

## Chapter 4

# Storage of Seeds

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## Introduction

In the simplest form of seed storage, mature seeds are held for a short period until weather or other factors permit sowing or planting. In the more comprehensive view, there are at least 3 objectives for storing seeds, and each of them dictates different strategies and procedures. These objectives may be described as storage for the following periods:

1. Very short periods (overwinter) between collection and sowing
2. Several years (10 or less) to ensure a reliable supply of seeds in the absence of annual crops
3. Long periods (10 to 50+ years) for germplasm conservation

The strategies employed will depend on all of the factors that influence seed longevity. Some of these factors have been discussed in chapter 1, but now they will be explored in the context of seed storage only. Following this, recommendations will be made for storage procedures to achieve the objectives listed here.

## Factors Affecting Longevity of Seeds

### Seed Characteristics

**Basic seed physiology.** In his classic paper, Dr. E. H. Roberts (1973) divided seeds into 2 groups based on their storage characteristics: orthodox and recalcitrant. Orthodox seeds are those that can be dried to moisture contents of 10% or less; in this condition they can be successfully stored at subfreezing temperatures. Recalcitrant seeds, on the other hand, are those that cannot be dried below relatively high moisture levels (25 to 45%) and therefore cannot be stored below freezing. Current knowledge of seed physiology can allow additional classification of tree seeds into the following groups (Bonner 1990): (1) true orthodox, (2) sub-orthodox, (3) temperate-recalcitrant, and (4) tropical-recalcitrant. In addition, Ellis and others (1990) have proposed an intermediate storage class that falls between orthodox and recalcitrant.

*True orthodox seeds* can be stored for relatively long periods at subfreezing temperatures—if their moisture contents are reduced to about 5 to 10% (wet weight basis). [Throughout this chapter, seed moisture will be expressed as a percentage of wet weight in keeping with the international protocol (ISTA 1993).] Most species of the economically valuable tree genera of the Northern Temperate Zone are classified as having true orthodox seeds: fir (*Abies* P. Mill.), alder (*Alnus* P. Mill.), birch (*Betula* L.), ash (*Fraxinus* L.), larch (*Larix* P. Mill.), spruce (*Picea* A. Dietr.), pine (*Pinus*

L.), sycamore (*Platanus* L.), cherry and plum (*Prunus* L.), Douglas-fir (*Pseudotsuga* Carr.), hemlock (*Tsuga* Carr.), etc. Many valuable genera of the tropics and subtropics are also true orthodox: *Acacia* L., *Albizia* Durz., many other Fabaceae, *Casuarina* Rumph. ex L., *Eucalyptus* L' Her., mesquite (*Prosopis* L.), and teak (*Tectona* L.f.). The time limits for storage of true orthodox seeds under optimum conditions is not really known. Eliason and Heit (1973) reported 86% germination in red pine (*Pinus resinosa* Soland.) samples stored for 42 years. Martin (1948) found that herbarium samples of velvet mesquite (*Prosopis velutina* Woot.) germinated quite well after 44 years. Barnett and Vozzo (1985) found that slash pine (*P. elliotii* var. *elliotii* Engelm.) still germinated at a rate of 66% after 50 years of storage at 4 °C. Other examples of storage data for true orthodox tree seeds are found in table 1.

*Sub-orthodox seeds* can be stored under the same conditions as true orthodox seeds, but for much shorter periods. The reasons for their decreased longevity are not completely known. However, indirect evidence suggests that some causes are high lipid contents—as in hickory (*Carya* Nutt.), beech (*Fagus* L.), walnut (*Juglans* L.), and some pines (*Pinus* L.) and thin fruits or seedcoats, including some maples (*Acer* L.), poplars (*Populus* L.), and willows (*Salix* L.) (Bonner 1990). Retention of viability for more than 10 years would be rare for sub-orthodox species with current storage technology (table 2).

*Temperate-recalcitrant seeds* cannot be desiccated but can be stored at or slightly below freezing. Genera with temperate-recalcitrant seeds include buckeye (*Aesculus* L.), chestnut (*Castanea* P. Mill.), oak (*Quercus* L.), and redbay (*Persea* P. Mill.). Some, but not all, of these species can be stored for 3 to 5 years at near-maximum moisture contents (30 to 50%) and low temperatures (−3 to +4 °C) (table 3).

