Florabank Guidelines

Module 9

Seed Drying and Storage

David Merritt[1], Anne Cochrane[2], Lucy Commander[3,4], Karen Sommerville[5], Elinor Breman[6], Alice Quarmby[7]

[1] Kings Park Science, Department of Biodiversity, Conservation and Attractions, WA

[2] Department of Biodiversity, Conservation and Attractions, WA

[3] The Australian Network for Plant Conservation

[4] The University of Western Australia, Crawley WA

[5] The Australian PlantBank, Australian Institute of Botanical Science, Royal Botanic Gardens & Domain Trust, Mount Annan NSW

[6] Millennium Seed Bank, Royal Botanic Gardens, Kew, Wakehurst, West Sussex UK

[7] Swainsona Seed Services, Nairne SA

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Key points

- Ensure that seeds are fully mature at the time of collection
- Avoid exposing freshly collected seeds to high temperatures and high relative humidity
- Dry and clean seeds as soon as possible after collection
- Store seeds under dry conditions at a constant, cool temperature

Introduction

The principle aim of storing seeds is to maintain their quality and viability from the time of their collection up until their use some time in the future. Good practices for storing seeds focus on controlling the storage temperature and the seed moisture content. Appropriate post-harvest handling and storage will avoid wasting the initial resources expended collecting, and the loss of valuable, and in some cases, irreplaceable seeds. Seed storage need not be expensive and can be achieved effectively at relatively low cost, particularly for the short-term. Good quality native seeds should remain viable for a considerable period of time if some basic rules are understood and followed (Smith et al. 2003; Merritt 2006; Crawford et al. 2007; Offord & Meagher 2009; De Vitis et al. 2020).

The storage temperature chosen will vary according to the type of seed, the purpose for which seeds are stored and the expected duration of storage. Generally, the longer the intended storage period, the cooler and drier the storage conditions should be (Figure 1).

Storage length and purpose	Short (≤ 5 years) Restoration/Revegetation	Medium (5-10 years) Restoration/Plant breeding	Long term (>10 years) Conservation
Size of collection	Large or small scale	Large or small scale	Small scale
Drying conditions	Air-conditioned room ~23°C; Ambient conditions (indoor or outdoor) if relative humidity <50%	Relative humidity 15-20%; Temperature 15-20°C	Relative humidity 15-20%; Temperature 15-20°C
Storage conditions	Air-conditioned room ~23°C, or refrigerator or cool room (5-15°C); Ambient relative humidity <50%	Refrigerator or cool room (5-10°C); Relative humidity 15-20%	Freezer (minus 20°C)
Storage containers	Calico bags. Woven polypropylene (WPP) bags. Wool bales. Opaque plastic drums with a sealing ring.	As per short-term storage for large collections and if RH of room 15-20%. Press sealed or heat-sealed plastic bags for small collections if RH of room 15-20%. Air-tight, sealed laminated foil bags or glass jars for small collections and if RH not controlled (e.g. a refrigerator).	Air-tight, sealed laminated foil bags. Glass jars.

Figure 1. Seed drying, packaging, and storage conditions (temperature and relative humidity (RH)) appropriate to seed collections of differing scale, and purpose.

Seed storage behaviour

Seeds of different species have different storage characteristics that lead to differences in their longevity under various storage conditions (Walters 2015). There are three broad categories of seed storage behaviour – **orthodox**, **recalcitrant**, and **intermediate** (Hong and Ellis 1996). These categories describe in general terms the tolerance of seeds to drying and their longevity at different storage temperatures.

Orthodox seeds have a low moisture content at maturity and can be further dried to very low moisture content (<5% of fresh weight) without loss of viability. In this state, seeds can be stored at low or sub-zero temperatures without damage. In these seeds, a reduction in moisture content is more influential in extending seed longevity than temperature. Some 90% of the world's seeds are orthodox (Wyse and Dickie 2017), and they are commonly found in seasonally dry or arid climatic regions.

Recalcitrant seeds do not tolerate desiccation and are short-lived, sometimes surviving only a few days or weeks following dispersal. Recalcitrant seeds may be dispersed within fleshy fruits (e.g. *Syzygium* species), or from dry, **dehiscent** fruits (e.g. *Archidendron hendersonii, Castanospermum australe*), but all commonly have high moisture contents at shedding. Plants that produce recalcitrant seeds may be found in a range of environments but occur most commonly in wet tropical and subtropical forests (Tweddle et al. 2003, Wyse and Dickie 2017). These types of seeds are more difficult to store than orthodox seeds, requiring storage at high moisture content, and becoming non-viable if dried below about 40-50% of fresh weight. Long term storage of recalcitrant seeds is only possible through cryopreservation (refer Martyn Yenson et al. 2021). See Box 1.

