Guide to handling of tropical and subtropical forest seed

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SEED STORAGE

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Chapter 3: Planning and Preparation of Seed Collections
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SEED STORAGE

8.1 Introduction

The main purpose of traditional seed storage is to secure the supply of good quality seed for a planting programme whenever needed. If sowing time follows immediately after seed collection and processing, seeds can go directly from the processing unit to the nursery, and storage is not needed. This is, however, rarely the case. In seasonal climates with a relatively short planting season, sowing time is normally determined by the wish to have plantable size seedlings at the beginning of the planting season. Hence, seeds must often be stored during the period from harvest to sowing. That is short term storage of less than a year.

Many species produce seed (or good seed crops) at long intervals, ranging from a few years to many years. To assure seed supply during the period between two good seed crops, a seed stock should be established (Wang 1975). Even where fruiting is regular and abundant every year, it may be more cost efficient to collect surplus seed to cover several years' supply rather than to undertake collection every year.

Hence, a seed store serves as a buffer between demand and production and has a regular turnover: seeds are stored during periods of seed availability and shipped to nurseries or other recipients when required to raise plants. A new type of seed store has arisen during the last few decades, *viz.* stores for conservation of genetic resources. In these so-called gene banks, seeds (and sometimes other propagation material) are stored for long periods at very low moisture content and temperature (cryopreservation). The techniques applied for storage at ultra low temperatures are quite different from conventional seed storage. Being outside the scope of operational forestry they will only be mentioned briefly in this chapter.

The quantity of seed of a particular species that must be stored in a conventional seed store is based on (i) the demand for seed of that species within the area for which the seed depot provides seed, i.e. local, domestic and/or for export, and (ii) the interval between times when (good quality) seeds can be collected. Storage is thus closely connected to planning of seed collection as outlined in chapter 3. The period of storage is often limited by the technical and physiological storage potential, i.e. the length of time seeds of a particular species will survive under the available storage conditions. To maintain viability over a prolonged period it is important that the optimal storage environment for the species is met, as far as possible. However, even under the best storage conditions, some species will only survive a very short time. Deterioration may be delayed by adopting the best possible storage conditions, but long term storage is not possible for those species.

This chapter initially deals with the physiological nature of seed storage, then outlines practical guidelines for maintaining high viability during storage. Eventually, practical aspects on seed turnover and seed store records are discussed in relation to seed store management.

8.2 Ecophysiological Role of Storage

Normally seeds go through a physiologically inactive (quiescent) period from their dispersal until they can germinate. An exception is viviparous seed, e.g. seeds of several mangrove species, which often germinate before they are released from the mother tree. For other seeds, the interlude between dispersal and germination depends on the deposit site (whether favourable to germination or not) and on possible dormancy. If the deposit site is unfavourable to germination, seeds may either die or stay dormant for a period, waiting for conditions to improve. The length of time seeds can stay viable in the natural environment depends on the seeds themselves and the conditions around them. Some seed types do not have the ability to stay alive for a long time. These so-called recalcitrant seeds have short physiological storability, which can only be slightly extended by storing them under controlled conditions. Orthodox seeds, on the contrary, have a long storage potential and may under favourable storage conditions build up large soil seed banks, which may stay viable for decades. Soil dwelling predators, micro-organisms and a relatively high soil temperature and humidity around the seeds are the principal factors contributing to seed deterioration in the field. Only seed with special protection, e.g. hard coated legumes, can survive long periods of storage under field conditions and build up large soil seed banks. In Australia, seeds of *Acacia suaveolens* buried in the soil were estimated to have a half-life of approx. 11 years, with approx. 4% of the seed from an individual years' production being viable after 50 years (Auld 1986). In South Africa, up to 50,000 seeds of *Acacia saligna* were found accumulating in the soil under a parent tree. This is equivalent to the total production in 5 years (Holmes *et al.* 1987)[1]. Although most non-hard-coated orthodox seeds easily deteriorate under natural conditions, they have a long storage potential and can often maintain viability for many years when stored under optimal conditions (Willan 1985, Doran *et al.* 1983).

8.3 Classification of Seed Storage Potential

A large variation in storability is encountered between species. In seed handling terminology, seeds have traditionally been grouped into two main groups according to their physiological storage potential *viz.* recalcitrant and orthodox seed (Roberts 1973a). Orthodox seed encompasses seed that can be dried to low (2-5%) moisture content and can, with low moisture content, be stored at low

[1] An extensive review of soil seed banks is given in Leck et al. (1989).

temperature. Viability is prolonged in a predictable manner by such moisture reduction and reduction in storage temperature. Seeds of recalcitrant species maintain high moisture content at maturity (often $>$ 30-50%) and are sensitive to desiccation below 12-30%, depending on species. They have a short storage potential and rapidly lose viability under any kind of storage conditions. A number of other characteristics of the two groups are listed in table 8.2.

Although the terms 'orthodox' and 'recalcitrant' are relatively well established, storage physiology of seeds seems to cover a more or less continuous spectrum, ranging from extremely recalcitrant, which lose viability in few days, to extremely orthodox the viability of which under optimal conditions counts in decades or centuries (Farrant *et al.* 1988). Recalcitrant seeds vary with regard to temperature; tropical recalcitrant seeds are normally sensitive to low temperature, whereas temperate recalcitrant seeds can be stored at temperatures slightly above freezing. This climatic distinction is, however, not always valid. For example, in Kenya recalcitrant species of *Cordia* and *Vitex* tolerate storage temperatures of +2°C (Schaefer 1991), and Bonner (1996b) reports low temperature tolerance in recalcitrant subtropical *Citrus* spp.

A group of species which can be dried to a moisture content low enough to qualify as orthodox, but are sensitive to low temperatures typical for orthodox seeds has recently been termed 'intermediate' (Ellis *et al.* 1990). An example of such a species is *Swietenia macrophylla*. Other examples are given in section 8.6.2. Further transition groups within the main classes, sometimes termed sub-orthodox and sub-recalcitrant, demonstrate the continuum in the range of storage behaviour. For example, orthodox seeds generally respond to reduced moisture content with extended viability (within the normal range of m.c. with an approximately doubled storage life for every 1% reduction of m.c. (Harrington 1972)). That holds for moisture reduction down to 4-5%, depending on species. Further desiccation does not increase storability but the seeds are generally not adversely affected by a lower m.c. as long as they are humified before imbibition. However, some orthodox species do not tolerate moisture content below a certain minimum, regardless of storage temperature. Dickie and Smith (1995) found that critical moisture content, below which viability was impaired, was 5% and 7% for *Agathis australis* and *A. macrophylla* respectively. These species were classified 'sub-orthodox'.

	Orthodox seed	Intermediate seed	Temperate recalcitrant seed	Tropical recalcitrant seed
Storage moisture content	Low	Low	High	High
Storage temperature	Low	High	Low	High

siological storage classes as related to temperature and moisture content

	Orthodox	Recalcitrant
Natural occurrence	Dominating strategy in arid and semi-arid environments, and pioneers in humid climates. Also prevalent in temperate and tropical high altitude species.	Prevalent in warm humid climates, especially climax forest species of tropical rain forests and mangroves. Also some temperate and few dry zone species.
Families and genera where the particular storage behaviour prevails	Myrtaceae, Leguminosae, Pinaceae, Casuarinaceae.	Dipterocarpaceae, Rhizophoraceae, Meliaceae, *Artocarpus*, *Araucaria*, *Triplochiton*, *Agathis*, *Syzygium*, *Quercus*.
Seed moisture content and temperature during storage	Tolerant to desiccation and low temperatures. Conventional storing 5-7% m.c. and 0-5°C. Cryopreservation 2-4% m.c. and -15°C to -20°C.	Intolerant to desiccation and low temperatures (except some temperate recalcitrant species). Tolerance level dependent on species, normally min. 20-35% m.c. and 12-15°C for tropical species.
Potential storage period	With optimal storage conditions several years for most species; for some several decades.	From a few days for extremely recalcitrant species to several months for more tolerant ones.
Seed characters	Small to medium size seed, often with hard seed-coat.	Usually medium to large size and heavy seeds; this partly attributed to a high m.c.
Maturation characters	Accumulation of dry weight ceases before maturation. Decline of moisture content typically to 6-10% at maturity. Little variation between individual seeds.	Accumulation of dry weight up to the time of seed dispersal. Little or no maturation drying, m.c. at maturity 30-70% with large variation between individual seeds.
Dormancy	Dormancy often occurs.	Dormancy absent or weak. Maturation and germination often more or less continuous.
Metabolism at maturity	Not metabolically active when shed.	Metabolic when shed.

