SEED STORAGE PRACTICES FOR NATIVE HAWAIIAN PLANTS

An On-Line Manual

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PART I.

I. INTRODUCTION

INTENDED AUDIENCE

This manual is intended for plant propagators, conservationists, land managers, and others in Hawai'i who need to store seeds of native Hawaiian plants, but do not have access to specialized facilities or staff. The techniques it describes require only simple facilities. Most equipment is available from normal vendors of horticultural supplies or household ware. These techniques are not necessarily the best available techniques when unlimited resources are available. They do, however, enable the end users of the seeds to store seeds for their own programs locally without the assistance of a major centralized facility. Although the manual is not intended as a handbook for gene banking of Hawaiian plant seeds, those interested in gene banking will find much of interest in it. They may wish to pursue some of the references mentioned in the bibliography.

Since the manual is written for users in Hawai'i, it uses English measurement units (metric equivalents are given), and native Hawaiian plants as examples. Users from other regions should have little difficulty making adaptations for their areas. The type of facilities described in this manual are appropriate to many smaller regional or local plant conservation programs that need to store seeds but do not have access to a centralized seed storage facility.

ROLES OF SEED STORAGE IN PLANT CONSERVATION

Seed storage can be a useful tool in many areas of plant conservation. Proper storage techniques make it possible to sow and plant when convenient instead of having to schedule around seed availability. For this purpose, short-term storage techniques that keep seeds viable for a year or two are adequate.

Storage techniques can make it possible to carry over bumper crops of seeds from year to year, or to save propagation material from plants that do not set seed every year. This requires medium-term techniques to keep seeds viable for several years.

Stored seeds can be an important backup for plants that are threatened with extinction in the wild. For this purpose, long-term storage over many years is the goal. Long-term storage is also appropriate for germplasm conservation in seed storage facilities. A valuable feature of seeds for this purpose is that each seed is a genetic individual, but requires far less space and upkeep than an individual in the greenhouse or in tissue culture. Unlike money, seeds in a storage facility are a diminishing asset, so storage conditions that best minimize deterioration are critical, and there must be plans for periodically refreshing the collection.

This manual addresses mainly short- and medium-term seed storage, but much of the material applies equally well to long-term storage.

Until recently, seed storage has not been an important technique for conservation of Hawaiian plants. The main reason was the belief that seeds of native Hawaiian plants were difficult to store. In Honolulu, the average temperature and relative humidity are 75°F/24°C and 70% respectively, with only small year-round variation. The warm temperature and high humidity create poor conditions for seed storage, and most seeds stored under such ambient conditions deteriorate rapidly. There was a widespread belief that seeds of Hawaiian plants, like seeds of many other plants from humid tropical areas, would not tolerate the drying and chilling techniques commonly used to store seeds of temperate plants.

However, this is not true. Recent unpublished studies at the United States Department of Agriculture National Center for Genetic Resources Preservation (USDA NCGRS, formerly National Seed Storage Laboratory, USDA NSSL) at Ft. Collins, Colorado, the University of Hawai'i Center for Conservation Research and Training (UH CCRT) in Honolulu, Hawai'i, and the National Tropical Botanical Garden (NTBG) on Kaua’i showed that seeds of most native Hawaiian plants tolerate both desiccation and freezing, and that their storage life can be greatly extended by properly controlling storage temperature and seed moisture level.

These findings not only demonstrated that seeds of many Hawaiian plants could be stored for years, they showed that they could be stored using already established techniques. There is an abundant, readily accessible literature on these techniques, and they are neither difficult nor expensive to implement. See the sections of this manual entitled References Cited and Useful Sources of Information. With the help of these and this manual, users of seeds should be able to develop their own seed collection and management programs.

LONGEVITY OF STORED HAWAIIAN PLANT SEEDS

Studies on longevity of Hawaiian seeds under storage are currently under way at NCGRP and CCRT. Only preliminary results are available, so this manual does not include projected longevities. This information will become available later. While absolute storage longevities are not currently available for most Hawaiian seeds, it is possible to give estimates of how much different storage methods can extend their longevity.

Stored at prevailing temperature and humidity conditions in Hawai'i, seeds of many native plant species deteriorate noticeably within a few weeks. While the same basic methods can be used to store the great majority of seeds, the actual longevity varies greatly with species. Some, such as Cyrtandra spp. are short-lived under even good storage conditions, while others, such as Acacia koa and some Malvaceae, can probably be stored frozen for decades or longer.

Results to date suggest that even short-lived seeds such as Broussaisia arguta and Metrosideros polymorpha can be stored for at least several years in refrigerated or frozen storage. Many species have expected storage longevities of a decade or more if frozen. The actual storage life for a given seed lot of any species will depend on many factors, such as ripeness of the fruits at collection, weather during the seed formation period, handling after collection, etc. It is necessary to test the seeds to determine their quality when first collected and monitor their condition in storage.

Effects of temperature and seed moisture level (or, equivalently, the relative humidity at which the seeds are in equilibrium with their surroundings) on seed longevity are well-understood for those agricultural seeds that can tolerate drying and freezing. For these, a seed viability equation has been developed to predict storage life at different combinations of storage temperature and seed moisture level. For a fuller explanation, see **Seed Storage Behaviour: A Compendium** (Hong et al. 1996).