Intermediate seeds have characteristics that fall somewhat between orthodox and recalcitrant seeds. The intermediate category covers a diverse range of storage behaviours (Walters 2015), but in general these types of seeds can usually be dried to a moisture content of around 10-15% of fresh weight without loss of viability, but their longevity can be reduced if dried further, or if stored at sub-zero temperatures.

Box 1. Seeds requiring special handling

While the majority of seeds can be stored dry in a freezer (orthodox), some seeds don't tolerate drying and some don't tolerate storage at sub-zero temperatures.

Seeds that don't tolerate drying are found most often in rainforest and mangrove environments and are more commonly produced by trees than by shrubs, climbers or herbs. Recent research on Australian rainforest plants shows that over 40% of tree species have seeds that are desiccation sensitive. Such species are very common in the families Araceae, Lauraceae and Sapotaceae, and in some genera in Myrtaceae (e.g. *Syzygium*).

Determining conclusively whether seeds are desiccation sensitive requires a series of germination tests with progressively drier seeds. In lieu of this, following the steps below will help you identify the majority of species in this category (Sommerville et al. submitted).

1. Check for information on the species in the Kew Seed Information Database. Species listed as recalcitrant don't tolerate drying. Species listed as intermediate will tolerate some drying but usually not to the extent necessary for freezing.
    a. If no data is available on your species, check for data on any other species in the genus. This is a good indicator of how your species will behave, providing it has a similar seed structure.
2. Check for information on storage potential in Hamilton et al. (2013), Sommerville et al. (submitted), Floyd (2008) and Dunphy et al. (2020).
3. Look at the seed's physical characteristics
    a. Seeds are usually tolerant of drying if they:
        i. Have a hard and impermeable seed coat (requiring scarification to germinate). These seeds are very common in Fabaceae and Malvaceae.
        ii. Weigh <20 mg. This applies to species in a variety of families including Apocynaceae, Cunoniaceae, Euphorbiaceae, Lamiaceae, Moraceae, Myrtaceae, Pittosporaceae, Rubiaceae, Solanaceae and Urticaceae.
        iii. Have a moisture content <20% of fresh weight at dispersal.
    b. Seeds are usually sensitive to drying if they:
        i. Have a moisture content >50% of fresh weight at dispersal.
        ii. Begin germinating within the fruit or within a week or two in temporary storage (e.g. *Araucaria bidwillii*, *Syzygium* spp.).
        iii. Have a large embryo (with no endosperm) and a papery thin seed coat (e.g. *Argyrodendron* spp., *Litsea* spp., *Pennantia cunninghamii*, *Syzygium* spp.).
        iv. Have a green embryo and a papery thin seed coat (e.g. *Archidendron hendersonii*, *Castanospermum australe, Micromelum minutum*).
    c. Rainforest seeds are often at least partially sensitive to drying if they:
        i. Are encased in a woody endocarp (e.g. *Cryptocarya* spp., *Endiandra* spp., *Planchonella* spp.).

Rainforest seeds that cannot be dried may be germinated and maintained as seedlings for 1-3 years (Dunphy et al. 2020). Seeds of some species may also be stored for short periods (several months or more) in a refrigerator at 4°C.

Rainforest seeds that tolerate drying, but not freezing, are more difficult to distinguish by physical characteristics. However, seeds with a high oil content (either in the whole seed or just the embryo) often fall in this category. These seeds can be stored in a refrigerator at 4°C. To test whether a species has freezing sensitive seeds, freeze a subsample of your seed lot (post drying) then compare germination after thawing to germination of a sample held at 4°C.

Seed longevity in storage

There is no easy, reliable method of determining the longevity of a seed collection at the time of storage. For orthodox seeds, longevity varies by at least four orders of magnitude between species (Probert et al. 2009) and is influenced by factors including the environmental conditions during seed development, seed maturity at collection, post-harvest handling practices, and genetics. Research into seed longevity has identified plant families that commonly produce longer- or shorter-lived seeds, as well as seed or environmental characteristics associated with longevity. Research on Australian species suggests that serotinous species (species storing seeds in the canopy), and woody trees and shrubs, have significantly longer-lived seeds than species of herbaceous habit or geosporous species (those species that release seeds into the soil seedbank) (Probert et al. 2009; Merritt et al. 2014). In addition, seeds that possess physical dormancy, and seeds with large embryos with little endosperm, are also commonly longer-lived (Probert et al. 2009; Merritt et al. 2014). Some of the longer-lived seeds are found in species of Casuarinaceae, Myrtaceae (e.g. *Calothamnus, Eucalyptus* and *Melaleuca*), Fabaceae (e.g. *Acacia* and *Jacksonia*), Sapindaceae (e.g. *Dodonaea*), and some Proteaceae (e.g. *Banksia* and *Hakea* but not *Telopea*), whilst some of the short-lived species include daisies (Asteraceae), grasses (Poaceae), and orchids (Orchidaceae) (Hay et al. 2010; Merritt et al. 2014). With respect to environmental characteristics, seeds from cooler and moist environments, including those from alpine areas, tend to be shorter-lived than seeds from warmer and drier environments (Probert et al. 2009; Satyanti et al. 2018).