Table 8.2. Some features of orthodox and recalcitrant seed

species. Few recalcitrant species are found in dry areas in the tropics; exceptions are *Dobera glabra* and *Azadirachta indica* (Schaefer 1991), although the latter shows a more orthodox behaviour towards the dry zone (see below). The members of Leguminosae occupy mainly dry areas or pioneer niches in the humid tropics. They are, albeit with few exceptions, accordingly almost exclusively orthodox.

8.4 Factors Affecting Seed Longevity

The period seeds will remain viable in store (their longevity) is determined by their genetic and physiological storage potential and by any deteriorating events or damage prior to or during storage, as well as by the interaction between individual factors.

1. Genetic. Storage potential is heritable. Species and sometimes genera typically show an inherited storage behaviour, which may be either orthodox or recalcitrant. Accordingly, each species is likely to respond identically to a given set of storage conditions (Bonner *et al.* 1994). Large genetic variation may, however, occur within species, sometimes ranging from orthodox to recalcitrant, more often expressed as different longevity of seeds from different provenances, individuals or clones when stored under similar conditions. Roberts and Ellis (1977) suggest that within a species there may be at least a 7 fold genotypic variation in seed longevity. Genetic variation within species may occur on different levels, e.g. land races, provenances, individuals and clones.

Large variations have been found in neem (*Azadirachta indica*) throughout its range of distribution. Neem is indigenous to South-East Asia (India, Thailand and Burma) where it grows under relatively humid conditions. The species has been introduced into many dryer areas of Asia, Africa and Latin America. It has been shown that indigenous SE Asian provenances are more desiccation sensitive (recalcitrant) than many African and Latin American land races (Lauridsen and Souvannavong 1993). Significant difference in storability between different genotypes has also been shown in neem. Seeds from 10 different mother trees from East Kenyan provenances showed a decline in viability after 4 months from 78 → 60% for the most orthodox seed lot, and from 79 → 4% for the most sensitive seeds (Oloo *et al.* 1996). On provenance level, Emmanuel and Dharmaswamy (1991) found large variation in storability of Indian teak (Tectona grandis). Finally, significant clonal variation in storability was found between six clones of Picea sitchensis exposed to various lengths of accelerated ageing (Chaisurisri et al. 1993).

Genetic influence on storability may be directly related to progressive ageing, or it may be indirect, ascribed to different susceptibility to factors, which may ultimately lead to loss of viability. For example, inherited variation in seed-coat morphology may cause variation in susceptibility to physical damage during processing, which in turn may influence storability. In herbaceous cowpea (Vigna unguiculata) genetic variation in relative susceptibility to attack by bruchid beetles has been revealed (Adjadi et al. 1985). Variation of that kind is also likely to occur in leguminous tree crops. Whether the bruchids remain active during storage (storage at ambient temperature) or the attack ceases (e.g. cold storage, CO_2 or insecticide treatment) the genetic difference in susceptibility may be reflected in different longevity, albeit in the latter case only via pre-storage deterioration (see below).

2. Developmental. Immature seeds generally have a shorter storability than seeds picked at full maturity (Seeber and Agpaoa 1976). However, early-collected seeds may be able to attain full maturity, including normal storability, if allowed to after-ripen as described in chapter 6. Possible reduction in storability thus depends on the stage of development at collection plus possible after-ripening. The physiological cause of reduced storability may be ascribed to failure of accomplishing essential stages of late maturation events, e.g. incomplete embryo development, inadequate protection from desiccation, or inadequate formation of storage proteins or chemical compounds necessary for storability (Hong and Ellis 1990). For example, in *Taxus brevifolia* the embryo grows in size right up to the stage of full maturity, and only fully mature seeds tolerate desiccation to a level necessary for storage (Vertucci *et al.* 1996).

Developmental stage is especially evident and important in recalcitrant seed. Firstly because dry weight continues to accumulate up to the time of seed maturity, so that seeds collected just before natural shedding may be underdeveloped. Secondly because the processes of maturation and germination are more or less continuous. If germination does not occur, deterioration proceeds rapidly, making late collection equally unsuitable (Berjak and Pammenter 1996).

Stage of development interacts with environmental factors before and after storage. For example, immature seeds tend to be more prone to processing damage (mechanical or heat) and may be more susceptible to infection.

3. Environment. For practical purposes, environmental factors can be grouped into those acting before and those acting during storage.

Pre-storage deterioration is of paramount importance for seed longevity because it influences the initial viability. Optimal storage conditions can only maintain viability, never improve it (Delouche and Baskin 1973). If viability has been reduced from say 95 → 70% prior to storage, even the best storage conditions cannot bring it back to 95%. Seed deterioration may start already in the field and is influenced by handling from collection and transport through processing. Any minor damage which occurs during handling may adversely affect storability. As recalled from chapter 6, most small injuries do not cause an immediate loss in viability, but may affect long term storability (Moore 1972). Possible damage leading to reduced storability has been discussed in relation to the individual seed-handling procedures in chapters 4, 5, 6 and 7.

Pre-storage may strongly influence the response to storage conditions. E.g. according to Delouche *et al.* (1973): 'Vigorous, high quality seed of most species store surprisingly well even under relatively adverse conditions, while badly deteriorated seeds store poorly even though conditions are quite favourable'. On the other hand, the effect or degree of effect of pre-storage injuries is not always expressed but influenced by the storage environment. For example, minor damage to the seed-coat that may serve as entry points for fungal attack is obviously only of importance under storage conditions where fungi are active (> 5-7% m.c.).

Loss of viability during storage can be caused instantly by insect or fungal attack or by progressive natural deterioration (ageing), the nature of which is described in section 8.5. Any of these events are influenced by the storage environment. Temperature and humidity are the most important factors in seed storage. Non-dormant seeds may germinate if their moisture content is above 30%. Rapid deterioration by micro-organisms can occur if moisture content is 18-30%, and seeds with a moisture content above 18-20% respire and metabolise actively. Metabolising seeds may be damaged by accumulation of toxic metabolites or heat if improperly ventilated. Certain seed insects are active at a moisture content of less than 10%, and damage by fungi may occur down to 4-5% (Bewley and Black 1994). For all the above reasons, it follows that the higher the storage moisture content, the more rapid the deterioration of the seed will be.

The above events are all closely connected with temperature, and the most rapid deterioration occurs at high temperature and humidity. Conversely, reduction of one, either temperature or moisture content, reduces deterioration. As a rule of thumb for orthodox seed, it has been suggested that a 1% reduction in moisture content or a 5.6°C reduction in temperature tends to double the seed longevity in storage (Harrington 1972).

4. Initial viability. Seed lots with high initial viability also have a higher longevity in storage than seed with low initial viability. The progression of natural ageing with resultant loss of viability is not linear over time but typically follows a sigmoid pattern (fig. 8.1). Loss of viability is initially slow, followed by a period of rapid decline. The higher the viability when the seed lot enters into storage, the longer the seed will keep viable under a given storage environment. For example, a seed lot with an initial viability of 100% may take several years to lose 50% of its viability in storage, while the same seed lot having deteriorated during a few weeks of sub-optimal conditions to say 80%, may reach 50% viability in much shorter time. The different rate of loss of viability during the storage period emphasises the importance of storage at the best conditions available as soon as possible after collection. That becomes especially important for species that rapidly lose viability at e.g. ambient temperature but respond greatly to improved (e.g. cold) storage conditions.

