service Endangered Species Act of 1973, a certain limitation to genus-level diversity exists; therefore, drying responses in terms of germination percentages are mainly observed at species-specific levels. Each sample of seeds received at the SCL was split between ultra-dry and conventional desiccation treatments, allowing for a robust comparative analysis. Refrigerated and frozen storage at the SCL are named after the seed storage behaviors (namely orthodox and intermediate) such as intermediate seeds are only stored at 5°C and orthodox seeds are only stored at -18°C. Storage units are plastic boxes containing the seed vials stored in alphabetical order (genus) and are labeled according to the seed desiccation protocol (Appendix 1a). Two storage units were defined for seeds in orthodox storage, and three units were defined for seeds in intermediate storage (Table 1). Each orthodox or intermediate conventional storage unit represents a desiccation method with an electrolyte, providing a specific RH at a given temperature. All samples were expected to reach an equilibrium of ~20% RH in storage following the seed desiccation guidelines from the CPC. Ultra-dry storage units represent the desiccation treatment over silica gel in a sealed container at ambient temperatures (~20°C). The ratio between silica gel and seeds was at least 1:1 to reach a desiccation and long-term storage RH of ~10% in orthodox and intermediate storage. All desiccation treatments lasted 30 days before placing the seeds in glass vials with rubber gaskets for long-term conservation. Three vial sizes were used for long-term storage, the majority being small (47mm long with an internal diameter of 13mm). Only a few vials were

Table 1: Storage units at each temperature and seed desiccation protocols for ultra-dry and conventional treatments.

	Ultra-dry seed desiccation ~10% RH in storage	Conventional seed desiccation ~20% RH in storage
Intermediate storage (5˚C)	C10	C20 (dried at 33.5% eRH ~20°C with MgCl2) C33 (dried at 33.5% eRH ~5°C with MgCl2)
Orthodox storage (-18˚C)	D8	D20 (dried at 43% eRH ~20°C with K2CO3)

medium (67mm long with an internal diameter of 19mm) or large (93mm long with an internal diameter of 25mm) due to the size and number of seeds in the accession (i.e. *Santalum ellipticum*). The first 10-25mm of each vial contained the seeds. Activated silica gel (ultra-dry) or mung beans (conventional) equilibrated in drying cabinets at ambient temperatures were added on top of the seeds to fill the vials in case of failure of the rubber gasket and to create a buffer to minimize headspace and possible uptake of moisture with the air inside the container (Appendix 1b). All vials were stored at either 5°C (intermediate storage) or -18°C (orthodox storage) in conventional refrigerators and freezers. Silica and mung beans were replaced as routine viability testing was performed to ensure the maintenance of the eRH. Viability tests were performed on routine intervals across 25 years. Before long-term storage, an initial test of a subset of the processed seed lot was performed to assess fresh seed's viability. Another viability test was conducted after desiccation to assess if seeds are still able to germinate and record their reference germination rate. Viability tests were plated on polystyrene disposable sterilized Petri dishes with a 1% agar solution or germination paper and housed in a Percival E36V growth chamber at >50% RH and ranging from diurnal temperatures of 22-26°C to noctural temperatures of 15-19°C under an 11h light/13h dark photoperiod. Germination pre-treatments were performed to help specific seeds overcome dormancy complications. Seed pre-treatments involve, but are not limited to, soaking the sample in water, gibberellic acid, or potassium nitrate 24 hours before viability testing. Physical dormancy-breaking protocols include mechanical treatments like scarification, creating an opening in the seed coat to allow moisture imbibition. Germination was observed and scored weekly according to the ISTA's seed testing rules, i.e., when the radicle is at least 2mm long and cotyledon production is observed. Seed accession for ultra-dry treatments lasted from 1997 to 2015. The

cessation of adding taxa to the ultra-dry treatment after 2015 was most likely due to the controversy surrounding the possible deleterious effects of over-drying.

4.2 Current investigations

All seeds originally accessioned into long-term storage of ultra-dry and conventionally dried treatments remained in their original vials and units at constant temperatures within orthodox and intermediate storage. Viability tests were performed routinely, and additional tests were scheduled before the analysis based on the remaining availability of seeds in storage and pre-scheduled testing intervals. Germination tests are conducted as seed accessions are listed on the SCL's monthly viability testing reports. The majority of the re-scheduled tests for this analysis were conducted between August and December, 2023 and followed the same viability testing procedure (plated on polystyrene disposable sterilized Petri dishes with a 1% agar solution or germination paper and housed in a Percival E36V growth chamber at the same parameters). These tests provided additional germination data, thus increasing sample size and viability intervals reaching 25 years in storage. Moreover, it refined the analysis using optimal germination techniques (adequate substrates and dormancy-breaking protocols) developed over 30+ years of Hawaiian seed banking. Eighty-four species are represented in the ultra-dry analysis across 31 families (Appendix 2). The Campanulaceae is the most represented family, encompassing 19 taxa. Almost all species are endemic or indigenous, with only one species, *Solanum americanum*, naturalized in the Hawaiian Islands. Thirty-three species have a conservation status of threatened or endangered, and three species, *Tetramolopium consanguineum subsp. leptophyllum, Cyanea superba subsp. superba and Delissea rhytidosperma* are extinct in the wild, providing only preserved seeds from cultivated stock.

4.2.1 Desiccation methodology review

Seed desiccation protocols have evolved since the initial seed sampling. The information on saturated salt solutions from the SCL's lab protocols archives (Seed conservation laboratory, unpublished) was used to recreate and quantify the original drying methods. No seeds were used, and only the drying environment conditions were reported, given that seeds reached eRH within their drying environments. BlueMaestro disc-mini-Bluetooth hygrometers (Appendix 3) were used to record the temperature and RH of the drying environments. Investigations were conducted from November, 2023 to February, 2024. A range of 28-150g of magnesium chloride (MgCl2) was added to several glass Petri dishes and saturated with deionized (DI) water. They were placed in an electronic drying cabinet at ambient

temperature (~20°C) or in a sealed container ~5°C to recreate the drying conditions for C20 and C33 storage units. The drying conditions for unit D20 were tested with 50g of potassium carbonate (K2CO3) in a sealed container with a fan attached to the lid. While the amount of electrolyte was measured in grams, the amount of DI water added to create a saturated solution varied with the type of salt to obtain a "slush-like" consistency as indicated by the SCL lab protocols. The temperature and RH were measured at least two days after the experiment to account for hygrometer equilibration. The consistency of the saturated salt solutions was assessed and maintained regularly, and DI water was either added or removed via pipetting. Ultra-dry desiccation protocols were tested using different silica gel reactivation methods. Manufacturers suggest different methods for reactivation, in either a commercially available oven or microwave. Both methods were tested with silica gel from different manufacturers (Dry&Dry, Acros Organics, and a mix of silica used in the SCL). An initial RH measurement was only possible for the sample from the brand Dry&Dry as the container had not been opened. All other silica was previously regenerated. Orange (activated) silica from each sample was placed in 2 Petri dishes of 50g each and left in the incubator at >50% RH for seven days. Deactivated weights were taken, and a 32g sample was sealed in a 225ml Snapware® Pyrex® tempered glass container at ambient temperature to record MC prior to regeneration. Each sample was reactivated according to the manufacturer's instructions. Silica was placed in an open 1.9L Pyrex® tempered glass container (microwave method) or on a metal baking sheet (oven method) and sealed in a glass container at ambient temperature after reactivation to cool. Activated weights were re-taken, and a 32g sample from each manufacturer was placed in an additional 225ml sealed container with a BlueMaestro hygrometer to record RH and temperature the following day.

4.3 Assessment of relative humidity

A few vials of seed accessions were selected and eRH measurements were recorded between April and August, 2024. This selection only included seeds germinating during their last viability test to ensure that the sample was still viable. No germination threshold was defined given that the sample with the lowest germination rates across both treatments (Deschampsia nubigena, <5% germination) was still considered viable. The vial selection prioritized the earliest seed accessions with +14 years of viability data. Several vials were not found in storage due to seed depletion or withdrawal from the SCL. Hence, two exceptions were made: a sample from 2010 (*Clermontia hawaiiensis*) in intermediate storage and one from 2015 (*Lobelia grayana*) in orthodox storage. Contents of the vials were transferred into 225ml Snapware® containers with a BlueMaestro hygrometer to log eRH. These determinations were carried out within a controlled environment

maintained in equilibrium with ~35-45% RH at 20°C (+/-2°C). Sealed vials were left at room temperature, and the transfer was made as quickly as possible (< 5 minutes) to minimize moisture uptake. Initial RH measurements indicated that the volume of headspace left in the sealed container was critical after including the seeds and silica/mung beans. As vials in storage were completely filled, a hygroscopic material was needed to fill the void space not to influence RH. Soft plastic from Ziploc® bags was crumpled and pressed to ensure the minimum void space. The sealed containers were double-bagged externally in larger Ziploc® bags to prevent additional moisture exchange in storage. Temperature and RH were recorded after at least four days to allow for equilibration. Additionally, RH experiments with laminated aluminum foil packets were performed since it is currently the preferred method for spatially effective long-term seed storage at the SCL and in numerous other seed bank facilities. Several foil packets of seeds, not included in the ultra-dry analysis, were pulled from refrigerators and freezers and left at room temperature for 1-2 hour(s) before opening to avoid condensation. Once the hygrometers were inserted, foil packets were sealed again with a heat seal as fast as possible. Hygrometers were also placed in foil packets of post-dried fresh seeds when moved to long-term cold storage from the drying cabinets in which they were desiccated for 30 days at ~30% RH and ~20°C. Seeds are packed in cotton paper envelopes or placed directly in the foil packets according to their size and amount. Foil packets used at the SCL are either small (10x12cm), medium (10x14cm) or large (16x24cm) and the space filled by the seeds can vary. Measurements were diversified across all packing formats and assessed after seven days.