Seed drying prior to storage

Drying is the first and most important step in slowing the rate of deterioration of orthodox seeds. The importance of drying seeds prior to storage cannot be stressed too highly. A small change in seed moisture content has a large impact on the longevity of seeds – as a general rule, the storage life doubles for every 1% reduction in moisture content. Drying also allows orthodox seeds to survive sub-zero storage temperatures and minimises damage to seeds via fungal and insect attack. Seeds that are collected at the point of maturity should be dried as soon as possible before processing.

Seed moisture content depends upon the relative humidity and temperature of the surrounding environment - this is the basic principle of seed drying. Most seeds absorb or lose water until equilibrium is reached with the moisture in the surrounding air (Figure 2); the exception being seeds that have water impermeable seed coats (i.e. physical dormancy or combinational dormancy) (see Module 11 – Seed Germination and Dormancy). Whilst these seeds can lose water to a dry atmosphere and can be dried effectively for storage, they will not absorb water from a moist atmosphere. All types of orthodox seeds should be ideally dried at a cool temperature and a low relative humidity. Ovens and high temperatures should not be used to dry seeds prior to storage.

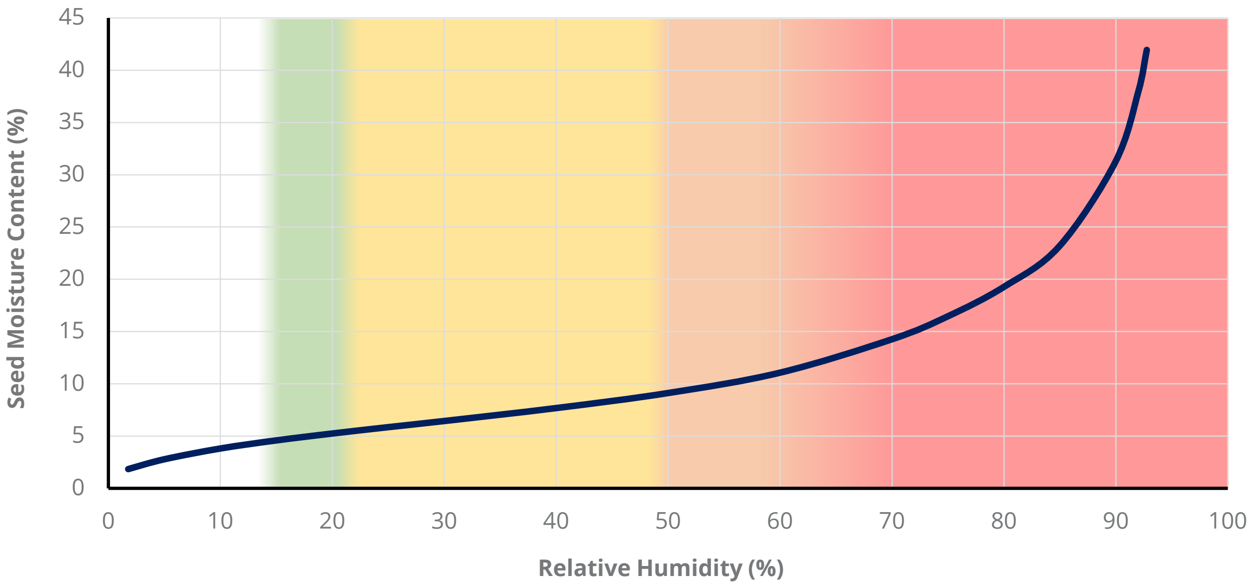

Figure 2. Most seeds are hygroscopic and will gain or lose water from or to the surrounding atmosphere. An isotherm, as illustrated, describes the relationship between seed moisture content and the relative humidity (RH) of the surrounding atmosphere, at a given temperature, in this case 23°C. Seed moisture content increases rapidly above 60-70% RH. To maximise longevity, orthodox seeds should be dried (or stored) at 15-20% RH. Drying seeds below 15% RH does not benefit longevity and may risk damage from over-drying. Drying or storing seeds at 20-50% RH is suitable for short-term storage, provided temperature is <25°C. Seeds will age rapidly at RH >60% and should not be held under such conditions unless they are recalcitrant.

Air drying of seeds can achieve variable moisture content between 6-12% of dry weight, depending on the relative humidity (RH) of the air (i.e. the prevailing environmental conditions). Artificial drying is preferable as it achieves a lower seed moisture content in a consistent and repeatable manner, however artificial drying methods can be costly and the method(s) used should be guided by the anticipated use of seeds and length of storage (Martyn Yenson et al. (in prep).; Figure 1).