Many native Hawaiian plant seeds screened so far show similar patterns. Using this information, we can use the seed viability equation make a table to estimate the potential longevity of stored seeds at different combinations of temperatures and moisture levels. See Table I: Relative longevity of seeds under different storage regimes. In theory, a seed that remains viable for a month when air dried and stored at room temperature should remain viable for 6.3 months if dried to CCRT moisture levels and stored at room temperature, 305 months if dried to NCGRP levels and frozen, etc.

TABLE I. Relative longevity of seeds under different storage regimes.

	Room Temperature 75°F 23.9°C	Refrigerated 39°F 3.9°C	Frozen 0°F -17.8°C
Air dried in Hawai'i (70% rel. hum.)	1	8.4	freezing damage
UH CCRT[1] drying levels	6.3	53	195
USDA NCGRP[2] drying levels	9.8	82	305
CPC[3] drying recommendation	12.5	105	389
IPGRI[4] drying recommendation	29	242	896

The drying protocols for the moisture levels in the leftmost column are:

[1]UH CCRT
University of Hawai'i Center for Conservation Research and Training: 30% relative humidity at 75°F (24°C). Described later in this manual in Section III under Preparation for Storage: Adjusting Seed Moisture Level.

[2]USDA NCGRP
United States Department of Agriculture National Center for Genetic Resources Preservation: 23% relative humidity at 41°F (5°C). From the USDA NCGRP website at:
http://www.ars-grin.gov/ars/NoPlains/FtCollins/nsslmain.html.

[3]CPC
Center for Plant Conservation: 19% relative humidity at 59°F (15 °C). From **Guidelines for the Management of Orthodox Seeds** (Wieland 1995).

[4]IPGRI
International Plant Genetics Resources Institute: 13% relative humidity at 68°F (20°C). From **Seed Storage Behaviour: A Compendium** (Hong et al. 1996).

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There is currently some question about whether the effects of temperature and seed moisture level do multiply at lower temperature and moisture levels, and whether lowering moisture levels below 8% (equivalent to 23% relative humidity at 5°C) continues to yield improvement in storage life for all seeds that tolerate drying. More research is needed to answer these questions. The answer is likely to be different for different species of seeds.

Not all seeds can be stored under these conditions. The next section considers classification of seeds by their storage properties.

II. STORAGE PROPERTIES OF SEEDS

CLASSIFICATION OF SEEDS BY STORAGE PROPERTIES

For storage purposes, seed scientists divide seeds into three standard categories based on their tolerance to drying and low temperature. These categories are called orthodox, recalcitrant, and intermediate.

Orthodox seeds can be dried to very low moisture levels. Once desiccated, they withstand freezing. They are straightforward to store and, under proper conditions, often can be stored for a long time. There is abundant literature on storage of orthodox seeds. **Seed Storage Behaviour: A Compendium** (Hong et al. 1996) contains an extensive bibliography. Within a wide range of moisture levels and temperatures, the seed viability equation accurately predicts the storage life of orthodox seeds. Most temperate crop seeds are orthodox. Fortunately, most native Hawaiian plant seeds screened so far appear to be orthodox.

Recalcitrant seeds will die if dried below a critical moisture level, and cannot tolerate low temperatures. They are usually short-lived and therefore not easy to store. Mangoes are a familiar example. While techniques exist for storing some recalcitrant seeds, each species requires its own method. Good general methods to predict storage life do not exist; longevity is usually short in any case. Among native Hawaiian plant seeds, few have been shown conclusively to be recalcitrant, although the list may grow with more screening.

Intermediate seeds can tolerate some combinations of desiccation and low temperatures, but tolerate freezing poorly. There is in fact a gradient from orthodox to recalcitrant, with no sharp boundaries between the categories.

The traditional three-way classification of seeds by storage properties into three categories, orthodox, intermediate, and recalcitrant, was based largely on research on seeds from temperate domestic plants. As more research is conducted on seeds of wild plants and tropical plants, scientists are finding many seeds that, while clearly not recalcitrant, do not fit well into the other two classes. Some have called these “not recalcitrant seeds.” More recently, Pritchard (2004) proposed revising the storage classification of seeds into three groups that he tentatively calls type I, type II, and type III. Type III seeds correspond to recalcitrant seeds in the traditional classification. Seeds in type I are classified as orthodox. However, in Pritchard’s classification, many formerly orthodox seeds are in type II, along with the former intermediate seeds. These former orthodox seeds do tolerate freezing, but, unlike true orthodox seeds, they store better at temperatures above freezing than below. Many nominally orthodox Hawaiian seeds may fall into type II in Pritchard’s classification.

Type II seeds can be stored by drying them to low moisture levels, then storing them refrigerated. Storage of type II seeds is currently an important area of research. There is evidence that the harmful effects of storage at low temperatures may be reversed at very low temperatures around -92º F (-70º C). At present, little literature outside of professional journals uses the revised classification. Most literature continues to use the three traditional categories.