4.4 Statistical analysis

Raw germination data was extracted from the SCL database as .xls files. These data were cleaned before their integration into the statistical analysis using RStudio 5796.4.1.0. Variables that could affect the results and skew the analysis were removed, such as inadequate testing methods (improper substrate, taxa incorporated into inadequate storage temperature, failure to treat dormancy complications) and human errors (fruits sown, test prematurely discarded, mishandling of Petri dishes). As temperature plays a significant role in seed longevity, orthodox and intermediate storage were analyzed separately. The cleaned datasets were separated into two CSV files by storage temperature, each containing four unique columns: Taxon Name, Testing Interval in Years, Percent Relative Humidity, and Overall Germination Rate. Percent Relative Humidity and Testing Interval in Years were incorporated as independent, predictor variables, with Overall Germination Rate as the response variable. Taxon Name was integrated as a random effect grouping variable to account for species-specific longevity, dormancy and vigor differences.

A Linear Mixed-Effect Model (LME) was the best fit for the analysis, as it allows for the inclusion of fixed and random effects. The ”Overall Germination Rate” contained longitudinal data with repeated observations of the same subject over time. Once the raw data was refined to include an equal number of tests between the reference level of ultra-dry samples at ~10% RH and conventional drying at 20%+ RH, a LME was run to assess the effect of RH and storage time in years on longevity. The same model structure was applied to seeds in orthodox and intermediate storages. For seeds in orthodox storage, the fixed effect of RH and the random effect of Taxon Name were converted to categorical factors with 2 and 65 levels, respectively. Germination rate, expressed as a percentage, and time in years were expressed as continuous numeric values. An additional column with the year variable grouped as intervals of 1-5, 5-10, 10-15, 15-20, and 20-25 was added and converted to a categorical factor to enhance data visualization. The same conversions were applied in intermediate storage, where the fixed effect of RH and the random effect of Taxon Name were converted to categorical factors with 2 and 68 levels, respectively. For both analyses, the packages “tidyverse”, “ggplot2” and “scales” were used to call the “ggplot” function for the fine-tuned boxplot visuals of the data with the newly created categorical year interval plotted on the x-axis against Overall Germination Rate on the y-axis. A scatter plot for each species’ germination rates was created with a fitted line to observe viability trends across time. The LME with the time in years as a continuous variable and no interaction term was favored in both orthodox and intermediate analyses for its simplicity and fit. The “performance” and “car” packages were loaded to assess the quality of the model, calling the “check_model” function to produce diagnostic plots for the linear model. The “nlme” package was loaded to call the “lme” and “varldent” functions to compare models under different assumptions (homoscedasticity and heteroscedasticity) and variances across RH levels. The “ANOVA” function was used to ensure the best fit and compare the two models with a log-likelihood ratio test. As longevity models are intrinsically non-linear, a quadratic model with a binomial term was used to account for the parabolic trend in the data, and an ANOVA was conducted to compare the models’ fit against the simple, linear model. The “dplyr” package was called to plot the quadratic model to visualize the sigmoidal curve in the Overall Germination Rate of our taxa. The rand function was called to assess the significance of the random effect variable. The “merTools” package was loaded to call the functions “predictInterval,” “REsim,” and “plotREsim” to predict model intervals, simulate random effects, and plot the estimates. The package “sjPlot” was then loaded to call the functions “plot_model” and “tab_model” to generate tables with the predicted estimates.

5. Results

5.1 Statistical analysis

Violation of constant variance and deviation from normal distribution was observed in both analyses visualized through faceted scatter plots that were generated for every species in intermediate storage (Appendix 4) and orthodox storage (Appendix 5). A negative coefficient of the effect of Time in Years on Overall Germination Rates was observed in both analyses, and this decline was significant ($p<0.05$). The Interclass Correlation Coefficients (ICC) measures the total variance of germination rates that is explained by the random intercept Taxon Name. It was above 55% in both analyses.

5.1.1 Intermediate storage

Despite the linear LME exhibiting the best fit in the intermediate analysis, it is established that germination data are intrinsically non-linear. The quadratic model showed a p-value of 0.558 indicating a non-significance of the effect of ultra-dry seed storage on longevity compared with conventional drying (Table 2). The Intercept indicates a baseline germination rate of 53% for ultra-dry seed storage and shows that seeds can germinate after ultra-dry desiccation. The Conditional R^2 indicates that 61% of the variation in germination rates can be explained by the predictor variables (Relative Humidity and Years) and the random intercept (Taxon Name) while the Marginal R^2 indicates that 4% can be explained by the fixed-effects alone. The quadratic model captured the parabolic shape of the curved regression showing that germination percentages don't decline at a steady rate over time (Figure 1).

Table 2: Quadratic model summary table for intermediate storage analysis. RH combined compares the germination rates of conventionally dried samples with ultra-dried samples. The p-value (0.558) indicates a non-significance on germination rates between the two desiccation treatments. The low p-value of Year indicates that germination significantly declines over time in storage.

Predictors	*Estimates*	*std. Beta*	*CI*	*standardized CI*	*p*
(Intercept)	0.53	-0.04	0.46 – 0.59	-0.24 – 0.16	**<0.001**
RH combined [20%+]	0.01	0.02	-0.02 – 0.03	-0.05 – 0.10	0.558
Year scaled	-0.06	-0.19	-0.08 – -0.05	-0.24 – -0.14	**<0.001**
Year scaled^2	-0.01	-0.04	-0.03 – 0.00	-0.09 – 0.00	0.061
Random Effects					
σ^2	0.04				
$\tau_{00\ TaxonName}$	0.06				
ICC	0.59				
$N_{\ TaxonName}$	68				
Observations	1060				
Marginal R^2 / Conditional R^2	0.041 / 0.610				
Deviance	-95.585				
AIC	-55.690				
AICc	-55.611				

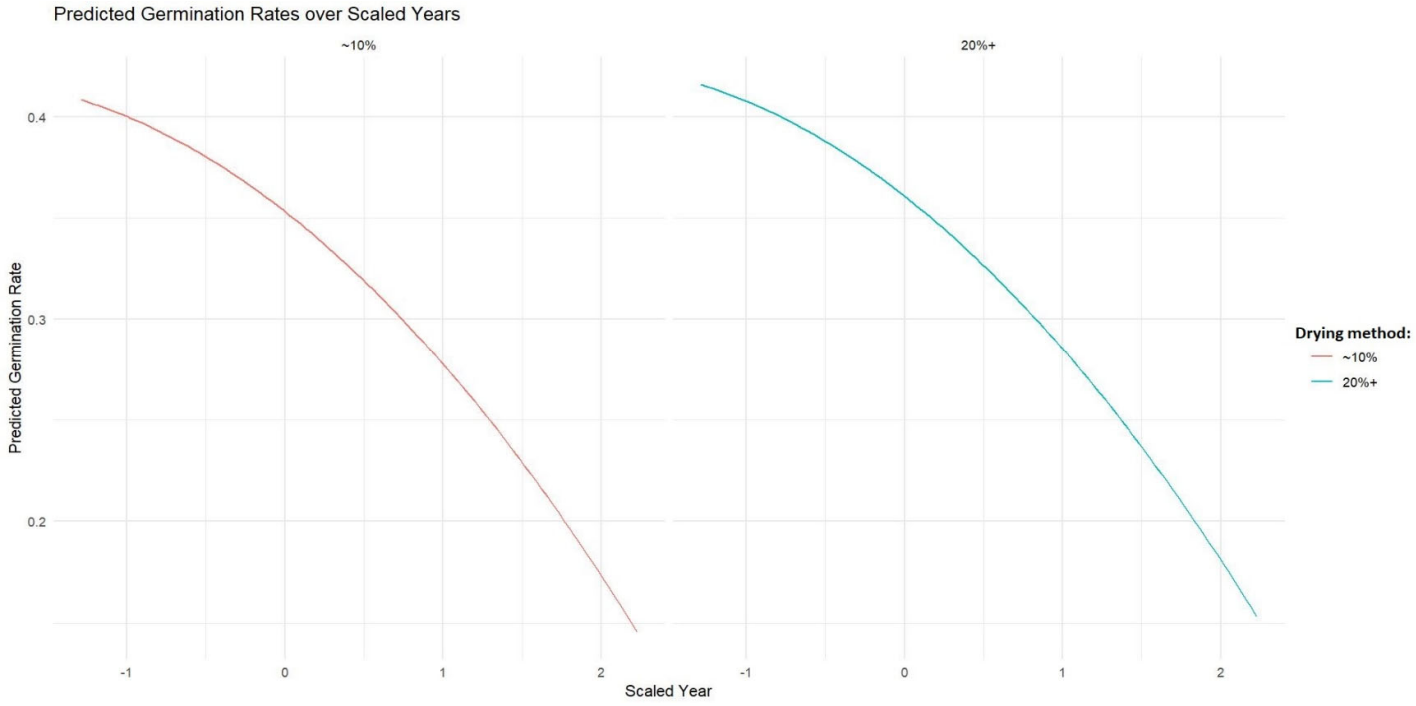

Figure 1: Predicted germination rates for seeds in intermediate storage over the scaled years generated by the quadratic model. Ultra-dried seeds (~10% RH) and conventionally dried seeds (20%+ RH). The curvature of the regression shows that germination rates don't decline at a steady rate over time.