Ideally, seeds are dried in a purpose-built room with a controlled environment (Figure 3). International guidelines for seed banking recommend seeds be dried under conditions of between 10-25% RH and 5-20°C (FAO 2014). Controlled environment drying rooms are commonly used for gene banks and are often set to maintain 15-20% RH and 15-20°C (Figure 1). Under these conditions, the seed moisture content will reduce to around 3-7% (of fresh weight). Alternatively, dehumidifiers installed into a well-sealed space can achieve a relative humidity of 25% or less. This type of environmental control for seed drying, whilst desirable, can be expensive, and may not be warranted for seeds that will be used within 5 years of collection (Figure 1). Seeds stored for <5 years may be dried at <50% RH.

Figure 3. Seeds drying in calico or paper bags, buckets, or trays in a purpose-built drying room (15°C and 15% RH). Seeds should be dried in porous bags or open containers and mixed regularly to allow even drying or, where space allows, dried in a thin layer that allows air movement across the surface. (Photos: L. Commander; D. Merritt)

Propagation igloos or greenhouses, solar drying boxes, forced air using fans, and drying cabinets with forced venting set to 30-35°C are all alternative means of achieving artificial drying which can be explored and applied at larger scales. There may also be opportunities for using an industrial drying service at agricultural premises. Be aware that long periods (e.g. months) in these higher temperature conditions may risk some loss of seed viability.

Drying over silica gel (silicon dioxide) is a practical solution for small quantities of seeds, and a practical solution for drying seeds in the field during collecting trips of a few days or more, particularly in regions of high humidity. The gel usually has an indicator dye that turns a different colour when saturated (Figure 4). At 25°C, a kilogram of silica gel can absorb about 75 grams of water. The more silica gel used, the lower the seed moisture content that can be achieved.

Figure 4. Silica gel with indicating dye. For these beads, a light orange colour indicatives active beads and a dark colour indicates saturated beads that require re-drying. (Photo: L. Commander)

For quicker drying, use equal weights of gel and seeds in a sealed container; however, be aware that over-drying can occur under these conditions. Alternative products to silica gel that act as desiccants include drying beads (Bradford et al. 2018), rice, and charcoal (Gold 2014).

In humid and tropical climates, seed drying is essential, as the ambient relative humidity may be as high as 75% or more. Seeds should be dried using forced ventilation or moisture extraction and a domestic fan or air conditioner can aid air circulation. In such scenarios, seeds should be sealed overnight (e.g. in plastic food containers or plastic drums) to prevent moisture uptake as the air temperature drops and relative humidity increases.

Although not ideal, if seeds appear to be immature upon collection (e.g. seeds are not at the point of dehiscence, and/or fruits are still green), then it is better to keep the seeds within the fruits, and even keep the fruits on the branches or stems if possible. This material should then be held under ambient conditions for 1-2 weeks, or until the fruits show signs of ripening (e.g. a colour change, or capsules open). The seeds can then be cleaned from the fruits and dried following the procedures for mature seeds.

As a quality control measure, it is useful to keep track of the temperature and relative humidity of the drying environment to detect any anomalies in conditions. Various types of data loggers that detect and record temperature and relative humidity can be used for this purpose.

Drying procedures for mature seeds

- Place cleaned seeds in porous bags (calico or woven polypropylene bag) or spread seeds out in an open container or trays. Seeds should ideally be laid out in a single or thin, even layer, or mixed regularly, for even drying. Place the seeds on open racks with enough space to allow for the free circulation of air between them.
- On a larger scale, seeds can be laid out on tarpaulins or shade cloth (if outside, these should be overlaid by shade cloth to prevent seed loss by wind).
- Dry the seeds until they have reached equilibrium with the drying environment. This can be determined by checking the moisture status of the seeds with a **hygrometer (Box 2)**, or by periodically weighing the seeds and observing when no further weight loss occurs. The time required for seeds to dry is largely dictated by their initial moisture content, size (smaller seeds dry faster than larger seeds), the thickness of the seed coat and the total volume of the collection. Between 1-4 weeks is generally sufficient for most seeds. For orchid and other micro-seeds, drying for one week is preferable due to their small size and short-lived nature.

Drying procedures for mature seeds using silica gel

- Place the silica gel in an airtight container with the seeds (Figure 5). Seeds and/or silica gel may need to be enclosed in a porous bag inside the airtight container to keep both separate – such separation is important due the rapid drying induced by silica gel.
- Use a weight of silica gel equal to the weight of seeds (1:1). Place the container in a room at approximately 15°C whilst the seeds are drying. It is preferable to air-dry the seeds prior to drying over silica gel to avoid drying initially moist seeds too quickly.

- Seeds should be mixed periodically to ensure even drying and the silica gel replaced or re-dried when the colour of indicator beads changes.
- Leave the seeds with the silica gel in the container until the moisture content is within the range required for storage. Moisture content of the seeds can be measured using a hygrometer (Box 2) and the relative humidity of the air in the container can also be monitored with a data logger.
- Silica gel or beads that have absorbed moisture (i.e. a colour change is apparent) can be re-dried by either heating in an oven above 100°C for several hours or by using a microwave oven on a medium power setting for 1 to 5 minutes (depending on the volume) with the silica gel placed in a thick glass container. After heating, the beads should be inspected for a colour change to ensure they are fully dried and then left to cool before being stored in an air-tight container until required.