*Tropical-recalcitrant seeds* have the same desiccation sensitivity of temperate-recalcitrant seeds and are also sensitive to low temperatures. Even short periods of exposure to temperatures below 10 to 15 °C can cause loss of viability (Berjak and Pammenter 1996; Chin and Roberts 1980). Included in this group of species are *Araucaria* Juss., *Hopea* Roxb., *Shorea* Roxb., ex C.F. Gaertn., and *Theobroma* L. Longevity of these seeds is usually measured in months, not years (table 4).

*Intermediate seeds* can be dried to moisture levels almost low enough to meet orthodox conditions (12 to 15%) but are sensitive to the low temperatures typically employed for storage of orthodox seeds. Viability is retained usually only for a few years. The research that led to the concept of

**Table 1**—Chapter 4, Storage of Seeds: storage test results for some true orthodox species

Species	Test conditions		Test results	
	Temp (°C)	Seed moisture (%)	Period (years)	Viability loss (%)
<i>Abies procera</i> Rehd.	0	9	7	11
<i>Acacia mangium</i> Willd.	4–8	—	1.2	6
<i>Acer saccharum</i> Marsh.	–10	10	5.5	5
<i>Alnus rubra</i> Bong.	2–4	5–8	4	0–13
<i>Araucaria cunninghamii</i> Aiton ex D. Don	–15	16–23	8	Little
<i>Atriplex canescens</i> (Pursh) Nutt.	[??]	—	4	Little
<i>Betula alleghaniensis</i> Britt.	3	—	8	2
<i>Casuarina equisetifolia</i> L.	–3	6–16	2	0–5
<i>Cercocarpus montanus</i> Raf.	5	8	6	7
<i>Cowania mexicana</i> D. Don	5	8	6	1
<i>Eucalyptus</i> spp.	3–5	4–8	5–20	—
<i>Grevillea robusta</i> A. Cunningham ex R. Br.	–6	6	2	<5
<i>Krascheninnikovia lanata</i> (Pursh)	5	—	2.5	<10
<i>Larix decidua</i> P. Mill.	2–4	7.5	14	27
<i>Liquidambar styraciflua</i> L.	3	5–10	9	3
<i>Paraserianthus falcataria</i> (L.) I. Nielsen	4–8	—	1.5	10
<i>Picea sitchensis</i> (Bong.) Carr.	2–4	7–9.5	13–24	0–11
<i>Pinus banksiana</i> Lamb.	2–4	11	17–18	0–8
<i>P. merkusii</i> Junghuhn & Vriese ex Vriese	4–5	<8	4	None
<i>P. ponderosa</i> P. & C. Lawson	0	8	7	None
<i>Tectona grandis</i> L. f.	0–4	ca.12	7	None
<i>Tsuga heterophylla</i> (Raf.) Sarg.	5, –18	8	2	None
<i>Ulmus laevis</i> Pall.	–3	10	5	None

Sources: Bonner (1990), Clausen (1967), Jones (1987), Springfield (1968, 1973, 1974), Tylkowski (1987), Wang and others (1993).

**Table 2**—Chapter 4, Storage of Seeds: storage test results for some sub-orthodox species

Species	Test conditions		Test results	
	Temp (°C)	Seed moisture (%)	Period (years)	Viability loss (%)
<i>Citrus limon</i> (L.) Burm. F.	–20	5	0.9	± 5
<i>Fagus sylvatica</i> L.	–10	10	5	34
<i>Gmelina arborea</i> Roxb.	–5	6–10	2	10
<i>Populus deltoides</i> Bartr. ex Marsh.	–20	6–10	6	21
<i>P. grandidentata</i> Michx.	–18	11–15	12	14–29
<i>P. tremuloides</i> Michx.	–18	6–8	2	1
<i>Salix glauca</i> L.	–10	6–10	1.2	0

Sources: Bonner (1990), Fechner and others (1981), Wang and others (1982).

intermediate seed behavior was done with coffee (*Coffea arabica* L.) (Ellis and others 1990), and although no forest tree species have been identified as intermediate as yet, there is a very good chance that some will fit this classification.

There are several genera that contain both orthodox and recalcitrant species. In the Northern Temperate Zone, maple

(*Acer*) is such a genus. Silver maple (*Acer saccharinum* L.) is clearly temperate-recalcitrant in nature (Tylkowski 1984), but red maple (*A. rubrum* L.) can be dried to 10% seed moisture content and is either true orthodox or sub-orthodox. Among tropical species, the genus *Araucaria* Juss. has a similar distinction. *Araucaria cunninghamii* Aiton ex D. Don is orthodox in nature, and *A. hunsteinii* K. Schum. &

**Table 3**—Chapter 4, Storage of Seeds: storage test results for some temperate recalcitrant species

Species	Test conditions		Test results	
	Temp (°C)	Seed moisture	Period (months)	Viability loss (%)
<i>Acer saccharinum</i> L.	-3	50	18	8
<i>Quercus macrocarpa</i> Michx.	1	44	6	None
<i>Q. pagoda</i> Raf.	3	35	30	6
<i>Q. robur</i> L.	-1	40–45	29	31–61
<i>Q. rubra</i> L.	-1 to -3	38–45	17	18–46
<i>Q. virginiana</i> P. Mill.	2	—	12	35

Sources: Bonner (1990), Schroeder and Walker (1987).