5.1.2 Orthodox storage

In addition to the non-linearity of germination data, the statistical analysis in orthodox storage exhibited slight heteroscedasticity showing a systematic change in the spread of the residuals over the range of measured values. Results from the log-likelihood ratio test produced a high p-value ($p>0.05$) showing that altering the model assumptions to fit homoscedasticity would not provide a better fit. The quadratic LME in the orthodox storage analysis rendered a better AIC than the linear model, and the low p-value ($p<0.05$) from ANOVA comparison of models (linear/quadratic) indicated that non-linearity was a significant issue. Results from the quadratic model rendered a p-value of 0.907 and showed a non-significance of the effect of ultra-dry seed storage on longevity compared with conventional drying (Table 3). The Intercept shows a baseline germination rate of 43% for ultra-dry seed

Table 10: Quadratic model summary table for orthodox storage analysis. RH combined compares the germination rates of conventionally dried samples with ultra-dried samples. The p-value (0.907) indicates a non-significance on germination rates between the two desiccation treatments. The low p-value of Year indicates that germination significantly declines over time in storage.

Predictors	*Estimates*	*std. Beta*	*CI*	*standardized CI*	*p*
(Intercept)	0.43	-0.12	0.37 – 0.50	-0.32 – 0.09	**<0.001**
RH [20%]	-0.00	-0.01	-0.03 – 0.03	-0.10 – 0.09	0.907
Year scaled	-0.10	-0.32	-0.12 – -0.09	-0.37 – -0.27	**<0.001**
Year scaled^2	0.04	0.12	0.02 – 0.06	0.07 – 0.17	**<0.001**
Random Effects					
σ^2	0.04				
$\tau_{00\ TaxonName}$	0.06				
ICC	0.57				
$N_{\ TaxonName}$	63				
Observations	774				
Marginal R^2 / Conditional R^2	0.083 / 0.607				
Deviance	-34.602				
AIC	4.361				
AICc	4.470				

storage at year 0. The Conditional R^2 indicates that 60% of the variation in germination rates can be explained by the predictor variables and the random intercept. The Marginal R^2 indicates that 8% can be explained by the fixed-effects alone, suggesting that 52% of the variance in germination rates is species-specific.

The quadratic LME captured the parabolic shape of the curved regression in orthodox storage (Figure 2).

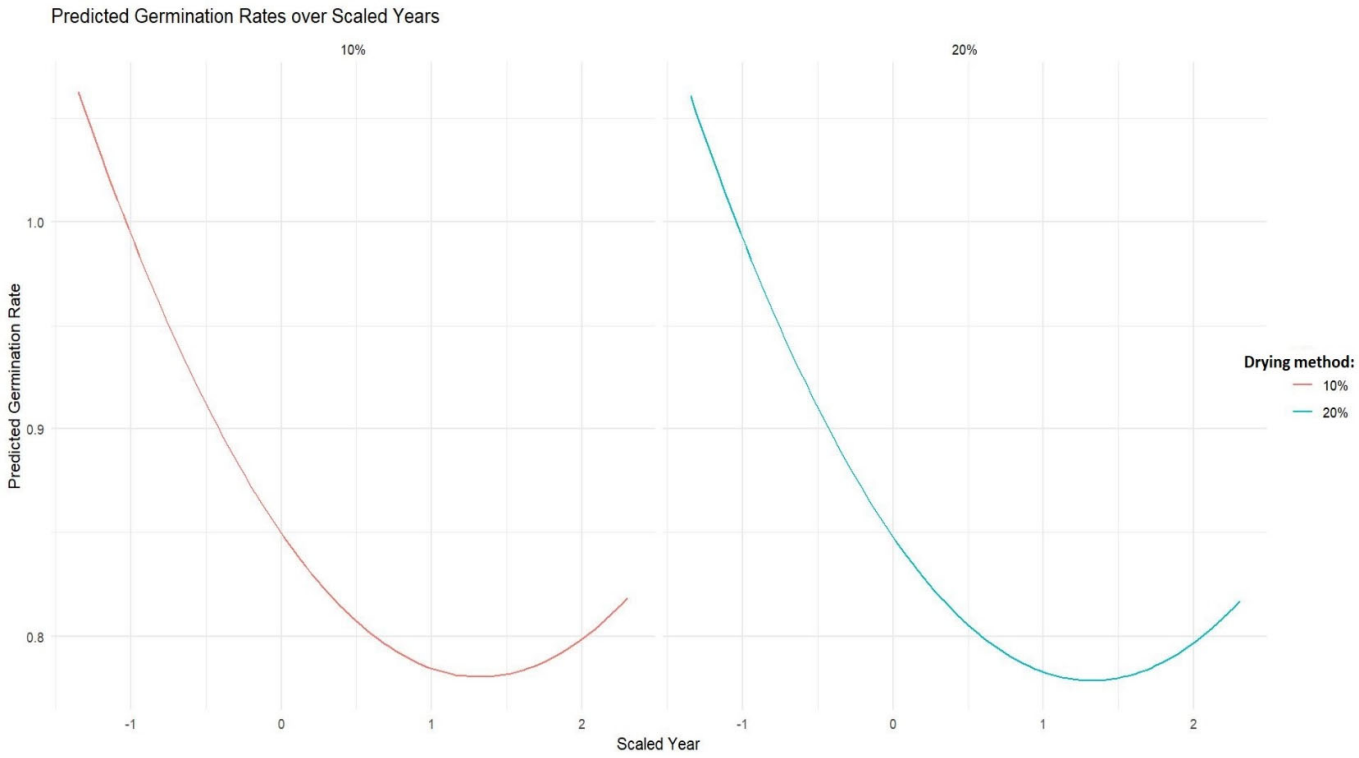

Figure 6: Predicted germination rates for seeds in orthodox storage over the scaled years generated by the quadratic model. Ultra-dried seeds (10% RH) and conventionally dried seeds (20% RH). The curvature of the regression shows that germination rates don't decline at a steady rate over time.

5.2 Desiccation methodology

Investigation into the methodology of conventional seed drying using MgCl2 as a desiccant showed RH between 35-37.9% across seven observations at ambient and cold (5°C) temperatures, regardless of the amount of electrolyte. While K2CO3 rendered the expected eRH (43%). The type of silica gel and reactivation method has been found to significantly impact the successive desiccation capacity of the beads. The RH of silica gel after microwave reactivation under the same parameters (defrost function for 12 minutes) showed a range of 7-24% according to its origin. Additionally, newly acquired silica gel from Dry&Dry showed 0% RH when tested in the 225ml sealed container. This silica desiccation capacity was not found in any samples once saturated and reactivated.

5.3 Relative humidity measurements

Readings from the hygrometers placed in sealed containers with ultra-dried and conventionally dried seeds showed significant RH fluctuations (Table 4). Over half of the ten vials in ultra-dry storage showed a higher RH (>10%) than expected. RH fluctuations below 10% were more prevalent in orthodox storage (0-8% RH) than intermediate (2-4% RH). None of the vials containing conventionally dried seeds in orthodox storage showed the expected value of 20% RH. An average of 41% RH was found among the tested vials with outliers from 30-57% RH (SD=9.6). Conventionally dried seeds in intermediate storage showed a more centered trend around the expected value such as the average RH which was around 27% (SD=5.1). Most ultra-dried species of selected vials with the lowest RH (<10%) were still able to maintain germination rates higher than 50% in their last viability test. Only two accessions, *Cyanea angustifolia* (23 years in intermediate storage) and *Vaccinium reticulatum* (24 years in orthodox storage) recorded germination rates of 44% and 20%, respectively. *Artemisia mauiensis* seeds showed similar germination rates between the desiccation treatments in both storage despite a significant variation in the recorded RH (0-35%). Despite the important variations in %RH between the two desiccation treatments, germination rates of species from the selected vials were similar, per the statistical analysis results. Dates of the latest viability tests ranged from 8-25 years. Therefore, fluctuations in the upcoming

Table 14: Latest germination rates and RH measurements for seeds of selected vials. Ultra-dry samples are referred to as C10 for intermediate storage and D8 for orthodox storage. Conventional samples are referred to as C20+ for intermediate storage and D20 for orthodox storage Highlighted: ultra-dry species with the lowest RH (<10%) and their respective germination rates

Species in intermediate storage	Years	Latest germination C10 (in %)	Latest germination C20+ (in %)	RH C10 (in %) / temperature (in °C)	RH C20+ (in %) / temperature (in °C)
Artemisia mauiensis	18	88	36	4.4 / 6.3	22.1 / 6.1
Clermontia hawaiiensis	10	92	78	3.2 / 5.2	26.5 / 4.6
Clermontia kakeana	26	88	100	14.3 / 6.2	24.8 / 5.8
Cyanea angustifolia	23	44	42	4.2 / 5.8	26.3 / 6
Cyperus trachysanthros	23	96	94	15.7 / 4.9	21.7 / 4.9
Deschampsia nubigena	15	5	3	20.0 / 4.2	23.1 / 3.4
Rumex albescens	21	72	54	15.0 / 5.6	29.0 / 5.6
Santalum ellipticum	14	84	72	1.9 / 4.5	23.5 / 4.2
Trematolobelia kaalae	23	82	88	15.6 / 4	33.0 / 4.3
Vaccinium calycinum	24	56	60	18.3 / 4.1	37.3 / 3.7