Table 2: Storage Characteristics of Native Hawaiian Plant Seeds gives seed storage classifications for Hawaiian plants for which data exist. Users of this manual from outside Hawai'i should check the IPGRI's **Seed Storage Behaviour: A Compendium** for information on the species which they wish to store. For instructions how to access the **Compendium** on the World-Wide Web, see Resources: World-Wide Web Resources in section V of this manual.

For many species there are currently no data. Without testing, there is no way to determine the storage category of a species beforehand. Nonetheless, with guidelines it is possible to make educated guesses. These guidelines are summarized in **A Protocol to Determine Seed Storage Behaviour** (Hong and Ellis 1996):

1. Large seeds which are dropped moist from perennial plants in habitats that rarely dry are likely to be recalcitrant.

2. Small seeds that dry naturally at maturity are often orthodox. The largest seed known conclusively to be orthodox is the cashew (Hong et al. 1996).

3. Seeds of Asteraceae/Compositae, Chenopodiaceae, Lamiaceae/Labiatae, and Solanaceae are orthodox; Brassicaceae/Cruciferae, Cucurbitaceae, Fabaceae/Leguminosae, Poaceae/Graminae, and Rosaceae are usually orthodox, with some exceptions; Sapotaceae are recalcitrant or intermediate. Note that plants within the same genus can show different storage behavior.

4. Seeds from achenes, many-seeded berries, many-seeded dehiscent capsules, many dry-seeded pods (but not arillate), many dry-seeded follicles, schizocarps, and urticles are orthodox. Seeds from most siliques and caryopses are orthodox. Note that pods containing 1-5 large seeds or many arillate seeds, berries containing 1-10 seeds, and capsules containing 1-5 seeds may show either storage behavior.

5. Plants growing in environments that are seasonally unsuited for germination usually produce orthodox seeds; some growing in habitats that are moist year-round produce recalcitrant seeds, but others produce orthodox seeds.

6. Dry seeds with hard coats, such as many Malvaceae, are usually orthodox.

Experience suggests that most small dry seeds of native Hawaiian plants are orthodox, or probably orthodox. Such seeds store well when dried at low humidity and refrigerated or frozen. When in doubt, these are good storage methods; at worst, they are almost always no worse than storage at room temperature and humidity

EFFECTS OF MOISTURE AND TEMPERATURE

The key to successful storage of orthodox seeds is control of seed moisture and storage temperature. Lowering either extends seed storage life. For an example, see Table 1: Relative longevity of seeds under different storage regimes. According to the seed viability equation, the effects of changing moisture and temperature multiply: If lowering seed moisture by X% extends storage life by a factor of 5, and lowering storage temperature by Y° extends storage life by a factor of 3, then lowering both at the same time will extend storage life by a factor of 5x3, or 15. For an explanation of the equation, see **Seed Storage Behaviour: A Compendium** (Hong et al. 1996).

For orthodox seeds, longevity continues to increase with lowered temperatures: Projected storage lives of some seeds stored over liquid nitrogen at -238°F (-150°C) can be hundreds of years or more. On the other hand, evidence is accumulating that lowering seed moisture below a critical level may cause desiccation damage. Currently, there is no consensus as to what this level is. Moisture and temperature interact; there is increasing evidence that the best storage moisture level is lower at high temperatures than at low temperatures. Both the best moisture level and the critical minimum moisture level probably differ somewhat from species to species. The difference between species is small enough that seeds of most species can be stored together under standardized conditions. For a review, see the 1998 special supplement to **Seed Science Research** entitled **Ultra-Dry Storage**.

As a criterion for seed storage, seed moisture content has some practical problems. It is difficult to measure in practice without a precision balance and a drying oven, and the measurement process destroys the measured seeds. Furthermore, it needs to be adjusted for seed oil content. Recent practice inclines toward controlling seed moisture level by drying the seeds to equilibrium with a known relative humidity inside the drying container instead of measuring moisture content directly (Wieland 1995).

III. PRACTICAL ASPECTS OF SEED STORAGE

Successfully storing seeds requires attention to the entire seed storage process starting from seed collection and continuing to regeneration of the stored seeds.

COLLECTION

Successful seed storage starts with collection. If seeds are not mature and healthy when stored, their germination rate will be poor, their seedlings will have poor vigor, and their storage life will be short. Nothing can be done after collection to improve seeds that were of poor quality to begin with. Collectors should strive to collect mature seeds from healthy plants. This requires some judgment, since there are no hard and fast field criteria for recognizing when seeds reach their peak storage potential. For large seeds, collectors can cut open a few seeds in the field to confirm the existence of well-developed embryos.

Some seeds, if collected immature, can be ripened by storing the fruits in a cool, well-ventilated place. The guiding principle is to maintain the seeds in an environment similar to what they would be in if the fruit were still on the parent plant. This is similar to buying unripe produce and letting it ripen at home.

FIELD STORAGE AND TRANSPORT

Improper handling between collection and processing can greatly shorten seed storage life. Proper handling techniques after collection differ somewhat for orthodox and recalcitrant seeds, but some considerations are common to both. In general, procedures for field storage and transport of seeds are similar to those for fresh produce.