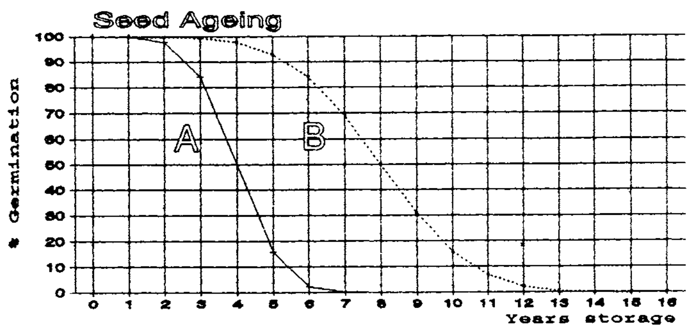


Figure 8.1. Survival or viability curves indicating decline in percentage of viable seeds in a seed lot during a storage period. Survival curves of seed lots of a given species tend to follow the same pattern under a given set of storage conditions. Hence curve A and B could be two species under similar storage conditions, or two seed lots of the same species exposed to different storage conditions. Compare fig. 8.4.

8.5 Seed Ageing

8.5.1 Physiology of stored seed

Metabolic activity requires available water. As orthodox seeds dry during maturity and later on during processing, the available free water is lost. The little water left in the seeds (e.g. 4-6% depending on desiccation rate) is 'bound' to macromolecules, i.e. it is immobile and does not enter into chemical reactions. Respiration ceases when moisture content has been lowered to below 18-20% (Bewley and Black 1982). Hence, in dry seeds there is practically no metabolism; the seed is alive without any measurable life manifestation (Bewley and Black 1994). In desiccation sensitive (recalcitrant) seeds moisture content is always high, and the seeds are concurrently metabolically active. The seeds continue to accumulate dry weight up to the time of dispersal, and germination events form a more or less continuum of the maturation process (Berjak and Pammenter 1996). Hence, recalcitrant seeds are metabolically active when shed and remain so throughout storage, but the **rate** of metabolism can usually be reduced by storing at reduced temperature and moisture content.

As long as free water is available, metabolism is strongly related to temperature. If moisture content and temperature are high, so is metabolism. Low temperature will drastically decrease metabolism but metabolic processes will still continue as long as free water is available. Even when moisture content has declined below the level where metabolic activities have ceased, both temperature and moisture content continue to influence seed longevity in storage through the ageing processes (see section 8.5.2).

8.5.2 Physiological changes during ageing

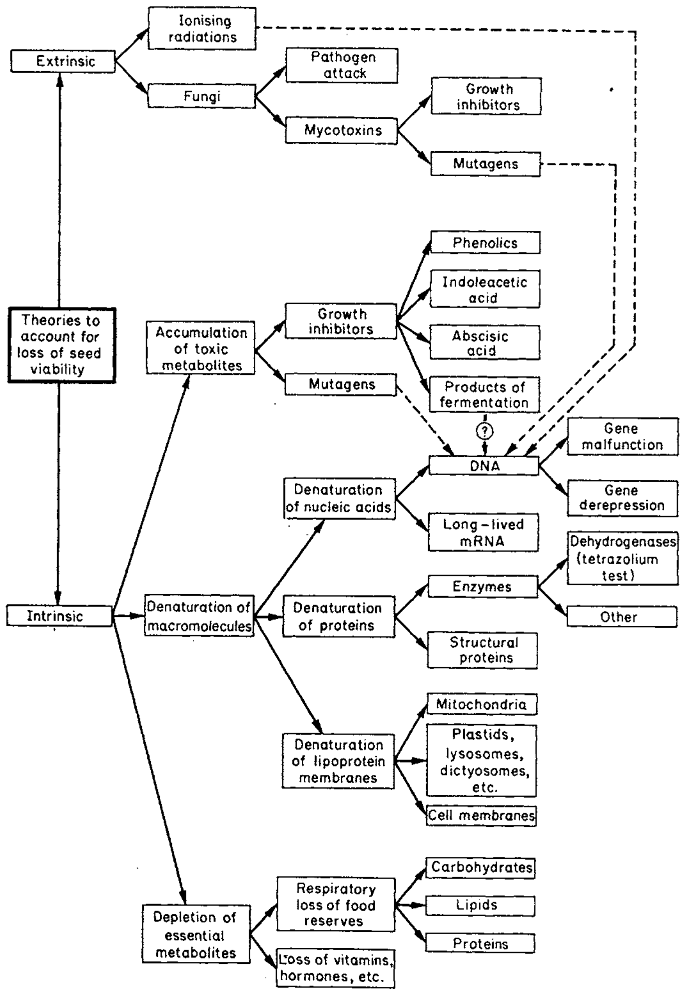
No matter how optimal storage conditions are, seeds will sooner or later die. Ageing denotes the progression of deteriorating events that take place within the seed and which ultimately lead to the death of the seed. The term 'progression' suggests that ageing takes place over a prolonged period, during which cytological and biochemical deterioration accumulate. Ageing does, accordingly, not include momentary loss of viability due to an instant damage, e.g. by temperature or mechanical impact (cf. chapter 6.9). Insect predation is also not considered as ageing, while fungal infection, being a more progressive process of deterioration, may be part of the ageing process (Roberts 1972); the distinction here is not clear. Causes of physiological ageing may be grouped into extrinsic factors, which are external factors influencing viability, and intrinsic factors, where the ageing is a result of events within the seed only. A summary of the two types of factors appears in figure 8.2. It should be noted that all extrinsic factors plus e.g. accumulation of toxic metabolites are of no or negligible importance in cold, dry storage of orthodox seeds. Discussion of the individual factors of ageing is outside the scope of this book. Interested readers are referred to e.g. Roberts (1972), Bewley and Black (1982), Heydecker (1972), Wilson and McDonald (1986), Berjak and Villiers (1972a,b,c, and d).

Seed deterioration starts immediately after maturity, but it only influences viability when progressed to an advanced stage because seeds are capable of repairing damage that has not reached a critical point (Berjak and Villiers 1972a). Minor damages may thus be reversible or may only be manifested if the seeds germinate under sub-optimal conditions (see discussion of viability-vigour below).

Ageing events can be slowed down by appropriate storage. Temperature and moisture content are the two major factors determining the rate of ageing. Oxygen pressure and light may have some influence on ageing in some instances. Their relation to ageing is as follows:

Temperature. Biochemical processes are generally slowed down at low temperature; the lower the temperature, the slower the process. That also includes processes leading to deterioration. Further, low temperature (< 8-10°C) inactivates most seed insects and storage fungi.

Figure 8.2.
Summary of factors and events of deterioration leading to seed ageing. From Roberts 1972.



Moisture content. Most biochemical and cytological deterioration is most likely to take place at high moisture content. Low temperatures are harmless to orthodox seeds with a low moisture content, but if moisture content is high (> 6-8%), seeds are prone to fatal damage by ice formation when exposed to sub-zero temperature (freezing).

Oxygen pressure. Denaturation of cell constituents (membranes, enzymes, DNA) only occurs under aerobic conditions (Roberts 1972, 1973b and c). Accordingly, high oxygen pressure promotes and low pressure represses denaturation of these constituents. Storage under low oxygen pressure, e.g. in vacuum or in CO_2 (see section 8.6) at temperatures where insects, fungi and micro-organisms are active prevents their development. Seeds stored at high moisture content (e.g. recalcitrant) do not tolerate low oxygen pressure because oxygen is necessary for respiration to sustain and for repair and turnover processes within cells (Roberts 1983).

Light. Ionising radiation has been mentioned as a factor influencing seed ageing in nature (Roberts 1972). For dry orthodox seeds there is, however, little evidence that light conditions play any role in seed longevity. For species with photo-dormancy (where light is necessary for breaking dormancy and for germination, see chapter 9), dark storage may prevent germination at high moisture content (Vazquez-Yanes and Orozco-Segovia 1996).

The rate of ageing and ultimately loss of viability varies tremendously between individual seeds in a seed lot. As there is no non-destructive way of measuring seed viability, viability of a seed lot is always measured on a representative sample (chapter 11). The decline of viability typically follows the pattern of fig 8.1. If the viability declines from 100% to say 99% in 3 months, and is 1% after 5 years, it means that the longevity of 1% of the seed lot was only three months, while another 1% remained viable for 5 years, a vast yet frequent pattern of variation in a seed lot.