Species in orthodox storage	Years	Latest germination D8 (in %)	Latest germination D20 (in %)	RH D8 (in %) / temperature (in °C)	RH D20 (in %) / temperature (in °C)
Artemisia mauiensis	18	66	62	0.0 / -16.1	35.6 / -16.4
Bidens sand subsp. sandvicensis	25	96	96	5.1 / -15.9	40.4 / -15.6
Cyperus trachysanthros	23	4	84	14.4 / -16.3	33.4 / -15.8
Lobelia grayana	8	18	22	25.8 / -14.8	44.0 / -15.3
Lysimachia mauritania	22	28	16	17.7 / -15.3	37.9 / -16.3
Metrosideros polymorpha	24	40	24	27.8 / -15.3	57.0 / -15.3
Peperomia tetraphylla	22	92	96	8.6 / -14.6	30.8 / -15.3
Rumex albescens	21	74	56	19.7 / -16	34.3 / -16.1
Trematolobelia kaalae	23	76	58	12.5 / -14.3	41.3 / -14.3
Vaccinium reticulatum	24	20	4	4.8 / -14.2	59.1 / -14.7

viability tests for the seeds that were accessioned more recently are still expected. The RH measurements of foil packets followed a similar trend. The ranges from 23-50% RH and 8-45% RH were found for intermediate and orthodox storage, respectively, despite all seed accessions following the same conventional desiccation protocols. Measurements of RH in vials and foil packets indicate that drying seeds at an eRH of >30% does not reach 20% RH in intermediate or orthodox storage.

6. Discussion

6.1 Interpretation

6.1.1 Statistical analysis

The data set in intermediate and orthodox storage includes a large range of values in the germination rates (from 0 to 100% germination) and variations in the year intervals of the viability tests across species. A certain violation of constant variance was expected, as well as a deviation from normal distribution, which were both observed in the statistical analyses. LMEs are usually the chosen models in analyses with ecological data where violation of assumptions is common due to missing random effects, and if model estimates are robust, assumptions remain unbiased (Schielzeth et al., 2020). As expected, the negative coefficient of the effect of year on germination rates in both analyses indicated a general decrease in viability for each additional increase in one year across both desiccation treatments. Therefore, improvements in viability testing protocols (e.g., dormancy-breaking techniques) over time may explain the trends in the facetted scatter plots. Seed dormancy often decreases spontaneously during storage (Pérez-García et al., 2009). Using alternate temperatures sometimes contributes to a perceived higher final germination percentage, although seeds inevitably age and lose viability post-storage under seed bank conditions (Pirredda et al., 2024). When dormancy is broken during storage or enhanced dormancy-breaking techniques are applied to a seed sample, the initial viability test is replaced as the reference value, and the new test is considered peak germination. This prevents data from exhibiting a false-positive trend in germination percentages over time. Considering dormancy while preserving seeds of wild species is particularly important when assessing longevity in conservation seed banking to prevent misinterpretations related to viability decline.

6.1.2 Findings

The failure to reject the null hypothesis implies that no significant effects of RH were found on seed longevities between desiccation treatments in each storage. It

is widely recognized that storing seeds in the dry state reduces the risk of pathogen growth, depletion of food reserves, and deleterious by-products with a diminished metabolism and lowered reaction rates (Vertucci & Roos, 1990). Following the natural dispersal of seeds from the parent plant, the time for orthodox and intermediate seed harvesting is at full maturation and prior to separation to ensure that the material is collected from the plant instead of from the ground where complications (predation, saturation, trampling) may occur. Seed maturity at harvest will predict the ability of the seed lot to maintain longevity. Maximizing seed longevity is crucial for genetic resource preservation (Mira et al., 2015). Seed storage behavior along with preharvest and postharvest treatments may result in significant differences across species and even in seed lots of the same collection event (Walters, 2013). A seed is a structure composed of complex substances such as carbohydrates, proteins, and oils, with some water in addition, variable in quantity, that can be added or removed (Thomsen & Stubsgaard, 1998). A minimum degree of tolerance to desiccation is required so that the seeds do not completely lose viability as seed MC begins to decrease. Fortunately, it has been estimated that only about 8% of the world's seed plant species produce desiccation-sensitive seeds (Wyse & Dickie, 2017). Published data report low desiccation injuries in orthodox seeds and issues mostly emerging from imbibition injury (Ellis et al., 1990) possibly avoided with seed priming prior to viability testing (Malagatti, 2023). A publication by Ellis and colleagues over 35 years ago found a doubling of longevity with each ~8% reduction in eRH of the weakly bound water fraction of the seed until reaching values between 10-11% RH (Ellis et al., 1990). Subsequent publications from Vertucci and Roos argued that interpreting isotherms at 20°C for an experiment conducted at 65°C leads to underestimating the critical water content and found the optimum water content to be between 19-27% RH (Vertucci & Roos, 1990). Results from Hong and colleagues after ten years of hermetic seed storage at -20°C suggest neither an advantage nor a disadvantage to seed survival of ultra-dry storage compared with conventional dry storage (Hong et al., 2005). Solberg and colleagues observed a certain variation in longevity and germination in a long-term viability study at sub-zero temperatures of crop species and cultivars. Despite most samples maintaining over 80% germination after 30 years, outliers were observed where germination dropped below 40%. The variation happened between species and within cultivars of the same crops (Solberg et al., 2020). Shorter-term studies from Yogeesha and colleagues found a sustained viability when ultra-dry seeds of China aster were stored at ambient temperatures (20-25°C) but no difference in germination between the ultra-dry and control samples of both species studied (China aster and onion) when stored at a controlled 15°C (Yogeesha et al., 2017). Cromarty and colleagues indicate that drying under the recommended threshold can substantially increase longevity for seeds that tolerate such desiccation (Cromarty et al., 1982). Alternatively, Walters and colleagues suggest

that storing seeds at RH lower than 15% will not increase shelf life and may accelerate deterioration (Walters & Engels, 1998). The difficulty lies in identifying the species most tolerant to desiccation without reducing vigor prior to long-term storage. The variations in desiccation treatments performed by gene banks can be explained by the greater conservation implications of certain species, as well as the type of research organism (temperate/tropical) since ecological aspects correlate with seed longevity and germination (Mira et al., 2015). Despite observing the generally sustained viability of seeds, differences in experimental designs and subsequent variation in longevities contribute to the uncertainty surrounding ultra-dry seed storage. Accelerating aging studies where seeds are exposed to suboptimal temperatures differ significantly from storage under gene bank conditions (Hay et al., 2022).

6.1.3 Influence of seed composition

Drying seeds of different species to the same RH can be achieved in a single environment by altering the drying treatment period, provided that the RH is sufficiently low for the species with the highest eMC (Cromarty et al., 1982). Results from this research and previous studies show that seeds will reach different internal MC when dried at the same eRH according to their internal composition (Ellis et al., 1990; Vertucci & Roos, 1990; Pérez-García et al., 2009). Seed composition significantly influences longevity, potentially affecting the results of ultra-dry experiments performed on distinct species and under varying experimental designs. Seed lipid content has been associated with lower longevity due to oxidative degradation in cell membranes (Pirredda et al., 2024). Research subjecting oily seeds to ultra-dry treatments suggest that lipid content may correlate with a higher desiccation tolerance due to their hydrophobic properties (Ellis et al., 1990; Vertucci & Roos, 1990; Xie et al., 2021). Pérez-Garcia and colleagues assessed the longevity of Brassicaceae after almost 40 years of storage at -10°C and found that all species maintained germination rates higher than 85% at MC between 1.5-3% (Pérez-García et al., 2009). This very low MC was most likely due to the high lipid content of Brassicaceae seeds, further supporting the higher desiccation tolerance of lipid-rich seeds compared to carbohydrates and protein-rich seeds. Research from Mira and colleagues on seeds of the same family indicated that longevity increased exponentially as water content decreased until a certain limit was reached. Desiccation from the recommended 25% RH to the critical MC found at 5.5% RH at 25°C (isotherm constructed at 25°C from storage at 45°C) increased seed longevity two-to seven-fold. Moreover, experiments with seeds stored at 35°C showed no viability loss for most species that had RH between 25-0.5% (Mira et al., 2015). Following this theory, the very low RH sometimes observed from the selected vials in this longevity study may directly result from seed composition, specifically lipid content. Seeds from the Asteraceae family (*Artemisia mauiensis*,

Bidens sandvicensis subsp. sandvicensis) exhibited RH<5% while maintaining germination after 18 and 25 years in storage. A thorough assessment of seed composition is recommended to support this analysis. The suggestion that further drying of seeds with high lipid contents can contribute to considerable benefits in long-term storage is also supported by Ellis and colleagues (Ellis et al., 1990).