Seeds should not be exposed to high temperatures. (In fact, to predict seed longevity, scientists use a technique called "accelerated aging" which relies on high temperatures and humidities to artificially speed aging of stored seeds.) Avoid leaving storage containers in the sun or inside closed vehicles. Orthodox seeds can be refrigerated while awaiting processing. So can some recalcitrant seeds.

During transport, seeds need to be well-ventilated. This is especially true for recalcitrant seeds, which metabolize rapidly. When still moist, orthodox seeds will also metabolize. If the seeds are kept in plastic bags, the bags should be open to allow air circulation. Seeds should not be packed so tightly as to create anaerobic conditions, since they deteriorate rapidly when metabolizing under such conditions. While recalcitrant seeds in particular should not be allowed to dry out, seeds and fruits in transport should not be damp, or they may suffer from fungus.

SEPARATION AND CLEANING

Cleaning will make seeds easier to store. Although hand cleaning is tedious, it will lead to potentially better seed quality and longer storage than mechanical methods since it is less likely to damage the seeds. Fruits and seeds vary greatly, so personnel will have to exercise ingenuity and judgment to extract and clean seeds. The following equipment is useful:

- Sink with running water.
- Spray bottles with adjustable nozzles.
- Counter or workbench.
- Sieves: A set of stacking graded sieves such as are used for soil testing are especially useful. These are available from Ben Meadows, Fishers, Forestry Suppliers, or Seedburo. See the section on Sources of Supplies. For Hawaiian seeds, we find the most useful sizes to be U.S. Standard #'s 5, 10, 18, 35, and 60, corresponding to hole sizes of 0.1574"/4 mm, 0.787"/2 mm, 0.394"/1 mm, 0.197"/0.5 mm, and 0.0098"/0.25 mm.

- For dry sieving of small quantities, a Keck Sand Shaker is useful. In areas with low humidity, small seeds may stick to the inside walls. If this is a problem, try wiping the walls with anti-static fabric softener sheets for clothes driers ("Bounce" is a popular brand).

- Kitchen sieves with different grate sizes will be useful for rough processing, to avoid wear on the more expensive graded sieves.

- Glass measuring cups, from large to small sizes: Pyrex 1, 2, and 4 cup measuring cups work well. Small, oily seeds may stick to the sides of plastic cups.

- Small trays, mixing bowls, strainers, colanders and other containers: ordinary kitchenware works well.

- Cutting tools: a sharp knife, a serrated edge knife, razor blades or a razor knife with disposable blades, fine-tipped pruning shears, large nail clippers ("Resco" dognail clippers are said to be especially effective).

- Cutting board.
- Hammer.

- Vise-grip pliers: useful for cracking seeds gently.
- Files, sandpaper, wire gratings, and other abrading tools.
- Filter funnels: Melitta #6 coffee funnels and filter paper are readily available in supermarkets.

- Magnifier lamp, headband magnifier, and 7-14x hand lens.
- Forceps, tweezers, hemostat, needle-nosed pliers
- Teasing needles or dental probes.
- Hand hair blow-drier, preferably multi-speed: Disable the heater unit.
- Small fan.

Seeds should be mature before extraction. If they are not, it may be possible to ripen them by leaving them in the fruits under cool, well-ventilated conditions until they mature. The conditions should simulate what the seeds would experience if left on the parent plant.

Begin extraction by removing coarse debris. If the seeds are damp, they may be easier to clean after drying. If necessary, dry them in moving air. Avoid using heat, which will speed drying but also shorten seed storage life. If the seeds are inside a fruit, the next step for most seeds will be removal from the fruit. After removal, the seeds can be sieved to remove more debris. A hair blow-drier or fan can be used to blow away light debris from heavier seeds. For most species, exceptionally small or light seeds are likely to be empty and inviable.

Small seeds in pulpy fruit can be extracted by mashing the pulp, mixing it with water, then pouring off the pulp while allowing the seeds to settle. Pyrex measuring cups work well for this technique. With practice, one can pour the slurry carefully, removing most of the pulp while losing few of the seeds. Seeds lost in pouring can easily be recovered by adding more water to the pulp and pouring again. Very small, oily seeds may float to the surface of the water. It may be possible to make them sink by spraying them with water from a spray bottle. A blender can be used to mash large quantities of pulp, but it is easy to overblend and damage the seeds. It should not be used for species for which there is no previous experience. For longest storage, hand processing is preferable.

If seeds are washed in water during cleaning, some may float. For many species, this is a good way of removing nonviable seeds; for others, floating is normal. After washing, dry seeds in moving air, avoiding use of heat.

TESTING BEFORE STORAGE

Freshly collected seeds should be tested before storage to confirm that they are viable and to give a base for future comparisons when testing for deterioration in storage. For small storage facilities, the most practical way to test seeds is to germinate them. For hints, see Danida Forest

Seed Centre Lecture Series Note C-8 and Technical Note 18 available from sources in Section VI under Useful Sources of Information. . Wild seeds are often difficult or slow to germinate. Testers should keep this in mind when choosing test methods. For Hawaiian species, see the instructions for Parts II and III of this manual.