**Table 4**—Chapter 4, Storage of Seeds: storage test results for tropical recalcitrant species

Species	Test conditions		Test results	
	Temp (°C)	Seed moisture (%)	Period (days)	Viability loss (%)
<i>Araucaria hunsteinii</i> K. Schum. & Hullrung	19	25–30	54	±30
<i>Azadirachta indica</i> Adr. Juss	26	10–18	56	65
<i>Dipterocarpus turbinatus</i> C. F. Gaertn.	16	41–44	161	47
<i>Hopea helferi</i> (Dyer) Brandis	15	47	37	2
<i>Shorea robusta</i> C. F. Gaertn.	13.5	40–50	30	60
<i>S. roxburghii</i> G. Don	16	40	270	±30
<i>S. talura</i> Roxb.	23.5	47	105	50
<i>Symphonia globulifera</i> L. f.	15	—	270	None

Sources: Bonner (1990), Bras and Maury-Lechon (1986), Purohit and others (1982), Tompsett (1987).

Hollrung is tropical-recalcitrant (Tompsett 1982). There are undoubtedly other genera, still unidentified, with these characteristics.

Placing seeds into these precisely defined groups of storage behavior is often tenuous, however, because recalcitrance is not an all-or-nothing characteristic (Berjak and Pammenter 1996). There is a great deal of natural variation, and species should be viewed as lying somewhere along a spectrum that stretches from extreme orthodoxy to extreme recalcitrance. Furthermore, as technology improves, a species may not be what it was once thought to be. *Fagus* L. was once thought to be recalcitrant, but with carefully controlled drying, seeds of this genus can attain low moisture contents and an extended storage life at subfreezing temperatures (Bonnet-Masimbert and Muller 1975; Suszka 1975) and should now be considered as sub-orthodox in storage behavior.

**Seed morphology.** Seed morphology is important to the storage life of seeds in the context of protection for the embryo. The hard seedcoats of species of the Leguminosae help maintain the low level of metabolism in these dry orthodox seeds by excluding moisture and oxygen. Hard, thick seedcoats, such as those of *Carya* Nutt., *Cornus* L.,

and *Nyssa* L., help protect the embryos from mechanical damage during collection and conditioning. The thinner or softer a seedcoat may be, the more likely that the seed has a shorter storage life because of rapid moisture uptake or bruising of internal seed tissues. Thin seedcoats may be a significant factor in storage difficulties of *Acer rubrum* L., *Pinus palustris* P. Mill., and *Populus* L. spp., but there is no direct evidence of this.

**Chemical composition.** General observations of seed behavior in storage has suggested that chemical composition is an important factor in longevity; for example, oily seeds do not store as well as starchy seeds. One can find support for this concept with the relatively poor performance in storage of *Carya* Nutt. spp., *Juglans* L. spp., and *Sassafras albidum* (Nutt.) Nees, all oily seeds, and the relatively good performance of *Celtis laevigata* Willd., *Fraxinus* L. spp., and *Platanus occidentalis* L., all starchy seeds (Bonner 1971). Exceptions to this rule abound, however. Oily seeds of *Liquidambar styraciflua* L. as well as *Pinus taeda* L., and many other conifers keep very well in proper storage. Within *Quercus* L., acorns of the black oaks, which are somewhat oily with very little carbohydrate, store longer than acorns of the white oaks, which are full of carbohy-

drates and very little lipid. Even among the black oaks, species with the highest lipid contents seem to store better, even though there is no evidence of cause and effect. One must conclude that among a wide range of species there is no compelling argument for gross chemical composition as the critical factor in seed longevity under proper storage conditions. There is some evidence, however, that suggests that the relative concentrations of particular carbohydrates play key roles in desiccation tolerance, a critical property in determining storage behavior of seeds (Lin and Huang 1994). This topic is obviously one that deserves more research.