6.1.4 Limitations

Seeds from crop species have been selected in other studies for analyses of ultra-dry storage. Hot-air drying is usually applied to agricultural seeds managed in bulk amounts used in short-term restoration efforts. Drying by raising the temperatures shortens the desiccation protocols, increases respiration and aging processes, and shortens seed lifespans (Thomsen & Stubsgaard, 1998). These methods employed by several studies with accelerated seed aging, do not constitute a sustainable protocol for germplasm of conservation importance. Empirical data from long-term viability studies are needed to make informed decisions about ultra-dry seed storage. This analysis's results don't provide a clear directional trend. The deficiency of the conventionally dried samples to reach 20% RH in storage made the comparative analysis more difficult as several samples reached RH above 40%, a value twice as much as what is generally recommended or expected for long-term storage. As it is recognized that under-drying is more deleterious than over-drying, germination rates from conventionally dried seeds in this analysis may be underperforming and, therefore contribute to the non-significance found for the effects of ultra-dry. The possibility that germination rates would be more distinct between desiccation treatments if all the samples were found to be in equilibrium with the expected RH is not ruled out. The trend in which this distinction would occur is unclear. The rates of the decline in seeds' longevities were similar across treatments for most species, as indicated by the results of the statistical analyses and the insignificant p-values. This observation supports the hypothesis that seed viability is sustained after over 20 years of cold storage under ultra-dry conditions. The failure of laminated foil packets to maintain integrity has been reported in a study by Hong and colleagues after a decade at ambient storage but not at subzero temperatures (Hong et al., 2005). It is unclear whether the RH recorded in foil packets is due to a decreased impermeability over time or insufficient seed desiccation. The same interrogative can be asked of samples stored in glass vials. The difference in desiccation capacity of silica gel can contribute to significant irregularities of eRH inside the vials once sealed for long-term storage. The permeability of drying cabinets and repeated opening/closing can be another factor disturbing the drying environment, and the time it returns to equilibrium varies. Similar issues can be considered as routine viability tests are performed and vials are opened. While the time for vials to stay open should be kept as short as possible, in practice, bottlenecks can happen and moisture uptake from the SCL environment

may not be uniform across all samples and seed sizes. If the effects of ultra-dry seed desiccation on viability over time have not indicated a clear tendency in this research, the indication that conventionally dried seeds aren't reaching targeted RH in storage may suggest that the current desiccation protocols underestimate the values obtained in storage. The SCL does not benefit from the controlled environment offered by drying rooms that may be found in other facilities. Ideally, drying rooms should be kept at similar parameters (RH especially) as those applied to seed drying. In practice, rooms in the SCL where seed manipulation occurs fluctuate between 35-40% RH at 20°C (+/-3°C). The disagreements regarding drying RH may be further explained, in part, by the differences in climatic regions and gene bank equipement.

6.2 Implications for *ex-situ* plant conservation

Plants provide fundamental support systems for life on Earth and are the basis for all terrestrial ecosystems (Brummitt et al., 2015). Decades of concerns about the environment and its biodiversity have led to the development of multitudinous conservation strategies, including the first National Parks and Botanical Gardens dating back from 150 to almost 500 years ago, respectively. From the early conservation efforts to the development of sustainability, ex-situ plant conservation evolved slowly until the general recognition of the concept of extinction and the prominent role of anthropogenic activity in accelerating extinction rates (Maunder et al., 2004). The ex-situ conservation of dormant seed collections constitutes a far more cost-effective and efficient way of storing genetic material for longer periods than botanic gardens, constrained by practical issues of space, continuous need for management, and risk of climatic or pathogenetic perturbation. Conversely, the technology of seed banking by which the seeds are dried and cooled can be widely applied to a range of species with little intervention to maintain large amounts of intraspecific diversity (Pritchard, 2004). Well-managed gene banks worldwide hold collections of a broad range of genetic resources with the overall objective of long-term conservation and accessibility of plant germplasm to plant breeders, researchers, and other users (FAO, 2014). While conservation facilities generally share the same goals, their operating systems may differ according to their climatic region and resources.

6.3 Implications for tropical seed banking

The Hawaiian archipelago is part of the Polynesia-Micronesia biodiversity hotspot, one of the smallest hotspots in terms of terrestrial land area and yet classified amongst the most endangered in the world (CIMCBC, 2007). A combination of

opposite forces originating from basalt eruptions of shield volcanoes, erosion, and ocean forces shape Hawaii's topography, offering a dynamic landscape of canyons and ridges of varied elevations and providing a unique variety of environmental conditions and resultant vegetation. Ex-situ seed banking is critical for plant conservation globally, especially for threatened flora in tropical ecosystems like Hawai'i (Chau et al., 2019). In tropical regions, less detailed knowledge is available on rare and endemic species, and their higher occurrence can cause uncertainties regarding proper conservation techniques (Brummitt et al., 2015). High endemism generally characterizes island systems due to their isolation. They are subject to higher extinction rates due to habitat conversion, invasive plant and animal predation, and changing local conditions (Rønsted et al., 2022). Endemic taxa may be intrinsically threatened due to their restricted distribution and are highly important to conservation prioritization (Işik, 2011). Hawai'i has more plant extinctions than other geographical regions (Humphreys et al., 2019). The number of federally listed taxa has increased by 56% over the last decade (Keener et al., 2018), and ex-situ species preservation might be the only way to prevent further extinctions. Contrary to previous understanding, not all species native to moist habitats and rainforests show recalcitrant seed storage behavior, but produce all three categories of seeds (Hong & Ellis, 1996). Most species native to the Hawaiian Islands produce seeds with a certain desiccation tolerance, allowing seed banking under conventional protocols. The peculiarity lies in the species that produce seeds with an intermediate behavior, withstanding desiccation but quickly losing viability when stored in conventional freezers at -18°C. This characteristic is more widely observed in Hawai'i than in other regional flora (Chau et al., 2019). Consequently, a specific storage protocol had to be defined for conventional seed banking, hence the intermediate storage methods employed at the SCL where temperature-sensitive seeds are stored at 5°C. If this storage is primarily specific to Hawaiian seed banking, other facilities may be resource-limited. Results from Hong and colleagues confirmed sustained viabilities of ultra-dry seeds stored at 20°C (Hong et al., 2005). This follows the initial theory behind the development of ultra-dry seed storage to reduce the need for refrigeration or completely avoid it (Ellis et al., 1990).

7. Conclusions

The inevitable decline of seed viability under gene bank conditions is the most critical factor determining long-term storage protocols. Improving post-harvest techniques constitutes a challenge to safeguard species diversity in conservation seed banks. Longevity is strongly influenced by the environmental conditions (RH and temperature) to which the seeds are exposed to, from their development to their inclusion in storage. Seed structure and chemical composition also influence longevity and are determining storage conditions. Mixed results from ultra-dry analyses under seed aging conditions have been observed, and results from long-term studies at low temperatures are scarce. The present longevity study found sustained viability and germination rates after more than 20 years of cold storage for ultra-dried seeds and seeds desiccated under conventional protocols. It revealed that eRH in long-term storage for seeds packed in glass vials or laminated aluminum foil packets don't always reach the targets (10 or 20%) and are generally more elevated. Whether this is a consequence of under-drying or storage issues remains somewhat unclear. If several samples of seeds initially ultra-dried exhibited RH>10%; they were still under the threshold for seed drying. Therefore, the comparative analysis between ultra-dry and conventional desiccation was still possible, and the RH thresholds were considered a range rather than an absolute value. The purpose of this research was to assess if ultra-dry seed storage had an effect on longevity. As seed viability inevitably declines in storage, ultra-dried seeds' germination rates were compared to conventionally dried seeds' germination rates for ~10-25 years. The hypothesis was that one group would have better sustained viability than the other. The similar germination rates over time of the two desiccation treatments did not contribute to the observation of that trend. Despite rejecting the null hypothesis and finding no significant difference among ultra-dry effects compared to conventional desiccation treatments, the results suggest that it is possible to dry below the standard recommended values without effectively killing seeds. This research also demonstrates that the expected humidity in storage is hardly achieved. Decreasing the current values of desiccation protocols may constitute an effective solution to maintaining eRH in storage, especially for gene banks with limited resources. Based on the results from this research and the prospect of future developments, setting the seed desiccation protocols to the same eRH of what is expected in storage may be a possible

improvement to the problems faced as it pertains to under drying in seed banks located in tropical climates

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Popular science summary

Seeds can be considered time capsules containing the potential for a new plant. Ex-situ plant conservation constitutes an effective and cost-efficient way of storing a large collection of dormant germplasm. Seed longevity in gene banks is a function of post-harvest conditions and seed properties. Influences on longevity by controlling the seed storage environment, primarily temperature and relative humidity are possible. Seeds are generally best stored in cool and dry environments; however, no scientific consensus has been reached regarding how to dry seeds to reach targeted equilibrium relative humidity. The theory indicates that the desiccation capacity of a seed depends on its chemical composition and the proportion of carbohydrates, proteins, and lipids. It has been found that seeds with higher lipid contents usually tolerate a strong degree of water removal. As protocols should be applied to most seeds for gene bank efficiency, standard desiccation values are recommended between 15-25% relative humidity. The potential for certain seeds to sustain longevity with lower relative humidity motivated the research surrounding ultra-dry seed storage, a method that involves drying below the standards considered “safe”. Below this value, seed viability may be enhanced or diminished. A clear trend has not yet been found regarding the effects of ultra-dry seed storage, and debates are ongoing. This research has not contributed to finding a clear verdict. Nonetheless, it demonstrates that the majority of seeds under ultra-dry storage could still maintain germination rates similar to those dried at conventional standards.

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I would like to express my infinite gratitude to my supervisors. Nathaniel Kingsley, for making this project possible and providing expert guidance through its achievement. His skills and knowledge have been instrumental in the completion of this work and his critical thinking allowed us to challenge our understandings and reshape our analyses. This research wouldn't have been possible without his involvement. Thank you for sacrificing your evenings and weekends to coding and debating our methods.

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Appendix 1a

Picture of the intermediate storage at 5°C in a commercially available refrigerator. Each box is referred to as a unit labeled according to the seed desiccation protocol. Each unit contains the sealed glass vials in which the seeds are stored and are arranged alphabetically at the genus level.