8.5.3 Viability and vigour

Before progressive ageing ultimately leads to the death of the seed, the accumulated deterioration is likely to affect the potential life processes of the germinating seed. The seed is said to have reduced vigour. Vigour or reduced vigour can be observed on the performance of the individual seed or seedling as related to:

1. Germination speed
2. Seedling establishment
3. Resistance to stress.

Aged seeds with reduced vigour typically germinate slowly, produce small or abnormal seedlings, and have little resistance to stress. In relation to the latter, ageing is generally most obvious when seeds germinate and seedlings establish under stressed conditions (Heydecker 1972). Hence, an aged seed lot that shows a relatively high germination under optimal conditions (e.g. a germination test) may germinate poorly under sub-optimal field conditions, whereas the difference may be much smaller for a fresh seed lot. See also discussion of vigour testing, section 11.7.1.

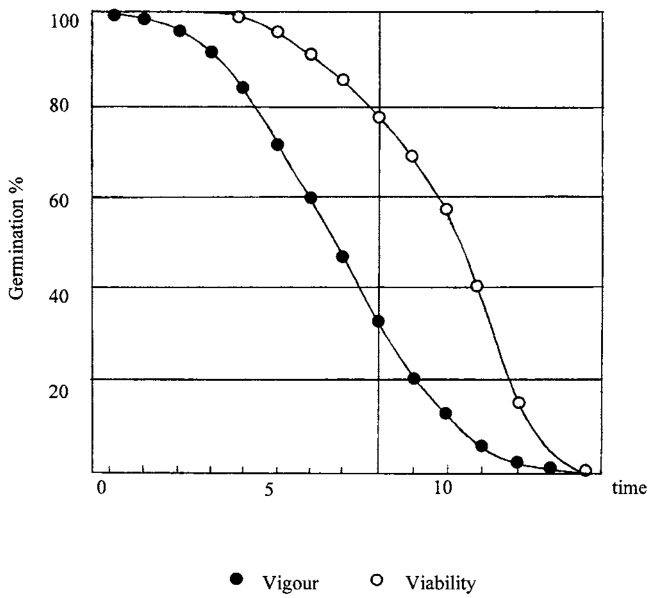
As ageing progresses, the seed loses its ability to germinate altogether, i.e. it loses its viability. Loss of vigour hence foreruns loss of viability. Viability is an either/or character, and can only be measured on a batch of seed, i.e. as the number of seeds that will germinate. Vigour is a continuous character observed on the individual performance. If the two features are to be compared directly, a germination test may be carried out under stressed conditions. The theoretical relation between vigour and viability is expressed in figure 8.3.

Reduction of seed vigour as preceding reduction of viability has two practical implications:

1. Seeds with a relatively high, yet reduced, viability under test conditions (e.g. 80%) may show poor germination and performance when grown under field conditions.

2. Seed lots in which the viability under test conditions is <50% may show deterioration beyond recovery for most of its seed. Therefore, seeds with very low viability should generally be discarded and not used for planting programmes.

Figure 8.3.
Relationship between viability and vigour as expressed by germination percentage. The time scale indicates the storage period (months or years) during which progressive deterioration occurs. The two curves may represent the same seed lot germinated under different conditions:
Under optimal germination conditions, the germination percentage will decline after a progressive storage period following the viability curve.
Under stressed germination conditions a much more rapid decline in germination appears indicating loss of vigour.
While only small differences in germination under optimal and stressed conditions can be observed during the first period of storage, the difference becomes very large later. After e.g. 8 (months) of storage 80% germinate under optimal conditions while, only 30% germinate under stressed conditions.



While the lifetime of an individual seed in a seed lot cannot be predicted, a seed lot usually displays a characteristic pattern as shown in fig. 8.1. The percentage of seed deaths per unit time through the total life span of the seed lot depends on species and storage conditions. Change in storage conditions appears on the survival curve as different steepness of the curve. Seed lots with different storage quality, e.g. due to different stage of maturity or damage during processing, will have different survival curves.

Seed lots of the same species with similar initial quality tend to show the same pattern of decline in viability over the storage period (i.e. similar viability curve) under the same set of storage conditions. Different storage conditions usually alter the viability curves: the poorer the storage condition, the steeper the slope of the curve. A situation with three different storage conditions is illustrated in fig. 8.4.

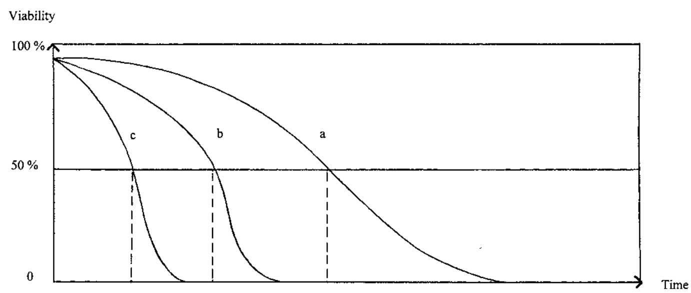


Figure 8.4.
Viability curves for three different storage conditions. 'a' represents the best, 'b' and 'c' less favourable storage conditions. Mean viability periods are found on the time scale by noting the distance from zero to the points where viability curves intersect with 50% viability level.

A seed lot which has aged before storage e.g. due to slow processing or temporary storage at sub-optimal storage conditions, might have entered the period of rapid decline on the survival curve. Even if it is stored under optimal conditions, it will lose viability fast. This illustrates the importance of fast processing and transference into the best possible storage, which is especially important for seeds that quickly lose viability under sub-optimal storage conditions.

Viability curves can be used for comparing different storage conditions or different seed lots under similar storage conditions. Further developed, viability curves can be used for predicting the storage life of a seed lot (see appendix A8.1)

8.6 Storage Conditions

Storage conditions should be designed to prolong the viability of seeds by reducing or limiting any factor that impairs viability. The general storage conditions should therefore aim at (1) reducing the metabolism of seeds, (2) keeping insects, fungi and other pathogens away, and (3) reducing general seed ageing. The general prescriptions for seed storage are summarized below.

- Store seeds at lowest possible temperature that will not damage the seeds
- Store seeds with lowest possible moisture content that will not damage the seeds
- Eliminate as many pathogens as possible before storage
- Protect seeds from pathogens during storage
- Store in the dark
- Store orthodox and intermediate seeds with low moisture content in airtight containers
- Store recalcitrant seeds in material permeable to gases but with retention of moisture

The storage conditions differ significantly between the two main groups, orthodox and recalcitrant seeds, and the two groups are considered separately.

8.6.1 Storage of orthodox seeds

Orthodox seeds are generally easy to store if basic processing and storage facilities are available. Most orthodox seeds will maintain a high viability under ambient temperature conditions, at least from harvest to first subsequent sowing season, if the seeds have been thoroughly dried before storage and are stored away from insects. Many orthodox seeds maintain viability for several years under these conditions. Long term storage - and storage of more sensitive orthodox seed - often necessitates improved storage conditions, where temperature and moisture content are controlled. It should be noted that several species formerly considered short-lived or recalcitrant have been shown to have extended viability and in reality to be orthodox provided their seeds are appropriately processed before storage (King and Roberts 1979). Triplochiton scleroxylon and orthodox provenances of *Azadirachta indica* are examples of species where storability has been greatly extended by improved harvesting and processing technique (see chapter 6.8.2).

Seed moisture and air humidity

Many orthodox seeds can be stored for long periods at ambient temperature provided their moisture content is low. At high moisture content and temperature a major cause of deterioration is mould. Although some fungi may survive low temperature and m.c., their activity rapidly declines below 10% m.c. and 10°C. In an experiment with *Bambusa tulda*, seeds stored with m.c. $<10\%$ at ambient temperature maintained 50% viability after 12 months whereas all seeds stored at higher m.c. lost viability completely in less than 4 months (Thapliyal *et al.* 1991).

Orthodox seeds should be dried down to at least 5-10% m.c. At that moisture content there is practically no metabolism and little or no fungal activity. Low moisture content should be maintained throughout storage, i.e. the seeds should be prevented from re-absorbing moisture. The physical relation between atmospheric water and seed moisture is presented in appendix A5.2. During seed storage, seed moisture comes into equilibrium with the humidity of the surrounding air. It should be recalled that seeds with high oil content have much lower moisture content than seeds with low oil content and high protein or starch content.