**Seed maturity.** Seeds of many orthodox species that are immature when collected (or extracted from fruits) are likely to fare poorly in storage (Stein and others 1974). Experimental evidence has demonstrated this fact for Scots (*Pinus silvestris* L.) (Kardell 1973), loblolly (*P. taeda* L.), longleaf (*P. palustris* P. Mill.), and eastern white (*P. strobus* L.) pines (Bonner 1991). The physiological basis for this effect is not known, but it seems logical that immature seeds have not been able to complete the normal accumulation of storage food reserves, develop all needed enzymes and/or growth regulators, or complete their full morphological development and cell organization. For species with seeds that are naturally dispersed while still physiologically immature, such as *Fraxinus excelsior* L., there is no apparent damage to storage longevity (Willan 1985). The ability to complete maturation naturally after separation from the mother tree has apparently evolved with these species. For conifers like the pines noted above, storage of immature cones for several weeks prior to extraction of the seeds appears to enhance seed maturity and viability retention during storage (Bonner 1991).

### Seed Handling Prior to Storage

Poor fruit or seed handling that damages seeds will often lead to reduced viability in storage, especially in orthodox seeds. The most common example of this is impact damage to seeds during extraction and conditioning. Seeds can be bruised by excessive tumbling of cones, running dry dewingers too fast or too full, or poor transport systems (Kamra 1967). During kiln drying of conifers, excessive heat while seed moisture is still high can easily lead to damage that will show up later as reduced vigor and viability in stored seeds (see chapter 3).

Another factor to consider in damage to seeds during extraction and conditioning is cracks or other breaches of the seedcoats that will allow microorganisms to enter. Cracks in seedcoats that occur during seed conditioning are

usually not visible to the naked eye but can be detected on radiographs (see chapter 5). This is one reason why hard-seeded legumes are usually not returned to storage after mechanical scarification. An exception to this is when seed burners are used for scarification (Lauridsen and Stubsgaard 1987). Seed burners tend to cauterize the breach in the seed-coat and kill surface contaminants.

Recalcitrant seeds, with their high moisture contents, are potentially very susceptible to damage during handling, but they seldom are subjected to rigorous cleaning or conditioning procedures. Furthermore, the most important group of recalcitrant species in North America—the oaks (*Quercus* L.)—have single-seeded fruits with rather strong outer covering structures and rather well-protected embryonic axes. Silver maple (*Acer saccharinum* L.), on the other hand, has recalcitrant seeds with a large embryo that is protected by a soft and pliable pericarp and is very susceptible to bruising during seed handling.

### Storage Environment

Storage environment is obviously very important in extending the life of seeds. The general objective is to reduce the metabolism of the seeds as much as possible without damaging them and to prevent attack by microorganisms. The ideal metabolic rate in storage will conserve as much of the stored food reserves in the seeds as possible, yet operate at a level that maintains the integrity of the embryos.

**Moisture.** Seed moisture is the most important factor in maintaining viability during storage; it is the primary control of all activities (table 5). Metabolic rates can be minimized by keeping seeds in a dry state. For true orthodox and sub-orthodox seeds, optimum moisture contents for storage are 5 to 10%. The normal practice with all orthodox tree seeds is to dry them to these levels and store them in moisture-proof containers that maintain them at these levels. Moisture in seeds (or any objects) will come to an equilibrium with the moisture in the storage atmosphere based on the differences in the vapor pressures and the chemical nature of the seeds (table 6). Proteins are the most hygroscopic, followed by carbohydrates, then lipids. These differences are reflected in the equilibrium moisture contents (the seed moisture content when equilibrium is reached) of various seeds (figure 1). Starchy seeds have higher equilibrium moisture contents than fatty seeds. For this reason, seed managers should know the dominant chemical constituents of the seeds they are storing.

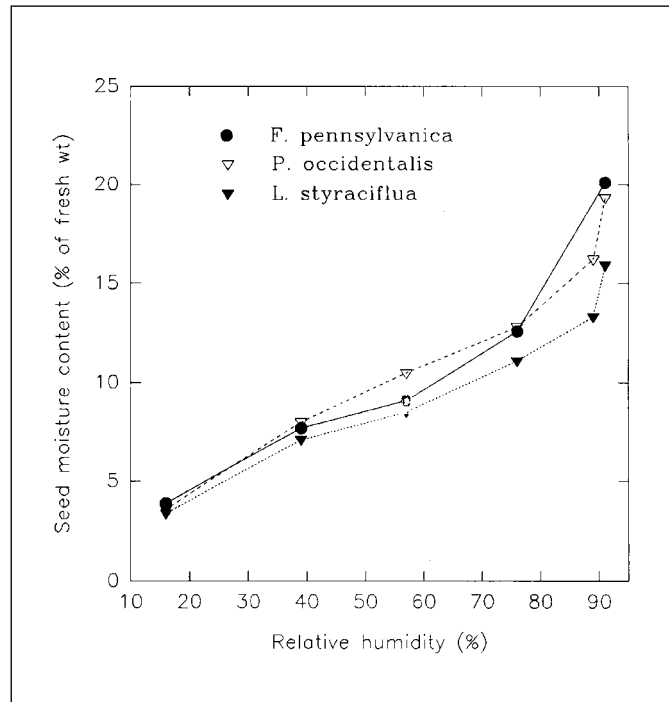
Recalcitrant seeds equilibrate in a similar fashion, but their naturally high moisture contents and rapid metabolism