Figure 5. Silica gel drum dryer containing seeds (left) and containing a hygrometer (right). (Photos: RBG Kew)

Measuring seed moisture content

Seed moisture content refers to the amount of water in the seed and is usually expressed as a percentage by weight. The standard method requires fresh seed samples to be weighed, placed in an oven at 103°C for 17 hours (low temperature method for oily seeds) or 130°C for 1 hour (high temperature method for non-oily seeds) and then weighed again (see ISTA 2020). This is a destructive method for measuring seed moisture content and may not be suitable for all types of collections (e.g. small numbers of seeds or seeds of rare species).

Moisture content via oven drying can be calculated on either a wet (fresh) weight or a dry weight basis, but the calculation should be consistently applied between species (the formula below describes moisture content on a wet weight basis).

$$\text{Seed Moisture Content (\%)} = \left(\frac{Weight\ Wet\ Seeds - Weight\ Dry\ Seeds}{Weight\ Wet\ Seeds}\right) \times 100$$

A non-destructive method of assessing seed moisture is to measure their equilibrium relative humidity (or eRH) with a hygrometer (Figure 6) (refer Box 2). These instruments measure the relative humidity in the atmosphere surrounding the seeds and can be used to determine when seeds have dried sufficiently to enable storage. These types of hygrometers are usually used in conjunction with a controlled environment drying room to ensure seeds are fully dried prior to packaging and storage.

Some products such as dry boxes have an inbuilt hygrometer. Alternatively, various types of data loggers or dial/analogue hygrometers can also be used to monitor or measure relative humidity of the drying environment – either of the ambient conditions or within a controlled environment drying room. Moisture indicator paper also provides some basic capacity to determine atmospheric relative humidity.

Box 2. Measuring seed moisture status with a hygrometer

It is convenient to measure seed moisture status by the equilibrium relative humidity method (eRH); that is, the relative humidity of air at equilibrium with seeds held in a sealed chamber. This method is rapid, and non-destructive.

Procedure

- Place a sample of seeds into the chamber of a hygrometer (Figure 6). Ensure that the chamber is fully filled with seeds - if there is a small volume of seeds, inert inserts may be required to reduce the size of the sample chamber.
- To avoid inaccurate readings, do not directly handle seeds, do not touch the inside surfaces of the sample chamber, and do not breathe on an exposed sensor.
- Allow the seeds to equilibrate (around 30 minutes on average) and record the end point RH and temperature displayed on the hygrometer.
- When seeds are sufficiently dried, the reading for RH should be between 15–20%.
- Clean the sample chambers with deionised water and/or alcohol and dry carefully after use.

Figure 6. A hygrometer (left to right) without seed; once seed has been placed inside the sample chamber; closed to take the measurement. (Photos: L. Commander)

Seed storage containers

Once seeds are dried, it is crucial to maintain that dryness by packaging them appropriately. Ideally, packaging should be done quickly after drying to prevent reabsorption of moisture, and if a controlled environment drying room is used, seeds should be packaged in the drying room. The storage containers should be equilibrated to the conditions of the drying room, prior to packing and sealing. Packaging seeds securely also prevents insect and disease contamination in storage. Table 1 details some of the most useful storage containers and their pros and cons (adapted from Offord & Meagher (2009)). A good overview of seed packaging containers can be found in the Millennium Seed Bank Partnership publication: *Selecting containers for long-term seed storage* (Manger et al. 2003).

Dried seeds can be sealed in airtight resealable bags, where appropriate. Heat sealers (Figure 7) commonly used in food packaging are relatively cheap and it is possible to obtain laminated plastic or foil bags in various sizes (Figure 8). Laminated foil bags provide a higher standard of storage than laminated plastic. Press-seal plastic bags will allow moisture in over a period of time, as well as being easily damaged unless of a durable quality, so other packaging is recommended.

Figure 7. Benchtop (left) and foot operated (right) heat sealer for sealing plastic or foil bags. (Photos: L. Commander)

Figure 8. Laminated foil bags for seed storage. (Photos: L. Commander)

Plastic containers are durable and useful for keeping small amounts of seeds protected in the short term. Glass jars used in preserving and bottling, especially those with rubber sealing rings under the lid, make ideal airtight storage containers for smaller seed collections, such as those for conservation purposes (Figure 9). Twist cap glass containers are also suitable, provided a torque meter is used to check they are properly sealed.

Glass containers are used in the Millennium Seed Bank, with the base collections stored in two containers for extra protection. Opaque plastic drums with a sealing ring in the lid provide some degree of airtight storage and can hold larger quantities of seeds - collections of tens of kilograms of seeds destined for restoration, for instance. When placing seeds in storage containers it is advisable to use a moisture indicator such as indicator silica gel to ensure the seal can be confirmed as remaining airtight, particularly in humid climates.