If space is limited, petri dishes lined with agar, filter paper, damp paper towels or blotter paper can be stacked. David Theodoropoulos of J. D. Hudson Seeds recommends 12 x 18 x 1" (30 x 45 x 2.5 cm) stainless steel cookie sheets:

"We have window glass cut to size to cover them, then epoxy an inch-wide square of rubber innertube at each corner to hold the glass up a bit and give some air circulation. Little peel-off rubber feet for the bottoms of the trays from the hardware store, and they are set. They can be stacked probably ten high (with double-thickness glass for strength), and with small seeds we have run up to 48 tests per tray on 2" (5 cm) squares of padding."

Note that some kinds of paper may contain residues that inhibit germination of sensitive seeds. Undyed unbleached paper is usually preferable. Theodoropoulos recommends Webril Handi-Pads, used by offset printers to clean plates. It is available from printer's supply shops and paper suppliers. He also recommends Kim-Pak, available from the same sources. The substrate should be damp, but not so wet that there is a film of liquid water. Whenever the seeds are checked for germination, germinated seeds should be removed.

PREPARATION FOR STORAGE

Adjusting Seed Moisture Level

Before being placed into storage, seeds should be dried to an appropriate moisture level. The proper relative humidity in the drying container depends on both the temperature in the drying container and the final storage temperature of the seeds. The following table, by Walters (2004) of the U. S. Department of Agriculture National Center for Genetic Resources Preservation, gives guidelines:

	Recommended Relative Humidity inside Drying Container		
Storage at:	59º F/15º C	41º F/5º C	0º F/-18º C
Drying Temperature			
77º F/25º C	28%	33%	46%
59º F/15º C	20%	26%	38%
41º F/5º C	14%	20%	32%

41º F/5 Cº is the temperature in the warm part of an ordinary refrigerator. 0º F/-18 Cº is the temperature in the freezer compartment of a refrigerator, or in a home freezer.

These guidelines are based on the principle that the relative humidity in the drying container should yield a seed moisture level equivalent to drying at 20-25% relative humidity at the final storage temperature. Research is still ongoing: Some other researchers recommend lower moisture levels, equivalent to as low as 10-14% relative humidity at storage temperature.

Seeds can be prepared for storage by drying them in sealed containers over saturated solutions of appropriate mineral salts. The salts will maintain a constant relative humidity of the surrounding air, which will dry seeds to a seed moisture content in equilibrium with the relative humidity. CCRT uses calcium chloride, which maintains a relative humidity of 30% at 77°F (25°C). Calcium chloride is inexpensive and readily available over the counter at hardware stores for closet dehumidifier units such as Damp-Rid and Damp-Check. It is non-toxic (it is an ingredient of some canned tomatoes) and easily disposed by washing down the drain.

To prepare the salt mixture, mix the salt with water to form a wet slurry. Place the slurry in an open container, and place the open container into a larger airtight container that will be used to dry the seeds. (The salt mixture should not come in contact with the seeds. It is used to control the humidity inside the larger container.) Spread the seeds in a thin layer in a small container and place it in the larger container, then seal the lid on the larger container. Allow enough time for seed moisture to equilibrate with the air inside the container. This may take several weeks. Circulating the air inside the container will speed the drying process. Seeds will dry more slowly at lower temperatures, but avoid using high temperatures to speed drying, since high temperatures will reduce seed storage life.

If mineral salts are not available, seeds can be dried by spreading them in a thin layer in an open container in a self-defrosting refrigerator. The action of the self-defrost unit will maintain a low relative humidity inside the refrigerator. It is difficult to control the exact relative humidity, but this method is satisfactory if better means are not available. (Relative humidities measured in several Honolulu refrigerators ranged from 10-40%, corresponding to seed moisture content of 6-11%.) After the seeds have dried to equilibrium with the humidity inside the refrigerator, seal the drying container tightly, take it out from the refrigerator, and let it come to room temperature before opening it to prevent moisture from condensing on the seeds. Seal the seeds in airtight containers and transfer them to storage.

Optimal moisture levels for room temperature dry storage are lower than calcium chloride can provide. Researchers are still trying to determine the optimal level. Current research suggests it corresponds to a relative humidity of 10-20% in the seeds' drying environment. In a laboratory, lithium chloride can be used to reduce relative humidity to 12%. For practicing growers, desiccated silica gel may be more practical. Monitor the relative humidity to insure that the seeds are properly dried. As a starting point, try drying seeds in a sealed container with an equal volume of desiccated silica gel for a week or so. Refresh the silica gel as necessary to achieve a final relative humidity of around 15%. Used silica gel can be recharged by baking in an oven. Instructions are usually packaged with the silica gel.

Silica gel is sold both plain and with an indicator added to show when it needs recharging. Indicator silica gel changes color as it absorbs water. It is relatively expensive, so most users mix it with plain silica gel. For sources, see Section VI: Resources: Sources of Supplies and Equipment.

Measuring Relative Humidity

Relative humidity within the drying containers can be measured easily using inexpensive colored humidity indicator strips. These are available from camera stores or from sources listed in the section of Sources of Supplies. While indicator strips are not precise, there are few other ways of monitoring relative humidity within drying containers which are more accurate, practical to implement, and economical at the same time.