The most effective way of preventing orthodox seeds regaining moisture is to store them in sealed, airtight containers or plastic bags. It has the following rationale: in open storage, the volume of the air is practically infinite as compared to the seed volume. Seeds may concurrently change moisture content by absorption until they are in equilibrium with the atmosphere. In closed sealed containers, the volume of the air is small as compared to the volume of the seeds. Even if the relative humidity of the air is high, absorption by the seed to reach equilibrium with the atmosphere surrounding them will not raise their moisture content significantly, simply because there is not enough moisture to absorb (Harrington 1972). The situation can be illustrated by the following example:

1 kg of pine seeds with an initial moisture content of 7% are stored in open containers in an atmosphere of 20°C and 70% RH. The seeds will absorb water until they are in equilibrium with the atmosphere, at m.c. of approx. 12%. If the same seeds are stored in sealed containers, the RH of the atmosphere will decrease concurrently with the absorption of water by the seed. The amount of water vapour at 20°C and 70% RH is approximately 10g/kg dry air. Dry air at 20°C weighs about 1.205 g/m^3. Supposing there is 0.5 litre (= 500 cm^3 or 5×10^{-4} m^3) free air above and between the seeds, this air weighs $1.205 \times (5 \times 10^{-4}) = 6.025 \times 10^{-4}$ g, which would hold a total of 6.025×10^{-6} g water. Compared to the moisture of the seeds (70g /kg at 7% m.c.) the amount of water in the air is negligible and would not raise the measurable m.c., even if it was all absorbed.

As long as storage containers are completely airtight, regaining of moisture is prevented. However, seeds may regain moisture if the sealing is not complete (e.g. permeable plastic bags or damaged rubber seals), or if the containers are frequently opened during storage. In both cases there is a risk that ambient air with high humidity replaces the air in the container with concurrent rise in seed m.c. Air humidity of the seed atmosphere may also influence seed viability, even where moisture content of the seeds does not rise drastically, through increased susceptibility to storage fungi. Since fungi develop on the surface of the seed, the high humidity may promote fungal attack even at relatively low seed moisture content.

Some practical precautions can be taken to keep humidity low during storage:

1. The initial moisture content should be low to avoid respiration (a by-product of respiration is water).
2. If small portions of seeds are likely to be removed from the storeroom frequently, seeds should be stored in smaller portions, e.g. small sealed plastic bags within a larger container. That will also facilitate the general handling since portions with definite weights (e.g. 50, 100 and 200 grams for small seeded species) can be weighed prior to storage and taken out as required.
3. Plastic bags or containers should be filled completely so that as little air as possible is stored with the seed (Boland *et al.* 1980). Vacuum packing or storing in CO_2 in polythene bags practically removes all air and makes the seed samples easy to handle.
4. If containers are not completely airtight, or if samples are frequently taken out e.g. for laboratory use, seeds may be stored with a desiccating chemical, e.g. silica gel or CaO, or in charcoal. 17 g CaO/100 g pine seeds were found suitable for long term (15 year, -4°C) storage in the Philippines (Seeber and Agpaoa 1976).

The necessity of preventing moisture absorption by airtight storage is obviously more urgent at high air humidity, e.g. ever-humid tropics or during rainy seasons. Cloth bags were thus found inferior to airtight containers during moist season storage in Nepal (Napier and Robbins 1988). As the RH increases with decreasing temperature, airtight storage is normally mandatory for cold storage. Storage in open containers at ambient temperature may be applicable for short term storage of some species, even where moisture content does increase. For example, Omram *et al.* (1989) found that seeds of *Casuarina glauca* and *C. cunninghamiana* could be stored in unsealed bags at room temperature for 8 months without significant loss of viability, albeit their m.c. increased from 5-6% to approx. 8 % during the period. The seeds lost viability in 20 months.

Temperature

Although low temperature is generally preferred in order to reduce ageing and prevent insect and fungal activity, many species can be stored at ambient temperature for long periods provided their moisture content is low and they are free from insects and mould.

Most legumes, eucalypts, and many pines and casuarinas have been successfully stored under ambient conditions for several months or years (Boland *et al.* 1980, Doran *et al.* 1983, Robbins 1983, Valera and Kageyama 1991, Turnbull and Martens 1983). Boland *et al.* (1980) state that most eucalypts can be stored up to 10 years at ambient temperature, albeit some loss of viability must be expected. Moisture content, air humidity and initial quality are of paramount importance. Since both seed insects and fungi are active under ambient temperature, special precautions may be needed, either eradication e.g. by fumigation or application of a pesticide, or both (see chapter 7).

The level of temperature at ambient conditions is obviously important; at the same moisture content a much faster decline in viability must be expected in lowland tropics with temperatures ranging 30-35°C, than in subtropical or highland conditions where ambient temperature may not exceed 20°C.

All orthodox seeds keep viability longer at low temperature, but since cold storage is expensive both in terms of establishment and operation, it may only be economical for a small portion of the total seed production, or only for long term storage (see section 8.7).

Cold storage is mandatory if the seeds are likely to lose viability at ambient temperature, i.e. for short term storage of sensitive seeds and any long term storage. In the Philippines, seeds of *Pinus merkusii* are reported to lose viability within 8 months when stored at ambient temperature. At 2°C they can be stored without significant loss in viability for up to 14 months (Seeber and Agpaoa 1976). Hence, any storage beyond a few months of this species must be under reduced temperature. At least two species of eucalypts, *E. deglupta* and *E. microtheca*, have short viability under ambient conditions and must be stored at low temperature (3-5°C) to maintain viability beyond two years (Boland *et al.* 1980). Generally, the lower the temperature the longer the viability. Most orthodox seeds maintain viability for decades under storage temperatures of -10 to -15°C. Such low temperature may, however, only be economical in special cases. Where availability of cold storage facilities is limited, only seeds that are likely to lose viability significantly during the potential storage period, are stored under cold conditions.

Atmosphere

Since orthodox seeds are preferably dried to moisture contents where they are no longer metabolically active, they do not require oxygen for their respiration. Bewley and Black (1994) found that reduction of O_2 pressure, e.g. by replacing oxygen with N_2 or CO_2 had little effect on seed longevity as long as temperature and moisture content were kept low. This is in contrast to earlier belief that reduction of O_2 level had a great influence on seed longevity (e.g. Goldbach 1979, quoted in Willan 1985). However, as seed insects and micro-organisms respire and hence need O_2 at a moisture content where the seeds themselves do not, replacement of the seed atmosphere with CO_2 is a common, effective and safe way of seed treatment (see chapter 7).

Containers

The type of storage container depends on storage conditions. If seed is stored with relatively high moisture content (e.g. > 10-12%) at ambient temperature, some metabolism may take place. Heat and water produced by respiration (whether from the seed themselves or associated organisms) must be removed by ventilation. Airtight bags or containers are therefore less applicable. Instead, seed should be stored in cloth (cotton, hessian) bags that allow some ventilation. Permeable bags may also be suitable for short term storage if air humidity is low.

Orthodox seed with low moisture content is preferably stored in airtight containers. The main purpose of airtight storage is to prevent moisture absorption of dry seeds. Sealed laminated plastic bags with minimal permeability to gases are superior to unsealed bags and thin plastic bags, especially when the seeds are stored at relatively high temperature (Thapliyal *et al.* 1991). It should be emphasised that a precondition for airtight or sealed storage is that seeds have been appropriately dried.

Glass jars, bottles, jerry cans, metal tins and drums are some of the storage containers available. These and others are listed in Appendix A8.2. The general requirement for storage containers for orthodox seeds is that they are airtight, are easy to fill and empty, have a suitable volume, can be easily cleaned, are of reasonably strong material that will not perforate or break during normal handling, and can be stored without unnecessary use of storage room.

Seed treatment

Generally, seeds are well protected from storage pests if stored under cold, dry conditions in airtight containers and especially if stored in CO_2. Several pathogens may, however, stay dormant under these conditions and become active if conditions become favourable e.g. after the seeds are removed from storage. If seed is stored under ambient temperature, fungal or insect attack may be a problem. Various seed treatment methods and their use are discussed in chapter 7.