Figure 9. Glass containers for seed storage. (Photo: L. Commander)

To reduce moisture uptake in any type of container, it is best to fill the container as full as practicable to reduce airspace. Always make sure lids are firmly closed and seals are intact. Small vacuum pumps that extract all the air from a storage container prior to sealing are not necessary and risk perforating laminated plastic or foil bags.

When storing very large quantities of seeds (e.g. tens or hundreds of kilograms of seeds for restoration) it may be impractical to use sealed bags or containers. Storing such large quantities of seeds in breathable bags (e.g. calico, woven polypropylene or paper) is possible, but they must be kept under dry conditions within a controlled environment room of low humidity and temperature. In the USA, climate-controlled warehouses are used to store vast quantities of seeds sourced from the wild or from seed production facilities for restoration (Figure 10). In Australia, large collections of seeds destined for use in restoration are increasingly being stored (on- or off-site) within insulated shipping containers with climate-control functionality.

Figure 10. Large-scale seed storage in a warehouse in Utah, USA. (Photo: L. Commander)

Table 1. Storage containers for packaging dried seed, or for storing seeds in a controlled environment room.

Container material	Advantages	Disadvantages
Glass containers	Airtight provided a rubber seal is used Transparent – moisture indicators can be added to check integrity of seal	Heavy – potential for breakage Not space efficient Not suitable for large quantities
Laminated foil bags	Lightweight Space efficient	Can be punctured by sharp objects Do not allow seed or moisture indicators to be seen Not practical for large volumes (i.e. large-scale restoration collections)
Sealed plastic containers or bags (non-woven)	Light weight Can be transparent	Rarely seal effectively Not space efficient
Breathable bags	Light weight Practical for large-scale restoration collections	Porous, with seed moisture content varying with storage conditions (i.e. can be problematic if RH is high)

Storage temperature

Once seeds are dried, packaged and labelled appropriately, they should be placed directly into storage at a temperature appropriate to the duration of storage.

Storage temperatures for dried orthodox seeds can include from room temperature (c. 23°C), air-conditioned rooms (15-20°C), fridges (c. 4°C), freezers (c. -20°C), or cryopreservation in liquid nitrogen (-196°C). Having access to a variety of facilities can provide flexibility for storing seeds for different lengths of time and for those with non-orthodox storage behaviour (Linington 2003). For orthodox seeds, the lower the temperature, the longer seeds will remain viable. The decision to store seeds at lower temperatures should be informed by the designated end-use of the seeds, the storage duration, and whether temperature affects dormancy loss/induction, and made on a species-by-species basis (Figure 1). Many Australian native species will store well at room temperature for many years, particularly those with water-impermeable seed coats (e.g. legumes / Fabaceae); many rainforest or fleshy fruited seeds (that may be recalcitrant) may last less than a year and should be stored more carefully or used immediately.

Room temperature storage

Many orthodox seeds that are dried appropriately can be stored at room temperature for short periods. However, to maximise viability retention under these conditions, it is still important that the moisture content remains constant by storing seeds in airtight containers/bags or through controlling and monitoring the relative humidity of the room.

Air-conditioned storage

Installing air conditioning allows for the reduction of the ambient temperature. To maximise the efficiency of an air conditioner, condition a small room rather than a large room, line and insulate the walls, floor and ceiling, and use double glazing or curtains on any windows.

Refrigerated storage

The most common options available for refrigerated storage are domestic refrigerators, commercial display or drinks refrigerators and walk-in cool rooms. Most refrigeration systems are not humidity-controlled and may deliver a relative humidity of 90% or more inside an unsealed storage container. It is therefore crucial that seeds are stored in airtight containers to ensure that moisture is not reabsorbed into seeds from the surrounding air. Laminated foil bags are recommended, as well as airtight glass containers. It is a good idea to put an indicator silica gel inside the containers to warn of any moisture entry. Refrigerated cabinets should be well drained and have good door seals. Walk-in cool rooms or chillers are used widely for storage of seeds in large quantities. If the power fails, do not open the fridge until power resumes to maximise the duration of cool temperatures.

Freezer storage

Of all the most practical options, freezer storage at -20°C will provide the maximum longevity for orthodox seeds and is recommended for long term conservation purposes and for restoration projects that are planned many years in advance. If storing seeds for less than 5 years, it is not usually necessary to use freezers. The moisture content of seeds placed into sub-zero temperature conditions should be between 3-7% fresh weight to avoid damage (see section on drying seeds and calculating seed moisture content). Freezers are not humidity controlled, so it is vital that seeds are stored in good quality airtight containers before placing into a freezer. Laminated foil bags are recommended, as well as airtight glass containers. As with refrigerator storage, the use of an indicator silica gel inside the containers can warn of any moisture entry. Chest freezers and upright freezers can be used for small quantities of seeds (Figure 11), but for larger quantities a walk-in freezer is desirable (Figure 12). If the power fails, do not open the freezer until power resumes to maximise the duration of cold temperatures.