Storage Containers

Plastic film cans and jars without gaskets may not be airtight. Airtight snap-top plastic vials or heat-sealed foil-lined plastic pouches such as are commonly used for stored foods are two convenient types of airtight storage container for small seed lots. Pouches can be sealed with an ordinary clothes iron. They can be opened and resealed several times. Rubber-gasketed glass jars work well for larger lots. For sources, see the section on Sources of Supplies.

STORAGE METHODS

Methods described in this section are appropriate for storing orthodox and some intermediate seeds. Recalcitrant seeds require special techniques. Contact Alvin Yoshinaga at alviny@hawaii.edu or at Lyon Arboretum for possible storage information. Storage of recalcitrant seeds is difficult, so the best policy is to sow them immediately instead of trying to store them. Dark storage of moist seeds may be useful for some species for short periods; for others, it will only stimulate the seeds to germinate.

This manual is mainly intended to serve users of working collections of seeds, such as growers and land managers with limited facilities and small budgets. It does not describe storage methods which require specialized facilities, such as cryopreservation over liquid nitrogen, embryo tissue culture, or other methods that might be used in major germplasm conservation facilities.

The three principal methods considered here are freezing, refrigeration, and room temperature low humidity storage. (We do not recommend storage at Hawaiian ambient room temperature and humidity for most seeds.) Of the three, freezing is expected to give the longest storage life for orthodox seeds. However, some facilities may not have access to freezers, and not all seeds store well when frozen. If freezing is impractical, refrigeration is a second choice. If no refrigeration facilities are available, or for seeds that cannot tolerate low temperatures, room temperature low humidity storage is a third choice.

FREEZING

Seeds are stored in airtight containers at -5 to 0°F (-20 to -18°C) after adjusting their moisture level. For small collections, an ordinary household freezer can provide the proper conditions. As the collection grows, a walk-in freezer may become more economical.

Seed Preparation

After cleaning, seeds need to be dried to proper moisture content before freezing to prevent ice formation. If seeds are dried at a relative humidity of 70% or greater, there is potential for freezing damage. For facilities that do have not equipment to measure seed moisture content, the most practical way to achieve the correct moisture level is to dry the seeds to equilibrium with a controlled relative humidity. Ongoing research suggests that drying the seeds at a relative humidity of greater than 65% may lead to freezing damage (Hong et al 1996), while drying at less than 20% relative humidity may result in damage from over-drying (Black, ed. 1998). (NCGRP prepares most of its materials by equilibrating them to 23% relative humidity at 41°F (5°C) for several weeks.) See section III on Preparation for Storage for instructions on how to dry seeds. After the seeds are properly dried, seal them in airtight containers and freeze them.

When taking out frozen seeds, allow the storage containers to come to room temperature before opening them, to prevent water from condensing on the seeds. Even in humid environments, it is unnecessary to redry the seeds each time that the container is opened before resealing the container unless the volume of air inside is much larger (>100x) than the volume of seeds.

Notes on Freezer Operation

For equal capacity, chest freezers are less expensive and use less energy than upright freezers. If power outages are a problem, consider providing a back-up power supply. If the freezer does warm to room temperature, there is no permanent damage to the seeds, except that they will age at their room temperature rate instead of their frozen rate. Storage temperature fluctuation is not in itself harmful, but should be avoided. When temperature fluctuates, seeds tend to age as if they were stored near the higher temperatures. Filling the empty space in the freezer with frozen plastic water bottles will buffer the effects of power outages and also reduce the operating costs of the freezer.

REFRIGERATION

Seeds are stored in airtight containers at ordinary refrigerator temperature (4-5°C (39-41°F)) after processing to adjust their moisture level. At one time this was the preferred method for storing orthodox seeds in central seed banks, but has been supplanted by frozen storage, which is more effective at slowing seed aging. For seeds that are not recalcitrant but not clearly orthodox, it is still the preferred storage method. Many Hawaiian seeds appear to have storage lives of at least several years under refrigeration, so it is a useful method for working collections and short-term storage.

Facilities with extremely limited resources can store the refrigerated seeds in non-airtight or even open containers if the refrigerator is self-defrosting. In Hawai'i, a self-defrosting refrigerator in normal everyday use will maintain a relative humidity between 20-40%. If nothing moist is stored inside and the door is rarely opened, the relative humidity inside will be near the lower of these levels. This should be satisfactory for most medium-term storage of orthodox seeds.

Seed Preparation

Seed preparation is the same as for frozen storage.

ROOM TEMPERATURE LOW HUMIDITY (ULTRA-DRY STORAGE)

Seeds are stored in airtight containers at room temperature after processing to adjust their moisture levels. This method is not as effective as low temperature methods for seeds that can withstand low temperatures. It is worth considering when maximum longevity is not important and refrigeration facilities are not readily available. For most Hawaiian seeds, low temperature methods are preferable.

Seed Preparation

After cleaning, seeds need to be dried for storage. The best moisture level for seeds stored at room temperature is lower than for seeds stored at low temperatures. Researchers are still trying to determine the best moisture level and corresponding drying relative humidity to achieve it; it is believed to be around 10-15% relative humidity for most seeds. (The 1998 special supplement to **Seed Science Research** entitled **Ultra-Dry Storage** reviews this question in detail.) For instructions on how to dry seeds, see Preparation for Storage in section III.