8.6.2 Storage of recalcitrant and intermediate seeds

Although the group is very diverse, species with recalcitrant or intermediate seed exhibit a number of common traits that justify discussion of their storage as a group. The storage behaviour ranges from the extremely recalcitrant and viviparous seeds of some mangrove species to seeds that tolerate at least some desiccation. A number of features are listed in table 8.2. Those directly related to storability are restated here:

1. Desiccation sensitive. Lowest safe moisture content is 60-70% for some extremely recalcitrant species and 12-14% for some intermediate species.
2. Chilling sensitive. Injury depends on species, moisture content and possible duration of chilling. For sensitive species chilling injury may occur below 20°C. Some species are tolerant of low temperatures (2-5°C).

3. Metabolic active when shed. The feature is partly connected to the high moisture content.
4. No dormancy. Germination processes start soon after shedding, and in some cases it is a direct continuation of the maturation process.

The delicate nature of recalcitrant seeds largely limits the manipulation of storage conditions and makes the potential for storage very limited even under the best conditions. Because of the narrow range of environmental conditions in which seeds remain quiescent, i.e. without germination and without rapid deterioration, the demands on storage conditions are often more onerous than those for orthodox seeds. Seed must be stored within a narrow range of moisture and temperature conditions. Although some progress has been made especially for short term storage, and for less recalcitrant and intermediate species, the essential feature of management of recalcitrant seeds is to keep storage period to a minimum. The general desire to reduce storage by a speedy delivery from processing to nursery becomes a must for these seed. However, where storage cannot be avoided (cf. section 8.7), storage conditions must be carefully balanced between reducing metabolism by reducing temperature and moisture contents, without hampering viability by too drastic a decrease in these factors. Storage conditions should basically aim at the following (King and Roberts 1979):

- Prevent desiccation
- Control microbial contamination
- Prevent germination
- Maintain adequate oxygen supply

Moisture content and humidity

Recalcitrant and intermediate seeds have a lowest safe moisture content (LSMC) below which desiccation damage occurs. It is normally beneficial to reduce moisture content as close as possible to that level, and maintain it throughout storage. LSMC varies considerably between species as indicated in table 8.3. Intermediate seeds can be dried to 12-17% m.c. and stored at least for several months. Intermediate seeds also show an increased storability at lower temperature.

Seeds of some dipterocarps show intermediate storage behaviour. Seeds of *Dipterocarpus intricatus*, *D. alatus* and *D. tubeculatus* can be dried to 10%, 17% and 12% respectively without great damage, and have prolonged storability when moisture content is reduced in the range of 6-20% (Tompsett 1992[2]). Otherwise most dipterocarps are quite desiccation sensitive. Among the dipterocarps, *Shorea* and *Parashorea* spp. tend to be more recalcitrant (LSMC 30-40%), *Hopea*, *Cotylelobium* and *Vatica* more desiccation tolerant.

[2] These seeds are categorized 'orthodox' in the terminology of Tompsett as he does not include the term 'intermediate'

Where germination can be prevented by other means, it has in certain species proven beneficial to store seeds fully imbibed rather than at low m.c. For example, Vazquez-Yanes and Orozco-Segovia (1996) found that photo-dormant seeds of four rain forest pioneer species stored better in an imbibed state under dark conditions than at any stage of reduced m.c. Photo-dormancy is confined to pioneer regeneration strategies. As most pioneers have orthodox seeds, the method of imbibed, dark storage is probably only applicable to relatively few species.

Seeds that are extremely desiccation sensitive, non-dormant and short-lived can neither be dried nor stored imbibed, and the only way of maintaining viability is to allow germination to proceed (see last paragraph of 8.6 'Storage of germinants').

Temperature

There is a large variation in temperature tolerance, but tropical species are generally susceptible to chilling injury at low temperature, varying from $<20°C$ for some species to $<5°C$ for less sensitive species. Chilling injury is closely connected to moisture content in the sense that seeds most sensitive to low desiccation are often also those most sensitive to chilling. Germination can sometimes be impeded or reduced by lowering the temperature. For seeds stored with high m.c. a balance must be found between germination and chilling injury, i.e. seeds must be stored at a temperature low enough to avoid or slow down germination, yet high enough to prevent chilling injury. This is not always possible. In an experiment with four recalcitrant species from Thailand stored under a range of temperatures, Corbineau and Come (1988) found that only one, *Symphonia globulifera*, did not germinate at low temperature and could be stored at 15°C.

Viability of many seeds is maintained longer if the seeds are stored at constant rather than fluctuating temperatures (Seeber and Agpaoa 1976). This is at least partly related to embryonic dormancy (chapter 9). On the forest floor, where the temperature is relatively constant, seeds of pioneer species may survive in a dormant stage until a gap in the canopy creates temperature fluctuations that trigger germination. Where air conditioned rooms are not available, diurnal temperature fluctuations may be minimised by storing the seeds in cellars or other underground storerooms. In the Philippines, Jacalne (1955, quoted in Seeber and Agpaoa 1976) recommends short term storage ($<$ 4 months) of *Swietenia macrophylla* seeds in closed cans mixed with charcoal and buried 40 cm underground in a shady place.

Chilling sensitivity seems, at least to some degree, to be restricted to intact seeds: cryopreservation of excised embryos of recalcitrant seeds has shown that several species maintained viability at ultra-low temperatures (Krishnapillay and Engelmann 1996).

Table 8.3. Lowest safe moisture content (LSMC) for some recalcitrant species (abbreviated from Tompsett 1992).

Comment	Species	Source*)	LSMC	Optimum Storage %	Days	Temp °C	Germ %	MC %
(I) SHOREA	*S. leptoderma*	Tompsett (unpub.)	>42	-	-	-	--	
(a) Section Shorea	*S. robusta*	Purohit *et al.* (1984, 1985b)	≤30	30	13	40	-	
		Tompsett (1985)	40	18	16	35	41	-
		Khare *et al.* (1987)	30	49	15	54	-	partial vacuum
	S. obtusa	Tompsett (unpub.)	c. 33	-	-	-	-	-
	S. sumatrana	Yap (1986)	>40	15	25	60	-	
(b) Section Pentacme	*S. siamensis*	Tompsett (unpub.)	51	59	16	66	51	-
		Panochit *et. al.* (1984)	-	56	15	83	40-48	-
(c) Section Anthoshorea	*S. roxburghii*	Sasaki (1980)	34	182	4	69	40	-
		Purohit *et al.* (1982)	-	105	23	50	-	-
(II) DIPTERO-CARPUS	*D. caudatus*	Tompsett (unpub.)	≤47	-	-	-	-	-
	D. obtusifolius	Tompsett (1987b)	50	-	-	-	-	-
	D. turbinatus	Tompsett (1987b)	45	161	16	8	41-44	-
	D. baudii	Yap (1981)	-	30	14	25	-	-
	D. humeratus	Maury-Lechon e*t al.* (1981)	-	56	15	10	26	-
	D. tuberculatus	Tompsett (1987b and unpub.)	12	425	-20	32	12	orthodox
	D. alatus	Amata-Archachai and Hellum (unpub.)	-	67	15	30	29	orthodox
		Tompsett (1987b and unpub.)	~17	939	-13	36	12	-
	D. costatus	Tompsett (unpub.)	>38	-	-	-	-	-
	D. intricatus	Tompsett (unpub. and 1987b)	10	1237	2	38	12	orthodox
	D. zylanicus	Tompsett (unpub.)	>50	100	21	53	39	~99% r.h.. CG
(III) HOPEA	*H. hainanensis*	Song *et al.* (1984, 1986)	30	365	18	80	35-38	-
	H. mengerawan	Tompsett (1986)	37	-	-	-	-	-
	H. utilis	Yap (1986)	>20	-	-	-	-	-
	H. helferi	Tang and Tamari (1973)	30	40	15	85	48	
	H. odorata	Tang and Tamari (1973)	45	-	-	-	-	-
		Mori (1979)	-	55	10-15	0-10	-	-
		Yap (1981)	-	30	25	50	-	-
		Kobmoo *et al.* (1988)	-	45	2	60	~53	
		Corbineau and Côme (1988)	<33	-	-	-	-	not stored moist
		Sasaki (1980)	-	45	4	5	53	-
	H. wightiana	Sasaki (1980)	-	60	4	5	-	-
	H. subalata	Sasaki (1980)	-	51	4	40	32-43	-
	H. nervosa	Sasaki (1980)	-	330	25	19	-	-
	H. ferrea	Sasaki (1980)	-	60	4	2	33-35	-
		Tompsett (unpub.)	30	300	16	40	30-50	PB then perl.
	H. parvifolia	Tompsett (unpub.)	-	47	21	45	41	99% r.h., CG