Figure 11. Upright freezers can be used for small quantities of seeds. (Photo: L. Commander)

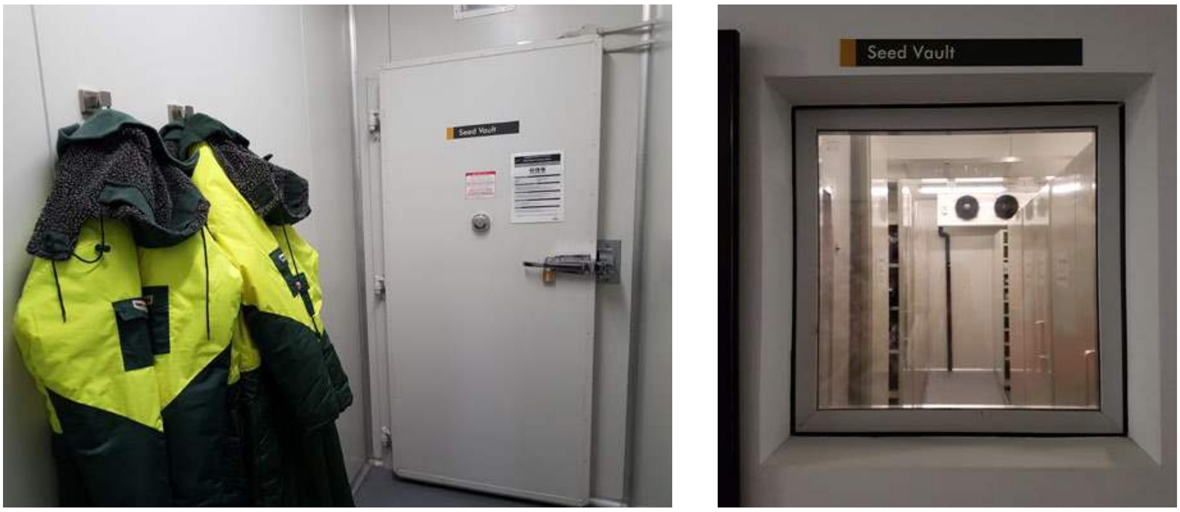

Figure 12. The walk-in freezer for seed storage at Kings Park and Botanic Garden. (Photo: L. Commander)

Cryopreservation

Cryopreservation is the storage of seeds at very cold temperatures (i.e. liquid nitrogen, at -196°C, or in the vapour phase between -140 and -192°C). This requires specialist knowledge and equipment, and is generally used for small quantities of seeds and for high value seeds such as those of threatened plant species (Figure 13). Cryopreservation provides the potential to store seeds for very long periods (decades, or more) and is generally the domain of botanic gardens and other conservation organisations. For further information see Martyn Yenson et al. (2021).

Figure 13. Cryopreservation requires specialist equipment. (Photo. L. Commander)

Insects and other pests

Airtight containers should prevent the contamination of seeds by insects as well as rodents. However, insect pests should be removed before storage (see Module 8 – Seed Processing). Cleaning seeds is a good way to remove insect contamination, but insects may still remain concealed within seeds and fruits, particularly as eggs. Carbon dioxide can be used as a fumigant. Airtight bags containing seeds can be filled with this inert gas for a few weeks to eliminate insect pests. Driacide (amorphous silica), diatomaceous earth and magnesite (magnesium carbonate) powders have been used to achieve successful control of insects in seed containers. As with all chemicals, safe handling practices should be followed, and warnings and information regarding use should be strictly adhered to. If seeds are to be stored at sub-zero temperatures, the freezing will eliminate insect pests. There is also evidence that insects are unable to survive in storage at relative humidity values below 35% (Bradford et al. 2018).

Monitoring seeds in storage

Meticulous record keeping is vital to ensure seeds remain valuable for future use as inadequate record keeping can place limitations on where and how seeds can be used. Labelling seeds and storage containers and monitoring of storage conditions and seed viability all help to safeguard the seed resource (see also Module 4 – Record Keeping).

Monitoring seeds in storage requires two basic considerations – monitoring the storage environment and monitoring the seed viability. The first is to monitor the storage environment and guard against fluctuating relative humidity and temperature conditions. Temperature and humidity gauges can be used for this purpose. Digital thermometers and relative humidity meters can be installed inside rooms or containers used for drying and storage (Figure 14). In addition, loggers may be used to record temperature and humidity to track fluctuations or ensure they stay constant over time. Monitoring systems are now available that can provide real-time monitoring to alert users to failures in environmental control.