Appendix 1b

Picture of the glass vials (large, 16x24cm) with rubber gaskets used to store the seeds of the ultra-dry analysis. The seeds are from *Gardenia brighamii* (Hawaiian name: nānū). On the left is the ultra-dried sample stored with silica gel. On the right is the conventionally dried sample stored with mung beans.

Appendix 2

List of species included in the ultra-dry analysis, their origin, conservation assessment, storage behavior and presence in the intermediate storage at 5°C and/or orthodox storage at -18°C (indicated by "X"). Conservation assessment data taken from naturalhistory2.si.edu. Species indicated as "rare" are recognized at the state level rather than at the federal level inder the USFWS Endangered Species Act.

Family	*Species*	Origin	Conservation assessment	Seed behavior	5°C	-18°C
Amaranthaceae	*Achyranthes splendens var. rotundata*	Endemic	endangered	Orthodox	X	X
Amaranthaceae	*Charpentiera tomentosa var. maakuaensis*	Endemic		Short-lived	X	X
Apiaceae	*Spermolepis hawaiiensis*	Endemic	endangered	Orthodox		X
Asteraceae	*Artemisia mauiensis*	Endemic		Orthodox	X	X
Asteraceae	*Bidens sandvicensis subsp. sandvicensis*	Endemic		Orthodox	X	X
Asteraceae	*Bidens torta*	Endemic		Orthodox	X	X
Asteraceae	*Remya kauaiensis*	Endemic	endangered	Short-lived	X	X
Asteraceae	*Tetramolopium arenarium subsp. arenarium*	Endemic	endangered	Orthodox	X	X
Asteraceae	*Tetramolopium consanguineum subsp. leptophyllum*	Endemic	extinct	Orthodox	X	X
Asteraceae	*Tetramolopium rockii*	Endemic	vulnerable	Orthodox		X
Begoniaceae	*Hillebrandia sandwicensis*	Endemic	vulnerable	Short-lived	X	X
Campanulaceae	*Clermontia hawaiiensis*	Endemic		Freeze sensitive	X	X
Campanulaceae	*Clermontia kakeana*	Endemic		Freeze sensitive	X	
Campanulaceae	*Clermontia montis-loa*	Endemic		Freeze sensitive	X	
Campanulaceae	*Cyanea angustifolia*	Endemic		Freeze sensitive	X	X
Campanulaceae	*Cyanea crispa*	Endemic	endangered	Freeze sensitive	X	
Campanulaceae	*Cyanea kauaulaensis*	Endemic	endangered	Freeze sensitive	X	X
Campanulaceae	*Cyanea lobata subsp. baldwinii*	Endemic	endangered	Freeze sensitive	X	
Campanulaceae	*Cyanea membranacea*	Endemic	rare	Freeze sensitive		X
Campanulaceae	*Cyanea superba subsp. superba*	Endemic	extinct	Freeze sensitive	X	
Campanulaceae	*Delissea rhytidosperma*	Endemic	extinct	Freeze sensitive	X	
Campanulaceae	*Lobelia dunbariae subsp. paniculata*	Endemic	rare	Freeze sensitive	X	X
Campanulaceae	*Lobelia grayana*	Endemic		Freeze sensitive	X	X
Campanulaceae	*Lobelia hypoleuca*	Endemic		Freeze sensitive	X	
Campanulaceae	*Lobelia niihauensis*	Endemic	endangered	Freeze sensitive	X	X
Campanulaceae	*Lobelia oahuensis*	Endemic	endangered	Freeze sensitive	X	X
Campanulaceae	*Lobelia yuccoides*	Endemic	rare	Freeze sensitive	X	
Campanulaceae	*Trematolobelia grandifolia*	Endemic	rare	Orthodox	X	X
Campanulaceae	*Trematolobelia kaalae*	Endemic		Orthodox	X	X
Caryophyllaceae	*Schiedea globosa*	Endemic	vulnerable	Orthodox	X	X
Caryophyllaceae	*Schiedea obovata*	Endemic	endangered	Orthodox		X
Caryophyllaceae	*Schiedea trinervis*	Endemic	endangered	Orthodox	X	
Caryophyllaceae	*Silene hawaiiensis*	Endemic	rare	Orthodox	X	X
Caryophyllaceae	*Silene lanceolata*	Endemic	endangered	Orthodox	X	X
Chenopodiaceae	*Chenopodium oahuense*	Endemic	endangered	Orthodox	X	X
Cucurbitaceae	*Sicyos pachycarpus*	Endemic		Short-lived		X
Cyperaceae	*Carex meyenii*	Indigenous		Freeze sensitive	X	
Cyperaceae	*Cyperus javanicus*	Indigenous		Freeze sensitive	X	
Cyperaceae	*Cyperus trachysanthos*	Endemic	endangered	Freeze sensitive	X	X
Cyperaceae	*Fimbristylis cymosa*	Indigenous		Freeze sensitive	X	X
Ericaceae	*Vaccinium calycinum*	Endemic		Orthodox	X	X
Ericaceae	*Vaccinium reticulatum*	Endemic		Orthodox	X	X
Euphorbiaceae	*Euphorbia herbstii*	Endemic	endangered	Orthodox		X
Euphorbiaceae	*Euphorbia skottsbergii var. skottsbergii*	Endemic	rare	Orthodox	X	X
Fabaceae	*Acacia koa*	Endemic		Orthodox	X	X
Fabaceae	*Canavalia galeata*	Endemic		Orthodox		X
Fabaceae	*Sesbania tomentosa*	Endemic	endangered	Orthodox		X
Fabaceae	*Sophora chrysophylla*	Endemic		Orthodox		X

Family	*Species*	Origin	Conservation assessment	Seed behavior	5°C	-18°C
Gesneriaceae	*Cyrtandra cordifolia*	Endemic		Orthodox	X	X
Gesneriaceae	*Cyrtandra dentata*	Endemic	endangered	Orthodox	X	X
Gesneriaceae	*Cyrtandra grandiflora*	Endemic		Orthodox	X	X
Hydrangeaceae	*Hydrangea arguta*	Endemic		Short-lived		X
Juncaceae	*Luzula hawaiiensis var. hawaiiensis*	Endemic		Orthodox		X
Liliaceae	*Dianella sandwicensis*	Endemic		Freeze sensitive	X	
Loganiaceae	*Geniostoma tinifolium var. tinifolium*	Endemic		Freeze sensitive	X	
Myoporaceae	*Myoporum sandwicense*	Endemic		Freeze sensitive	X	
Myrtaceae	*Metrosideros macropus*	Endemic		Orthodox	X	
Myrtaceae	*Metrosideros polymorpha var. glaberrima*	Endemic		Orthodox	X	
Myrtaceae	*Metrosideros polymorpha var. polymorpha*	Endemic		Orthodox	X	X
Myrtaceae	*Metrosideros polymorpha var. pumila*	Endemic		Orthodox	X	X
Papaveraceae	*Argemone glauca var. glauca*	Endemic		Orthodox		X
Piperaceae	*Peperomia latifolia*	Endemic		Orthodox		X
Piperaceae	*Peperomia tetraphylla*	Indigenous		Orthodox	X	X
Poaceae	*Deschampsia nubigena*	Endemic		Short-lived	X	X
Poaceae	*Eragrostis atropioides*	Endemic		Orthodox	X	X
Poaceae	*Eragrostis deflexa*	Endemic	rare	Orthodox	X	
Poaceae	*Panicum tenuifolium*	Endemic		Freeze sensitive	X	
Polygonaceae	*Rumex albescens*	Endemic		Orthodox	X	X
Primulaceae	*Lysimachia mauritiana*	Indigenous		Orthodox	X	X
Rhamnaceae	*Colubrina oppositifolia*	Endemic	endangered	Orthodox		X
Rosaceae	*Osteomeles anthyllidifolia*	Indigenous		Orthodox	X	X
Rubiaceae	*Gardenia brighamii*	Endemic	endangered	Freeze sensitive	X	X
Rubiaceae	*Kadua acuminata*	Endemic		Freeze sensitive	X	
Rubiaceae	*Kadua affinis*	Endemic		Freeze sensitive	X	X
Rubiaceae	*Psydrax odorata*	Indigenous		Freeze sensitive	X	X
Santalaceae	*Santalum ellipticum*	Endemic		Freeze sensitive	X	X
Solanaceae	*Lycium sandwicense*	Indigenous		Orthodox	X	X
Solanaceae	*Solanum americanum*	Naturalized		Orthodox		X
Solanaceae	*Solanum incompletum*	Endemic	endangered	Orthodox	X	X
Solanaceae	*Solanum sandwicense*	Endemic	endangered	Orthodox	X	X
Thymelaeaceae	*Wikstroemia uva-ursi*	Endemic		Freeze sensitive	X	X
Urticaceae	*Neraudia angulata*	Endemic	endangered	Freeze sensitive	X	
Urticaceae	*Touchardia latifolia*	Endemic		Freeze sensitive		X
Urticaceae	*Urera kaalae*	Endemic	endangered	Freeze sensitive	X	X

Appendix 3

Information sheet from the BlueMaestro Disc-Mini hygrometersused in this research. Obtained from the Bluemaestro website at **bluemaestro.com**