make it difficult for a true equilibrium to be reached with the atmospheric moisture. The large differences in chemical makeup of various species of oak lead to large differences in their equilibrium values (figure 2). Because all recalcitrant seeds are stored at high moisture contents, their equilibrium moisture contents are not as important in seed storage management as they are for orthodox seeds.

**Temperature.** Metabolic rates can also be minimized with low temperatures, both for orthodox and for recalcitrant seeds. The storage moisture content determines just how low temperatures can be set for seed storage. From freezing to  $-15^{\circ}\text{C}$ , 20% is the approximate upper moisture limit. Below  $-15^{\circ}\text{C}$ , the limit is about 15%; and in cryogenic storage in liquid nitrogen ( $-196^{\circ}\text{C}$ ), 13% is the limit. Therefore, true orthodox seeds maintained at moisture levels of 5 to 10% can be safely stored at just about any temperature. The longevity of orthodox tree seeds in liquid nitrogen is really not known, but short tests with several species suggest that they can survive for long periods just like orthodox agricultural seeds (table 7). It is not known if sub-orthodox seeds

have this same tolerance of low temperatures, but it is known that they can be stored for a few years at temperatures as low as  $-20^{\circ}\text{C}$  (table 2).

**Figure 1**—Chapter 4, Storage of Seeds: equilibrium moisture contents at  $25^{\circ}\text{C}$  for 3 orthodox species: American sycamore (*Platanus occidentalis* L.) has the lowest lipid content; sweetgum (*Liquidambar styraciflua* L.) the highest (from Bonner and others 1994; Bonner 1981).



**Table 5**—Chapter 4, Storage of Seeds: potential moisture damage thresholds

Moisture content (%)	Potential effect
> 30	Germination can occur
10–18	Active fungal growth
< 8–9	Insect activity reduced
5–8	Best range for sealed storage
< 5	Desiccation injury possible in some species

**Table 6**—Chapter 4, Storage of Seeds: equilibrium moisture content at 4 to  $5^{\circ}\text{C}$  and 3 relative humidities for some seeds

Species	20% RH	45% RH	95% RH
<b>Trees with orthodox seeds</b>			
<i>Carya ovata</i> (P. Mill.) K. Koch	—	10	15
<i>Juglans nigra</i> L.	—	11	20
<i>Liquidambar styraciflua</i> L.	—	8	20
<i>Liriodendron tulipifera</i> L.	—	10	19
<i>Picea abies</i> (L.) Karst.	6	8	—
<i>Pinus sylvestris</i> L.	6	8	—
<i>P. taeda</i> L.	—	10	17
<i>Prunus serotina</i> Ehrh.	—	9	17
<b>Crops with orthodox seeds</b>			
<i>Glycine max</i> (L.) Merr.	6	8	19
<i>Zea mays</i> L.	8	12	20
<b>Trees with recalcitrant seeds</b>			
<i>Quercus alba</i> L.	—	37	50
<i>Q. nigra</i> L.	—	17	29
<i>Shorea robusta</i> Gaertner f.	—	—	35