The second consideration is monitoring the viability of the seeds in storage. For details of seed testing procedures, refer to Module 10 – Seed Quality Testing. A pre-storage viability and germination test is ideal to provide baseline information regarding the quality of the seed collection, as well as for use in monitoring the effectiveness of the chosen storage conditions. Viability testing is increasingly expected of seed suppliers and it is good practice to provide end-users with appropriate information regarding the quality of their seed batches. When the seeds are removed from storage, repeating the viability and germination test under the same conditions will determine whether viability has declined in storage. However, regular monitoring of seed viability is resource intensive, consumes seeds and can be challenging with regards to the setting of testing schedules. Re-test schedules are ideally based upon the purpose of the collections and their expected longevity. An additional challenge with the germination test is that there may be issues with overcoming seed dormancy and lack of germination may not necessarily indicate lack of viability. This issue is covered in detail in Module 11 – Seed Germination and Dormancy.

Always avoid unnecessary fluctuations in seed temperature and humidity during handling by only working on small amounts of seeds outside the storage environment. If seeds have been stored in hermetically sealed containers and at low temperature, always allow seed packages retrieved from storage to adjust to the temperature of the working area before opening. This prevents moisture condensation onto cold seeds that may be trapped once the package is re-sealed. Ideally, seed packages are opened in a drying room to prevent any risk of moisture absorption.

Figure 14. A data logger can be used to display and record temperature and humidity in the seed store as well as during pre-storage drying. (Photo: L. Commander)

Seed store design considerations

Planning for seed storage requires a number of important considerations:

- The type (i.e. seed storage behaviour) and number of species to be stored and the expected storage duration.
- The size (volume and weight) of the collections and the space required.
- The resources required and available to construct and maintain the facility and to curate the seeds in storage.

Seed storage facilities require areas for receiving seeds, seed drying, seed cleaning, packaging and storage, dispatch, moisture content and germination testing, office space for record-keeping and storage space for collecting equipment. The use of shelving can maximise vertical space for storage, and shelving can be tailored to the number of containers of each type and size. Such a facility should also include precautions against unforeseen circumstances such as fire or theft through the installation of fire extinguishers, smoke alarms, and back-up generators, and through taking out insurance (see also Martyn Yenson et al. 2021).

The purpose for which seeds are stored is a fundamental consideration in seed store design (Figure 1), particularly as construction and running costs increase with increasing levels of environmental control. In this regard, flexibility in storage conditions can be created through having rooms maintained under different environmental conditions, and/or a range of fridges or freezers, as appropriate to the storage duration or end-use of the various collections. In some cases, the construction of insulated, climate-controlled shipping containers can provide effective storage facilities. New containers can be added as required, and this approach is becoming more common throughout the mining sector in Australia with the increasing demand for larger quantities of seeds for rehabilitation. For smaller operations that have limited resourcing and minimal space requirements (community groups, for example), using space in a community seed bank can be an alternative to establishing your own seed storage facility and may be more economical.

For seeds stored for conservation purposes, duplication is a key risk-management strategy. Individual collections can be divided and stored under similar conditions in two separate locations. This may be desirable for high-value seeds, in particular those of threatened species, for example. Conservation collections are also often split into active collections that are more frequently accessed (those used for viability monitoring, propagation, translocation, research, etc.) and a base collection (rarely accessed and held for conservation purposes) (see also Martyn Yenson et al. 2021).

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Glossary

Combinational dormancy: seeds with physical and physiological dormancy, i.e. a water impermeable layer in their seed or fruit coat and inability to germinate due to an inhibiting mechanism in the embryo, which prevents radicle emergence through the covering structures.

Dehiscent: fruits that open to release seeds.

Geosporous: species which store their seeds in the soil seed bank (cf. serotinous species).

Hygrometer: an instrument for measuring the humidity of the air or a gas.

Intermediate: seeds that display properties between orthodox and recalcitrant.

Orthodox: desiccation tolerant seeds, i.e. seeds that survive drying, and for which longevity increases with a decrease in storage temperature (cf. recalcitrant).

Physical dormancy: seeds with a water impermeable layer in their seed or fruit coat

Recalcitrant: desiccation intolerant seeds, i.e. seeds that do not survive drying (c.f. orthodox).

Serotinous species: species which store their mature seeds within fruits (e.g. *Banksia* cones) in their canopy for an extended period of time (cf. **geosporous species**).

Online resources

Center for Plant Conservation (2019) CPC Best Plant Conservation Practices to Support Species Survival in the Wild. https://academy.saveplants.org/sites/default/files/CPC-best-plant-conservation-practices-protocols.pdf

Royal Botanic Gardens, Kew, Millennium Seed Bank Seed Information Database. https://data.kew.org/sid/

Royal Botanic Gardens, Kew, Millennium Seed Bank Technical Information Sheets. http://brahmsonline.kew.org/msbp/Training/Resources

Royal Botanic Gardens, Kew, Seed Storage Predictor. https://seedcollections.shinyapps.io/seed_storage_predictor/

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