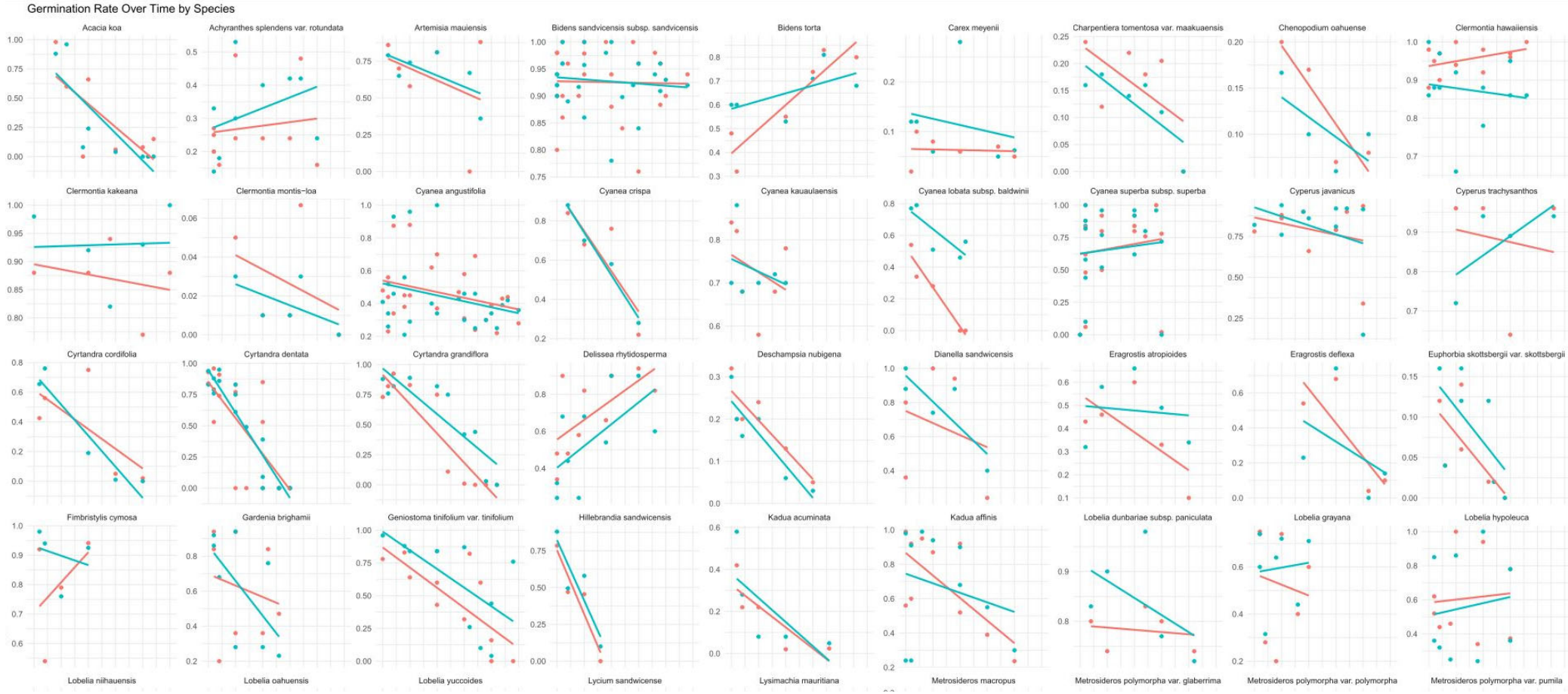
Disc Mini 3 in 1 – Temperature, Humidity and Dew Point Sensor and Logger

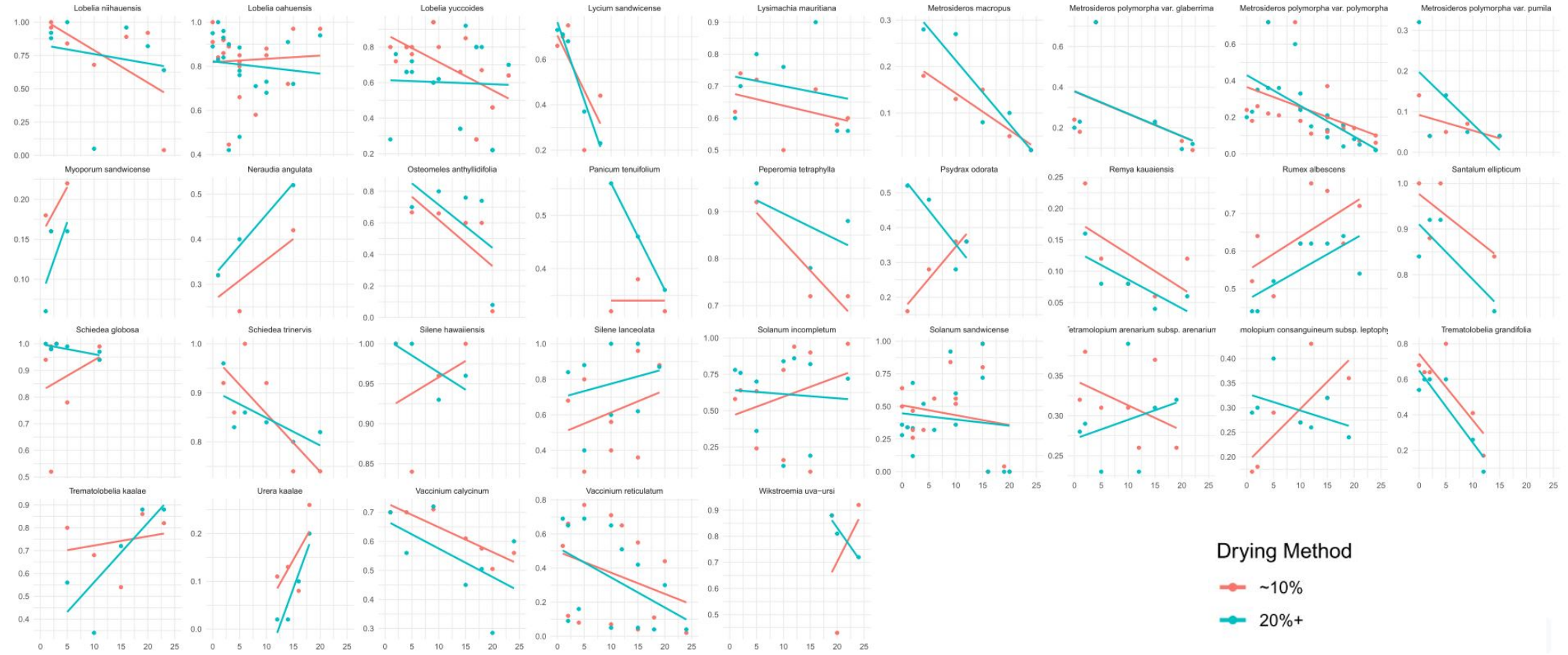
Product description

- Bluetooth 4.3
- Small size (33mm/1.30" x 9.5mm/0.37")
- Apps for iOS and Android
- Monitors temperature, humidity and dew point
- Sensor - Silicon Labs SI7020
- Accuracy +/- 0.4C, +/-4RH | Temp Range -40 to +125C
- Logging interval - 10s to 12hrs - user defined. Default 1hr
- ***9 - 12 months battery life***
- Battery size CR2032 (replaceable)
- Logging storage 6,000 timestamped readings (circular buffer)
- Range up to 250 feet (75 meters)
- FCC/CE and ROHS
- Blue Maestro Quality Guarantee
- Customization options available - contact us
- Bulk discounts available - contact us
- Learn the difference between the Disc Maxi and the Disc Mini

Appendix 4

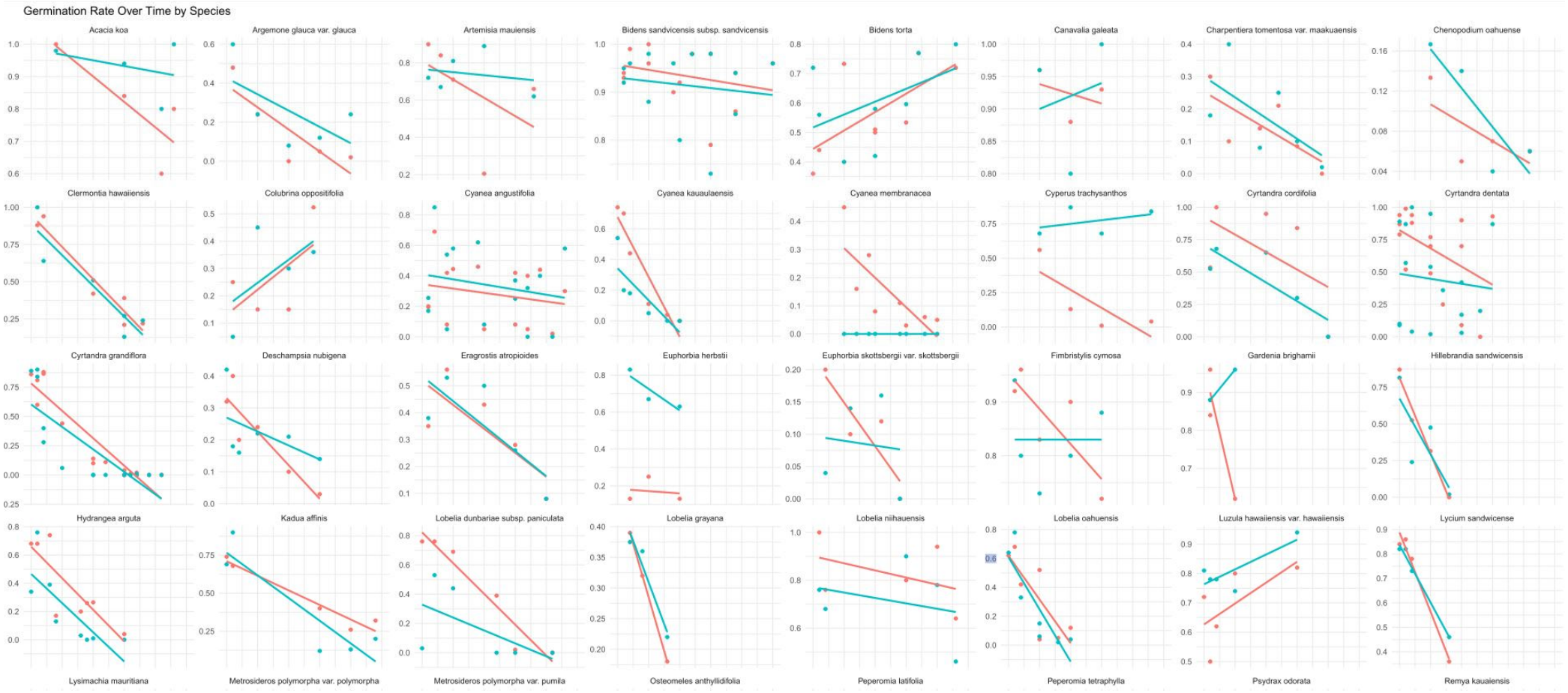
Scatter plots for each species included in the intermediate analysis. Percentage Germination Rates over viability tests intervals in Years. Species in C10 are represented by the red regression and species in C20+ are represented by the blue regression.