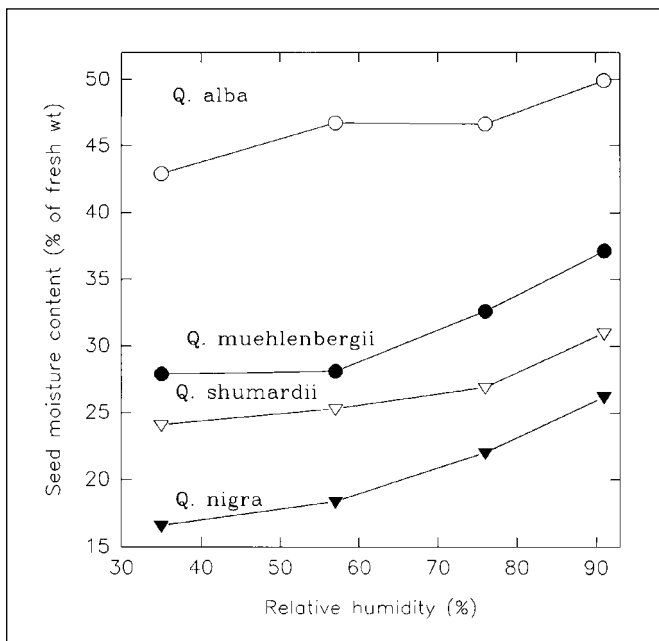
Sources: Bonner (1981), Bass (1978).

**Table 7**—Chapter 4, Storage of Seeds: cryogenic storage test results for some forest tree seeds

Species	Seed moisture (%)	Period (days)	Viability loss (%)
<i>Abies alba</i> P. Mill.	—	6	5
<i>Fagus sylvatica</i> L.	—	6	total
<i>Larix decidua</i> P. Mill.	—	6	5
<i>Picea abies</i> (L.) Karst.	—	6	1
<i>Pinus sylvestris</i> L.	—	6	0
<i>Populus tremula</i> H P. <i>tremuloides</i>	—	6	1
<i>Pinus echinata</i> P. Mill.	—	112	0
<i>Ulmus pumila</i> L.	—	112	0
<i>Abies concolor</i> (Gard. & Glend.) Lindl. ex Hildebr.	<13	180	0
<i>Pinus ponderosa</i> P. & C. Lawson	<13	180	0

Source: Bonner (1990).

**Figure 2**—Chapter 4, Storage of Seeds: equilibrium moisture contents at 25 °C for 4 recalcitrant oak (*Quercus* L.) species. White oak (*Q. alba* L.) has the lowest lipid content; water oak (*Q. nigra* L.) the highest (adapted from Bonner and others 1994).



If seeds have impermeable seedcoats that will inhibit the uptake of moisture and oxygen from the surrounding atmosphere, they can be stored for a number of years at room temperature. The primary examples of such storage come from seeds of the *Leguminosae* (Bonner 1990).

Recalcitrant seeds require different conditions. Temperate recalcitrant seeds can be stored at or just below freezing ( $-3$  °C) (table 3), but lower temperatures for just a few months will kill them (Bonner 1973), apparently due to intracellular ice formation. The lethal exposures for temperate-recalcitrant seeds are poorly defined and appear to be a

function of both temperature and length of exposure. On at least one occasion, sub-freezing temperatures for a week killed all *Quercus* acorns that were on the ground or still on the trees in central Louisiana. On the other hand, exposure to sub-freezing temperatures for 3 days on the ground, with a minimum of around  $-10$  °C at night, did not kill acorns of *Quercus pagoda* Raf. in Mississippi (Bonner 1992). This question will require research to provide a satisfactory answer.

Tropical-recalcitrant seeds have a much higher lethal minimum temperature than temperate species. Chilling damage and death will occur below 12 to 20 °C, depending on the species. Among the species included in this book, only certain *Araucaria* Juss. can be considered as tropical-recalcitrant species. Because there are no ice crystals formed at these temperatures, the chilling damage in these seeds must have a different physiological basis than damage in temperate-recalcitrant seeds.

A number of conifer species can be partially redried after stratification and returned to storage when planting is delayed. Seed moisture contents may be over 20% in such cases, so subfreezing temperatures cannot be used. Good results have been obtained by storing stratified seeds of ponderosa pine and Douglas-fir with seed moisture contents of around 26% at 2 °C for 9 months (Danielson and Tanaka 1978).

**Atmosphere.** Reduction of oxygen levels will slow metabolism and increase longevity of seeds, but it is not practical to regulate this factor precisely in operational storage situations. In past years, seeds of *Populus* L. species were often stored in vacuum desiccators to extend storage life; the beneficial effect in this case was reduction of oxygen for metabolism. (Proper drying and refrigeration have replaced vacuum storage for *Populus* now.) Recalcitrant



seeds, with their active metabolisms, require oxygen to such a degree that it is quickly depleted in airtight storage containers, and the seeds die. Any recalcitrant seeds must be stored in containers that afford free access to the surrounding atmosphere.