SPECIAL PRECAUTIONS FOR GERMINATING STORED SEEDS

Seeds that have been dried to low moisture levels may be damaged by taking up water too fast if they are wetted immediately after removal from storage ("imbibition injury"). To prevent this, spread the seeds in a thin layer and leave them in a sealed container with wet paper towels for a day or so to let the seeds absorb water from the air before allowing them to contact liquid water. The towels should not contact the seeds.

REGENERATION OF STORED COLLECTIONS

Seeds in storage are like a bank account that charges a service fee but gives no interest. Eventually, the percentage of viable seeds will drop to a point at which the seeds will have to be replaced. As the percentage of viable seeds drops, the remaining seeds will be increasingly selected for storage longevity and genetically decreasingly representative of the original parent population. For cultivated species, regeneration is recommended when germination goes down to 85% of the original rate. For guidelines for wild plants, see the **CPC Guidelines for Management of Orthodox Seeds** (Wieland 1995).

Periodic testing is the only practical way to check the status of the stored seeds. In large seedbanks, seeds of cultivated plants in frozen storage are typically tested after 10 years, while refrigerated seeds are tested after 5. Persons storing species for which there are little longevity data may wish to test more frequently. The most common way to test the seeds is by germinating a sample of the stored seeds. Note the special precautions for germinating stored seeds in the previous section.

There is currently discussion about appropriate sources of regenerated seeds. Seeds from wild plants will be from parents which have been selected for survival under natural conditions. However, they may be difficult to obtain. Some experts believe that properly produced seeds from first-generation F1 greenhouse plants from wild parents are satisfactory, and may store better than wild seeds since the parent plants are likely to be healthier than wild plants.

IV. ACKNOWLEDGEMENTS

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VI. RESOURCES

SOURCES OF SUPPLIES AND EQUIPMENT

The following list is provided for the convenience of those needing seed storage supplies. It is neither comprehensive nor intended as an endorsement for particular suppliers.

General Seed Processing Supplies

Southern Exposure Seed Exchange carries a line of stock oriented toward amateur cultivators of heirloom varieties of plants. The inventory includes airtight storage vials, processing tools, silica gel and foil-lined heat-sealed storage packets. Contact information:

Southern Exposure Seed Exchange
P. O. Box 460
Mineral, VA 23117

http://www.southernexposure.com
tel. (540) 894-9480, fax -9481.

Industrial-scale seed processing equipment is available from:

Seedburo Equipment Company
1022 W. Jackson Blvd.
Chicago, IL 60607

http://www.seedburo.com
tel. 800-284-5779 (USA)
312-738-3700 (international)
fax 312-738-3700

Germination Testing

The following dealer stocks germination testing supplies for small-scale tests. Their world-wide web site has useful information on germination techniques:

J. L. Hudson Seeds
Star Route 2, Box 337
La Honda, CA 94020

http://www.jlhudsonseeds.net

General Field and Laboratory Supplies

The following two dealers supply forestry equipment, including sieves and some labware:

Ben Meadows Company
3589 Broad St.
Atlanta, GA 30341

http://www.benmeadows.com
tel. 800-241-6401
fax 800-628-2068

Forestry Suppliers, Inc.
P. O. Box 8397
Jackson, MS 39284-8397

http://www.forestry-suppliers.com
tel.800-647-5368 (USA)
(601) 354-3565 (international)
fax 800-543-4203

The following is a dealer in general laboratory supplies:

Fisher Scientific
2000 Park Land Dr.
Pittsburgh, PA 15275-9943

http://www.fishersci.com
tel. 800-766-7000 (USA)
973-467-6511 (international)
fax (USA) 800-926-1166

Heat-Sealed Foil-Lined Storage Pouches

The Center for Plant Conservation recommends Type 321 Foil Seed Pouches made by Barrier Foil Products Co. There is no retail distributor in the United States. Orders for small quantities from England are prohibitively expensive. U. S. botanical gardens occasionally pool orders. Contact information:

Barrier Foil Products Co.
Hollands Mill
61 Shaw Heath
Stockport
Cheshire
SK38BH
United Kingdom

e-mail: BARRIERFOIL@aol.com
tel. 0161-480-4007
fax 0161-474-7412

In the United States, Fres-co produces pouches intended for food products. These are only available in bulk, and, due to their construction, are less convenient for seed storage than the Barrier Foil pouches. Contact information:

Fres-Co System USA, Inc.
3005 State Rd.
Telford, PA 18969-1033

http://www.fresco.com
tel. 215-721-4600, fax -4414

Small, lighter weight pouches are available in small quantities from Southern Exposure Seed Exchange, listed above for general seed processing supplies. Southern Exposure also stocks snap-lid airtight plastic vials.

Similar small pouches are also available from Seed Savers Exchange, an organization dedicated to conservation of heirloom vegetable varieties.