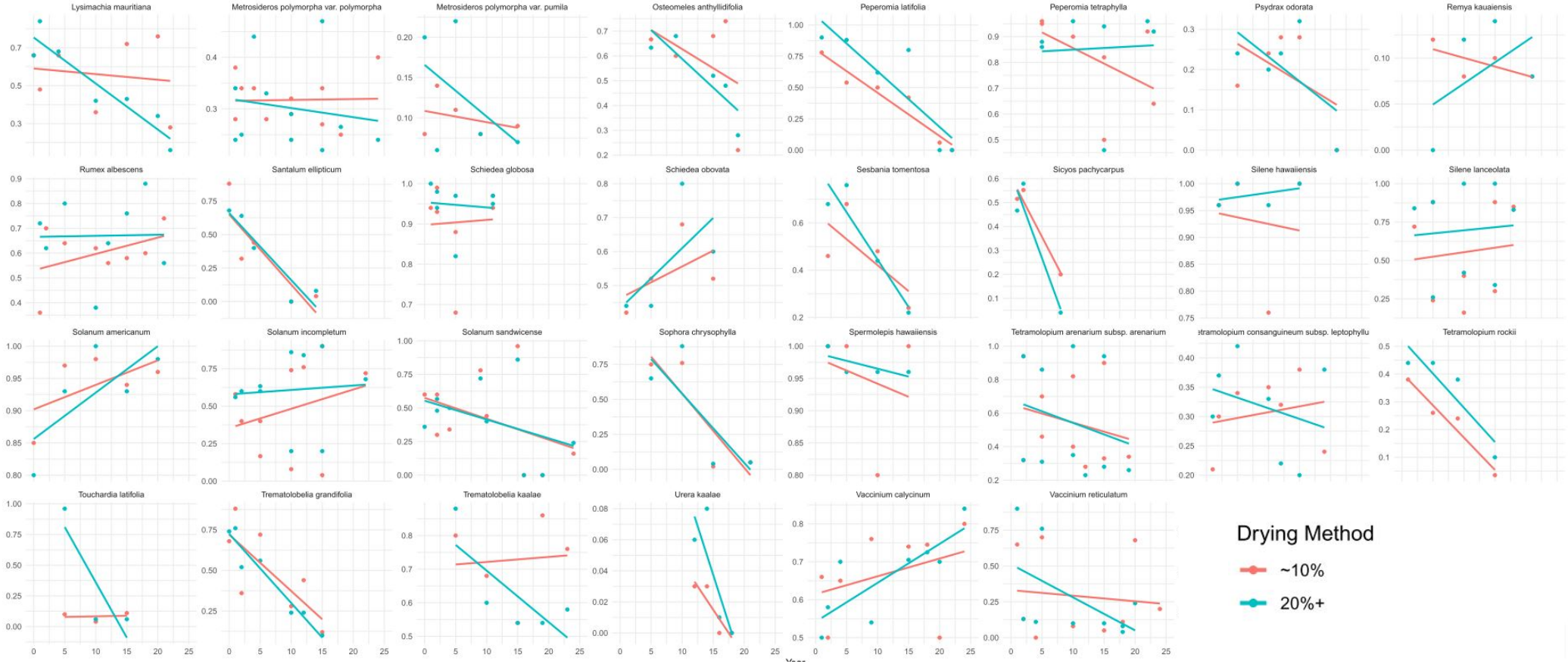

Germination Rate Over Time by Species
Acacia koa
Achyranthes splendens var. rotundata
Artemisia mauiensis
Bidens sandvicensis subsp. sandvicensis
Bidens torta
Carex meyenii
Charpentiera tomentosa var. maakuaensis
Chenopodium oahuense
Clermontia hawaiiensis
Clermontia kakeana
Clermontia montis-loa
Cyanea angustifolia
Cyanea crispa
Cyanea kauaulaensis
Cyanea lobata subsp. baldwinii
Cyanea superba subsp. superba
Cyperus javanicus
Cyperus trachysanthos
Cyrtandra cordifolia
Cyrtandra dentata
Cyrtandra grandiflora
Delissea rhytidosperma
Deschampsia nubigena
Dianella sandwicensis
Eragrostis atropioides
Eragrostis deflexa
Euphorbia skottsbergii var. skottsbergii
Fimbristylis cymosa
Gardenia brighamii
Geniostoma tinifolium var. tinifolium
Hillebrandia sandwicensis
Kadua acuminata
Kadua affinis
Lobelia dunbariae subsp. paniculata
Lobelia grayana
Lobelia hypoleuca
Lobelia niihauensis
Lobelia oahuensis
Lobelia yuccoides
Lycium sandwicense
Lysimachia mauritiana
Metrosideros macropus
Metrosideros polymorpha var. glaberrima
Metrosideros polymorpha var. polymorpha
Metrosideros polymorpha var. pumila


Lobelia niihauensis
Lobelia oahuensis
Lobelia yuccoides
Lycium sandwicense
Lysimachia mauritiana
Metrosideros macropus
Metrosideros polymorpha var. glaberrima
Metrosideros polymorpha var. polymorpha
Metrosideros polymorpha var. pumila
Myoporum sandwicense
Neraudia angulata
Osteomeles anthyllidifolia
Panicum tenuifolium
Peperomia tetraphylla
Psydrax odorata
Remya kauaiensis
Rumex albescens
Santalum ellipticum
Schiedea globosa
Schiedea trinervis
Silene hawaiiensis
Silene lanceolata
Solanum incompletum
Solanum sandwicense
Trematolobelia grandifolia
Trematolobelia kaalae
Urera kaalae
Vaccinium calycinum
Vaccinium reticulatum
Wikstroemia uva−ursi
Drying Method
~10%
20%+

Appendix 5

Scatter plots for each species included in the orthodox analysis. Percentage Germination Rates over viability tests intervals in Years. Species in D8 are represented by the red regression and species in D20 are represented by the blue regression.


Germination Rate Over Time by Species
Acacia koa
Argemone glauca var. glauca
Artemisia mauiensis
Bidens sandvicensis subsp. sandvicensis
Bidens torta
Canavalia galeata
Charpentiera tomentosa var. maakuaensis
Chenopodium oahuense
Clermontia hawaiiensis
Colubrina oppositifolia
Cyanea angustifolia
Cyanea kauaulaensis
Cyanea membranacea
Cyperus trachysanthos
Cyrtandra cordifolia
Cyrtandra dentata
Cyrtandra grandiflora
Deschampsia nubigena
Eragrostis atropioides
Euphorbia herbstii
Euphorbia skottsbergii var. skottsbergii
Fimbristylis cymosa
Gardenia brighamii
Hillebrandia sandwicensis
Hydrangea arguta
Kadua affinis
Lobelia dunbariae subsp. paniculata
Lobelia grayana
Lobelia niihauensis
Lobelia oahuensis
Luzula hawaiiensis var. hawaiiensis
Lycium sandwicense
Lysimachia mauritiana
Metrosideros polymorpha var. polymorpha
Metrosideros polymorpha var. pumila
Osteomeles anthyllidifolia
Peperomia latifolia
Peperomia tetraphylla
Psydrax odorata
Remya kauaiensis


Lysimachia mauritiana
Metrosideros polymorpha var. polymorpha
Metrosideros polymorpha var. pumila
Osteomeles anthyllidifolia
Peperomia latifolia
Peperomia tetraphylla
Psydrax odorata
Remya kauaiensis
Rumex albescens
Santalum ellipticum
Schiedea globosa
Schiedea obovata
Sesbania tomentosa
Sicyos pachycarpus
Silene hawaiiensis
Silene lanceolata
Solanum americanum
Solanum incompletum
Solanum sandwicense
Sophora chrysophylla
Spermolepis hawaiiensis
Tetramolopium arenarium subsp. arenarium
Tetramolopium consanguineum subsp. leptophyllu
Tetramolopium rockii
Touchardia latifolia
Trematolobelia grandifolia
Trematolobelia kaalae
Urera kaalae
Vaccinium calycinum
Vaccinium reticulatum
Drying Method
~10%
20%+

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