There have been extensive trials with storage of seeds in inert gases (Justice and Bass 1978), primarily crop species, but these procedures show no long-term advantage over good standard temperature and moisture conditions. One advantage of gas manipulation may be for transport of seeds in tropical regions where refrigeration may not be available. Success of this nature has been reported for shipment of Monterey pine (*Pinus radiata* D. Don) seeds sealed in atmospheres of nitrogen or carbon dioxide (Shrestha and others 1985).

Carbon dioxide can also be used to kill insect larvae in storage. Dry orthodox seeds can be placed in atmospheres that are 60 to 80% CO<sub>2</sub> for 4 weeks at room temperature to kill larvae. If seed moisture is below 8%, there should be no damage to the seeds for at least several years (Stubsgaard 1992). If there is enough moisture in the seeds to stimulate metabolism, the seeds will absorb the CO<sub>2</sub>. In small sample bags, the absorption will collapse the bag around the seeds as if a “heat-shrink” packaging process were in use. This same condition is often observed in plastic bags of seeds in moist stratification.

## Storage Facilities

### Cold Storage

Facilities for seed storage will vary by the amount of seeds to be stored and the projected length of storage. Small seedlots—a liter (quart) or less—can be stored in household refrigerators and freezers. Larger seedlots and quantities will require a walk-in refrigerator or freezer (figure 3). These units are usually assembled from prefabricated insulated panels and can be made almost any size to fit the owner’s needs. A suggested size for a nursery operation is one large enough to hold a 5-years’ supply of seeds. The cold storage at the USDA Forest Service’s W. W. Ashe Nursery in Brooklyn, Mississippi (figure 3) has a capacity of 1,584 m<sup>3</sup> (52,800 ft<sup>3</sup>). One cubic meter will hold from 125 to 140 kg (275 to 310 lb) of seeds. Many orthodox and sub-orthodox seeds show declining germination and vigor after a few years in storage at temperatures just above freezing (Bonner 1991; Zasada and Densmore 1977), so freezers maintained at about –18 to –20 °C are preferred for any storage of sensitive species longer than 3 or 4 months. Because it would be inconvenient to have separate facilities, most users just place all orthodox species in freezers. For reasons discussed

earlier, recalcitrant species must be stored at temperatures no lower than –3 °C. It is usually convenient to store recalcitrant seeds in the same facility used for stratification and seedling storage. Short-term storage of any redried stratified seeds as noted earlier should be done here also. All of these facilities should have backup generators and safety alarms in case of power failure.

For cryogenic storage, special tanks must be employed to hold the liquid nitrogen, and special equipment is needed to maintain its level. The tanks (figure 4) in place at the USDA National Seed Storage Laboratory in Fort Collins, CO, each have a capacity of 2,600 to 5,500 samples, depending on the size of the sample container. Samples are stored in sealed glass tubes and suspended above the liquid nitrogen in its vapor (temperature approximately –150 °C).

### Containers

Orthodox seeds should be dried to safe moisture contents (5 to 10%) and stored in sealed containers that prohibit absorption of moisture from the atmosphere. The containers used most commonly for tree seeds are fiberboard drums with a thin plastic coating on the inside (figure 5). These drums are available in sizes of about 0.5 and 1.0 hl (1.5 and 3 bu); they hold approximately 25 and 50 kg (55 and 110 lb) of loblolly pine seeds. Any large, rigid container can be used, as long as it can be sealed. The best practice is to insert a polyethylene bag liner for this purpose. It is also a good idea to do this with fiberboard drums, as repeated use of the drums over a number of years will cause breaks in their interior plastic lining. Glass containers, very popular in pre-plastic days, should not be used because of the danger of breakage. If they are used, plastic bags should be inserted to hold the seeds in case the glass is broken.

**Figure 3**—Chapter 4, Storage of Seeds: a large walk-in refrigerator for seed storage.





**Figure 4**—Chapter 4, Storage of Seeds: liquid nitrogen tanks for long-term storage of seeds for germplasm conservation at the USDA National Seed Storage Laboratory, Fort Collins, Colorado.