(IV)	*P. tomentella*	Tompsett (unpub.)	43	91	16	40	40-50	perl. (10 seeds per test)
PARASHOREA	*P. malaanonan*	Tompsett (unpub.)	42	-	-	-	-	
	P. densiflora	Yap (1981)	-	60	25	90	54	-
	P. smythiesii	Tompsett (unpub.)	c. 40	317	18	46	c. 45	perl.
(V)	*V. cineraea*	Yap (1981)	-	25	60	65-86	-	-
VATICA	*V. mangachapoi*	Song *et al.* (1983)	>31	-	-	-	-	-
		Tompsett (unpub.) >34	85	21	24	40-45		perl.
	V. umbonata	Mori (1979)	-	60	10-15	10-50	-	-
		Yap (1981) - 60	14	20	-	-		
		Mahdi (1987) 74	-	-	-	-	-	
	V. odorata	Tompsett (unpub)	41	100	18	38	40	9% r.h., CG
(VI) COTYLELO-	*C. burkii*	Tompsett (1986)	30	-	-	-	-	-
BIUM	*C. melanoxylon*	Tompsett (unpub.)	≥33	51	21	36	35	-
(VII) NEOBALANO-CARPUS	*N. heimii*	Yap (1981)	--	50	14	80	28-47	-
(VIII) DRYOBALA-	*D. lanceolata*	Tompsett (unpub.)	42	62	21	92	51	PB
NOPS		Schafalitzky de Muckadell and Pilim (1983)	60	-	-	-	-	-
	D. aromatica	Tang and Tamari (1973)	37	-	-	-	-	-
	Yap (1981)		34	16	14	50	38-40	
(IX) STEMONO-PORUS	*S. canaliculatus*	Tompsett (unpub.)	43	77	18-21	20	47	
(X) ANISOPTERA	*A. marginata*	Tompsett (unpub.)	-	83	21	40	41	~99% r.h. CG

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*) For references see Tompsett 1992

abb: CG = Conviron germinator; perl.: Perlite medium; PB = Polythene bags

Table 8.3. Lowest safe moisture content (LSMC) for some recalcitrant species. (abbreviated from Tompsett 1992).

Storage atmosphere

Although metabolism may be reduced by lowering moisture content and temperature, recalcitrant species generally remain metabolically active during storage. Consequently, as seeds need oxygen for respiration, they cannot be stored in an oxygen free atmosphere. When stored at high moisture content, ventilation is mandatory to prevent heating and anoxia, and to remove toxic metabolic gases (Tompsett 1992). Short fumigation in CO_2 may be applicable to kill seed insects, but the seeds must subsequently be stored in an atmosphere with oxygen (ATSC 1995).

Storage containers and storage media

Because of the active seed metabolism, containers that allow some ventilation or gaseous exchange are normally essential (Tompsett 1992). Gunny, cotton or hessian bags may be suitable for intermediate seeds with relatively low m.c. but less so for recalcitrant seeds at high m.c., since they tend to become overgrown with mould.

Polyethylene bags are suitable provided the material is thin and permeable enough to permit some gaseous exchange (King and Roberts 1979). A wall thickness of 0.1-0.25 mm was found suitable to prevent excessive moisture loss, yet allowing some ventilation (Bonner 1996b). Panochit *et al.* (1984) found that seeds of *Shorea siamensis* stored at ambient temperature in folded plastic bags experienced prolonged viability as compared to those in sealed plastic bags, presumably because the sealing reduced ventilation.

Storage in media with some moisture-retention capacity to prevent desiccation has been found suitable for some species. Song *et al.* (1986, quoted in Tompsett 1992) stored seeds of *Hopea hainanensis* in moist coconut dust, and perlite has been used successfully for a number of recalcitrant species. Schaefer (1991) stored seeds of *Podocarpus milanjianus* and *Prunus africana* in cold moist sawdust, which also helped to reduce fungal infection.

Application of natural germination inhibitors like abscisic acid (ABA, see chapter 9) to prevent germination in storage has largely failed to prolong viability (King and Roberts 1979). Storage of recalcitrant *Prunus africana* seed within the pulp, in which germination inhibitors occur, reduced viability of seeds significantly as compared to extracted seeds (Schaefer 1990).

Seed treatment

Control of insects, fungi and other pathogens is important since the seeds are stored at temperature and moisture content where pathogens remain active. Seeds should as far as possible be pathogen free when they enter into storage. Development of possible surviving germs may be restricted by application of a fungicide. An environmentally harmless method of germ eradication is CO_2 fumigation or short immersion into cold or warm water. CO_2 fumigation is the most popular method. Most recalcitrant species tolerate up to 10 days' fumigation (depending on metabolism). In Australia, 24 hours' immersion in cold water is used for seeds sensitive to CO_2 fumigation (ATSC 1995). For *Citrus* spp. 50°C for 10 minutes has been recommended (King and Roberts 1979).

Fungicides may be applied moist by immersing the seeds into a solution, or by dry treatment. In the latter case the seeds must be surface dry. See also chapter 7.

Hydration - dehydration

Storage life of some recalcitrant and intermediate seeds can be prolonged by a mid-storage hydration - dehydration treatment. The treatment apparently activates the innate repair mechanism during the hydration stage. The method was originally developed in India for prolonging the storage life of bamboo (*Dendrocalamus strictus*) seed (Sur *et al.* 1988). In a study of *Ailanthus excelsa* (Simaroubaceae), seeds stored 3 months at a moisture content of 14% were hydrated (soaked) for 48 hours in various liquid media reaching a moisture content of approx. 62%. The seeds were then re-dried at ambient temperature (33°C) for 72 hours, back to the original 14% m.c. The improvement in germinability after another 2 months' storage depended on soaking media. Soaking in water doubled the germination rate as compared to the untreated control (13 → 26%); soaking in 10^{-4} M Na_2HPO_4 improved germination to 44% (Ponnuswamy *et al.* 1991).

Storage of germinants

Although storage conditions should normally aim at avoiding germination, there are instances where viability is lost so rapidly at any seed storage attempt that storage as germinants has to be applied. West African *Butyrespermum parkii* lose viability completely in two weeks, and several dipterocarps share a similar fate, especially if collected under moist conditions where germination processes may already have been initiated on the parent trees. Vivipary is normal in e.g. the mangrove genus *Rhizophora* where a true seed stage does not occur, the dispersal unit is a seedling. Such seeds must be allowed to germinate, but for practical handling purposes reduction of the germination speed is often desirable. During such storage, there should be no attempt to reduce the moisture content, but growth rate is reduced by keeping the temperature at the lowest possible level.

In the storage experiment of Corbineau and Come (1988) (referred under 'Temperature' above in this section), the authors found that germination was high under wet storage even at low temperatures (for *Hopea odorata* and *Mangifera indica* even at 5°C *viz.* 95 and 80% respectively), but the germinated seeds soon died at low temperatures. Exposure to higher temperatures to avoid chilling injury increased both germination (to 100%) and growth rate.

Figure 8.5.
The seeds of the mangrove genus *Rhizophora* develop into seedlings while they are still attached to the tree; the Philippines.

8.7 Management of Seed Stores

Fresh mature seeds always have the highest physiological quality. If seeds can be sown directly after processing, it not only saves the cost of storage, but also gives the best germination and quality of seedlings (cf. discussion of viability and vigour section 8.5.3). Moreover, some types of dormancy problems are more easily overcome in fresh seeds (chapter 9). Storage can be avoided or kept at a minimum if:

1. Species fruit regularly every year.
2. Sowing time follows soon after processing.

Accordingly, seed must be stored if:

1. Fruiting is periodic at long time intervals.
2. Fruiting periods occur after the normal sowing season.

In the first case, seed must be stored for a period equivalent to the interval between good fruiting years. It should be recalled from chapter 2 that the best quality seed is normally collected in mast years. Since collection costs are also relatively lower for large crops than for poor crops, it may be more economical to collect large quantities of seeds in good seed years and store them for current supply until the next large crop appears. The balance between collection cost and storage cost must be found for species where seed production varies considerably from year to year. Long term storage obviously requires that seeds are physiologically storable for the appropriate period of time, and under the conditions available. *Acacia xanthophloea*, *Croton megalocarpus* and *Araucaria cunninghamii* are examples of orthodox species which fruit irregularly or at long intervals, and which can be stored under appropriate conditions during the interim period (Schaefer 1991, Keys *et al.* 1996).