Seed Savers Exchange
3076 North Winn Road
Decorah, IA 52101

http://www.seedsavers.org
tel. (563) 382-5990, fax -5872

Moisture Control Supplies

Calcium chloride is available from hardware stores and houseware suppliers as part of closet moisture control systems such as Damp-Check®, Damp-Rid®, or similar products. Silica gel is available from camera stores, craft supply stores, Southern Exposure Seed Exchange or Gaylord Brothers. Non-indicator silica gel is sometimes available for free from places that receive shipments of electronic equipment.

Humidity indicator strips are available from camera stores or, as item #62031, from:

Gaylord Bros.
P. O. Box 4901
Syracuse, NY 13221-4901

http://www.gaylord.com/archival.htm
tel. 800-448-6160
fax 800-272-3412

APPENDIX: SCREENING AND TEST PROTOCOLS

This section summarizes the procedures used to determine the storage properties of native Hawaiian plant seeds. The methods described are not necessarily the best ones for practical storage.

National Tropical Botanical Garden (NTBG)

Results citing NTBG as the source are not the results of a formal storage study, but an interpretation of NTBG's tests of its seed collection. NTBG dried the seeds at 15-25% relative humidity and stores them at 15-30% relative humidity, both at room temperature (presumably 55-84°F/19-29C°). They did not do germination tests of fresh seeds, but tested the stored seeds after they had been stored for a while. We (UH CCRT) have reviewed their test results and decided to classify all species which showed at least 5% germination after more than 1.5 years of storage as "not recalcitrant", and "orthodox" if they survived UH CCRT or USDA NCGRP freezing tests as well.

United States Department of Agriculture National Center for Genetic Resources Preservation (USDA NCGRP)
Formerly National Seed Storage Laboratory (USDA NSSL)

NCGRP has been conducting experiments to determine the relationship of storage temperature and humidity on aging rates of seeds of native Hawaiian plants. Seeds are deliberately stored at high temperature and humidity to artificially accelerate aging during storage. Results of the study can be extrapolated to predict storage longevities under actual storage conditions.

When seeds come in, a sample is germinated to determine the percentage of viable seeds. Most germination is done on damp blotter paper in petri dishes. Another sample is weighed, then baked and weighed again after baking to determine seed moisture content. A preliminary test for drying and freezing tolerance is run by drying some seeds at 13% relative humidity and 77°F (25°C) for a week, and then freezing some at 0°F (-18°C) for 16 hours before testing. Seeds which past these tests are classified as "not recalcitrant". The remaining seeds are stored at 59°F, 77°F, and 95°F (15°C, 25°C, and 35°C) at 12-13%, 32-34%, and 47-56% relative humidity, yielding nine possible temperature/humidity combinations. (Due to the small amount of material available for many species, not every combination is necessarily tested.) Samples of seeds are periodically removed from storage and germinated to measure viability. If enough seeds are available, they are also tested for tolerance to freezing over liquid nitrogen at -238°F (-150°C) .

University of Hawaii Center for Conservation Research and Training (UH CCRT)

CCRT has been conducting studies of storage lives of seeds in real time under practical storage conditions. Seven storage methods have been tested, though most species have not been tested with all of the methods. Note that these are experimental methods and not necessarily the ones which are best for practical storage.

In the individual species descriptions and graphs, the storage methods are referred to by their treatment numbers:

1. Open storage at room temperature and humidity
2. Room temperature, low humidity
3. Refrigerated storage - uncontrolled humidity
4. Refrigerated storage, low humidity
5. Frozen storage, low humidity

6, 7. Imbibed storage methods

The actual temperatures and humidities involved are as follows:

1. Room temperature and ambient humidity in Honolulu. Temperature, 55-84°F (13-29°C), relative humidity 58-98%.

2. Room temperature, low humidity. Inside a sealed jar with dehydrated silica gel. Temperature 55-84°F (13-29° C), relative humidity 10% or less.

2a. Room temperature, low humidity. As for #2, but 61-79°F (16-26°C).

3. Low temperature, reduced humidity. In an open jar in a refrigerator. Temperature 39°F (4° C), relative humidity approximately 20%.

4. Low temperature, low humidity. Inside a sealed jar with dehydrated silica gel in a refrigerator. Temperature 39°F (4°C), relative humidity 10% or less.

5. Frozen. Stored under treatment 4 until dried, then in a sealed jar with dehydrated silica gel in a freezer. Temperature 0°F (-18°C), relative humidity 30% or less.

6. Low temperature, imbibed. On 1% agar in petri dishes in a refrigerator. Temperature 39°F (4°C), relative humidity at saturation.

7. Room temperature, imbibed. On 1% agar in petri dishes in a dark cabinet. Temperature 55-84°F (13-29°C), relative humidity at saturation.

When seeds come in, a sample is germinated to determine the percentage of viable seeds. Most germination testing is in petri dishes on either 1% agar or filter paper. The remaining seeds are stored under the different storage treatments. After 3+ months of storage, samples of seeds are drawn and germinated to determine their viability. From the results, they are scored for drying and freezing tolerance. Seeds which survive storage treatment 2 are classified as “not recalcitrant”. If they survive treatments 5 or 5a as well as or better than warmer treatments, they are classified as “orthodox”.