**Figure 5**—Chapter 4, Storage of Seeds: fiberboard drums that are commonly used for storage of tree seeds.



Small seedlots can be stored in polyethylene bags or bottles (figure 6). All plastic is not the same, however; low-density polyethylene with water vapor transmission rates of 4 g/m<sup>2</sup>/day or lower at 25 °C is good for seeds (Lauridsen and others 1992). This requirement is met by polyethylene bags with a wall thickness of 0.075 to 0.1 mm (3 to 4 mils). As temperature is lowered, permeability of these materials decreases (Stubsgaard 1992). The common household freezer bags in the United States meet this thickness requirement, but most sandwich bags do not. Bags thinner than 0.075 mm should not be used, because they are too permeable to moisture vapor. For recalcitrant seeds, maximum bag wall thick-

**Figure 6**—Chapter 4: Storage of Seeds, polyethylene bags and bottles that are commonly used for storage of small samples of tree seeds.



ness is 0.25 mm (10 mils); thicker plastics can limit gas exchange because they are impermeable to oxygen and carbon dioxide. There is no maximum thickness for orthodox seeds. Seeds with sharp points or appendages, such as *Fraxinus* L., *Taxodium* L.C. Rich, or *Carya* Nutt., can cause problems by piercing the bag walls and allowing moisture to enter. When storing these types of seeds, double bags can be used to reduce the problem. The same steps can be taken when emerging insect larvae from oak acorns eat holes in the bags. Information on vapor transmission rates of other packaging materials can be found in Lauridsen and others (1992).

### Moisture Control

Refrigerated storage units can be made with controlled humidity so that orthodox seeds can be stored in unsealed containers without danger of moisture absorption. At the low temperatures usually employed for tree seeds, however, this feature would be very expensive. It is much cheaper to dry the seeds and store them in sealed containers. If recalcitrant seeds are stored in the same facility as orthodox seeds, dehumidification could not be used because of desiccation damage to the recalcitrant seeds. Dehumidification is also a factor when seeds are stored in household refrigerators. Most currently manufactured refrigerators are frost-free, which means that the moisture has been removed from the inside atmosphere. In such units recalcitrant seeds will quickly become desiccated if care is not taken.

## Storage Recommendations

### Orthodox Seeds

All orthodox seeds should be stored in moisture-proof, sealed containers with seed moisture contents of 5 to 10%.

If the period of storage will be 3 years or less for true orthodox species, or 2 years or less for sub-orthodox species, temperatures of 0 to 5 °C are sufficient. For longer periods of storage for both types of orthodox species, freezers (−18 to −20 °C) should be used.

### Temperate-Recalcitrant Seeds

Temperate recalcitrant seeds should be stored with moisture contents at least as high as that present when the mature seeds were shed from the tree. (Refer to genus chapters in this manual for information on individual species.) This moisture level must be maintained throughout storage, which may require occasional rewetting of the seeds. Temperatures should range from 0 to 5 °C, although 1 or 2 degrees below freezing will not harm most species. Containers should be basically impermeable to moisture loss, but must allow some gas exchange with the atmosphere. Polyethylene bags with a wall thickness of 0.075 to 1.0 mm (3 to 7 mils) are suitable. Some oak acorns can be stored for 3 years in this fashion (table 3), but some viability will be lost. For other recalcitrant species, few data are available.

### Tropical-Recalcitrant Seeds

Storage of tropical recalcitrant seeds is done in the same manner as storage of temperate species, except that temperatures must be kept at a high level. There are differences among species but the lower limits are generally 12 to 20 °C. Successful storage for more than 1 year should not be expected.

### Cryogenic Storage

For long-term germplasm conservation programs, true orthodox and sub-orthodox seeds can be dried to moisture contents of 5 to 10% and stored in liquid nitrogen. Such programs require special equipment and procedures and are beyond the scope of this book.

## Other Management Considerations

The first step in planning for seed storage facilities or programs is to consider the objectives of storage. If storage is only needed for periods of 6 to 30 months, freezers may not be needed. If storage will be for longer periods, then at least some freezer capacity will be needed. If recalcitrant seeds will make up the bulk of the stored materials, then freezers will not be needed. If seeds and seedlings will be stored in the same facility, then space requirements will be very large. Overestimating storage needs can be a problem, but underestimating them is an even bigger one.

Germination should be retested on seedlots that will be stored for more than 5 years. After the initial test, tests should be carried out after 3 years and every fifth year thereafter. Seed vigor will decline before germination percentage (Hampton and TeKrony 1995), so tests on stored seedlots should include some measure of vigor or germination rate (see chapter 5). When total germination has declined 15% from its original level, plans should be made to use the seeds as soon as possible.

In long-term storage for germplasm conservation, genetic damage or shifts are always a consideration. A few studies have demonstrated some chromosome damage during storage of tree seeds of the following species: *Fraxinus americana* L. (Villiers 1974), *Pinus echinata* P. Mill (Barnett and Vozzo 1985), and *P. sylvestris* L. (Simak 1966). There is no evidence yet, however, that these aberrations cause damage that is transmitted to the next generation. While more research should be done on this question, especially in seeds stored cryogenically, there seems to be no cause for alarm.

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