If fruiting is regular and annual but takes place outside the normal sowing season, short term storage is applicable, i.e. from collection to sowing, which is obviously less than one year. For orthodox seeds such short term storage rarely implies problems if the seeds have been appropriately dried and can be stored free from insects and rodents. Storage at ambient conditions is often applicable.

However, deterioration of orthodox seeds during short term storage may occur for particularly sensitive species if moisture content cannot be kept sufficiently low, e.g. if the seeds are to be stored during the rainy season. That is typically the situation for species that fruit during the late dry season or early rainy season. In seasonal climates it is preferable to sow seeds in the nursery during the dry season so that plantable size seedlings are ready for out-planting when the rainy season commences.

During storage at ambient conditions in permeable bags or containers the seeds are likely to absorb moisture from the humid atmosphere. Some examples will illustrate the problem:

In Nepal fruits of *Prunus cerasoides* mature between March and May, just before the rainy season (June - Aug.). To obtain plantable size seedlings for outplanting next rainy season, the suitable sowing time is September, i.e. the seeds must be stored for almost 6 months. When stored in cotton bags, the moisture content raises to 12.5%, and the seeds lose viability in few months. *Alnus nepalensis* fruits in December-February and may be sown immediately at lower altitude. At higher altitude the seeds are preferably stored until September as for *Prunus cerasoides*. Seeds of *Alnus* are even more susceptible to regaining moisture than *Prunus* and have a short storability at high moisture content. In both examples appropriate storage in airtight containers is therefore mandatory (Napier and Robbins 1988).

Short term storage may also be necessary in frost prone areas where chilling injuries can kill newly established seedlings. In North India, seeds of *Toona ciliata* mature in June. Since the seeds generally have a short viability, they are commonly sown soon after harvest. However, seeds sown in July suffer frost injuries during the subsequent winter, so sowing is preferably postponed until late February-March. This implies that the seeds must be stored for an interim period of 8-9 months. The practical problem has been solved by storing the seeds at low (0°C) temperature in containers with a high moisture content (Chand and Bhardwaj 1996).

For species with recalcitrant seeds, fruiting during the early rainy season with concurrent germination and seedling establishment before the onset of the dry season is common. Again, seeds must thus either be stored over the first rainy season and then sown 2-3 months before the new rainy season, or they must be sown immediately and then kept as seedlings in the nursery during the dry season. Moisture absorption is obviously not a problem for recalcitrant seeds, but a generally short viability may make immediate sowing necessary. The seedlings must then be maintained in the nursery through the interim dry season, which necessitates access to water during the dry season. This problem has been encountered for semi-recalcitrant neem (*Azadirachta indica*) in East Africa, where it is a limiting factor for the cultivation of the species. To reduce the need for watering during the dry season, seedlings may be pruned or stored moist as stumps.

A primary purpose of seed stores for orthodox seeds is to serve as 'buffer', to ensure that seeds of any species and any provenance of the species collected are always available. This role is especially important for commercial seed stores. Such stores will typically contain seed lots of different age. Because of the natural decline in viability, there should be a regular turnover, so that old seed lots are used before new seed lots. The principle 'first in - first out' should be generally applied and only forsaken if special storage conditions are applied, e.g. storage of part of the seed lot under long-term storage conditions. In these cases it may be more feasible to use a more recent seed lot, stored under short term storage conditions, rather than preparing a new seed lot for long term storage by additional drying.

Seed lots should be used before their viability is reduced significantly (cf. discussion of viability and vigour section 8.5.3). The lowest acceptable viability varies, but generally seed lots with less than

50% viability should be discharged, i.e. seed lots should be used or disposed of before their germination approaches 50%. Storage of old, deteriorated seed is waste of storage capacity. Viability may be predicted by the aid of viability/survival curves (appendix A8.1) and verified by viability tests (chapter 11). With proper management the amount of discarded seeds should be very small.

It is important for the management of seed stores that a register is established which shows what is in stock and keeps track of the individual seed lot. Seed stock registration is part of the whole seed documentation system that will be presented in chapter 14. Only a few points shall be mentioned at this stage. A seed stock register may be kept as a separate manual file or a computer database system, or it may be linked to the full seed documentation system e.g. seed source registration, tree improvement and seed testing. Special databases for seed stock registration and management are SISTEM+ of the Oxford Forestry Institute and TREESEED of the Australian Tree Seed Centre (Filer 1988, Wolf and Turnbull 1982, ATSC 1995). Simple seed stock databases can also be set up in any ordinary database programme e.g. Dbase and Excel.

8.8 Storage Units

8.8.1 Store room capacity

As far as storage room is concerned, seed volume rather than weight is important. Most seed has a specific gravity of approx. 0.5-0.8. One kg of seed therefore typically takes up round 1¼ to 2 litres. Added to that figure should be air space between and above the seed plus the containers themselves which, depending on container type and seed size, for most types would reach a volume of some 2.5-3 litres per kg seed.

Freezers and refrigerators can usually be utilised to most of their full volume capacity. However, in store rooms sufficient room must be added for easy handling, e.g. working/walking space between shelves.

Seed stores should have a room capacity for the largest quantity of seed that is likely to be stored at any one time, e.g. storage of a bulky crop in a good seed year. However, for air conditioned and cold storage, excess storage adds cooling costs, and for these types of store room the capacity should fit the need as close as possible. For example, in NE Australia good seed crops of *Araucaria cunninghamii* only occur once every 6-10 years. The seeds of this species are large, and to be kept viable during the interim period between two good crops, they must be cooled down to -18°C. For the Queensland planting programme a cold store capacity of 100 m^3 is needed (Keys *et al.* 1996). Because seed is removed for sowing during the interim period, smaller storage units, which can be closed down as the amount of seed in store decreases, are suitable.

Seed suppliers normally handle a range of species with different storability and storage period. Although cold storage may be preferable for most species, it is often only economically feasible for a small part of the seed, *viz.* seeds with short viability under ambient conditions, and seed to be stored over a prolonged period of time. It is not economical to store seeds under expensive cold storage if it does not significantly prolong storability as compared to ambient conditions. Also the size of the seed influences storage economy. The basic cost of storing one litre of seed is the same whether it contains e.g. 1,500,000 seeds of *Anthocephalus chinensis* or 200 seeds of mahogany. For the former a large number of seeds can be stored in a refrigerator, while the latter may require a large expensive cooled storage room.

Hence, storage conditions should be differentiated according to species storability, potential storage period plus economic considerations. Survival curves and prediction of storage life under different conditions (appendix A8.1) are useful tools for deciding storage conditions and storage periods for different species in a seed procurement programme. Often 2-4 different storage conditions are appropriate, e.g. (1) Ambient temperature, (2) Air conditioned room (one or two different temperatures) and (3) Cold storage (one or two levels). At the Australian Tree Seed Centre four levels of storage temperature are used:

1. Air-conditioned 23-25°C, 35% Rh.
2. Air-conditioned 16-18°C, 60% Rh.
3. Cool room 3-5°C, ≈ 90% Rh.
4. Freezer -15 to -18°C.

Deep freezing is only used for long term storage for conservation and storage trials (ATSC 1995).

8.8.2 Location of seed stores

Although there are several advantages of placing seed stores close to the processing depot, e.g. reducing transport distance and keeping the whole process of seed procurement under one roof, there are instances where alternative location should be considered. The conditions of seed processing and storage differ in one factor viz. temperature: where processing for orthodox seed is most effective under warm condition, storage is better at lower temperature. It should be recalled that for orthodox seed storage life roughly doubles as the temperature declines 5°C (Harrington 1972), and where artificial cooling is to be applied to bring down temperature further, reduction in outside temperature will save cost of cooling. 5°C is under dry conditions equivalent to about 500 m increased altitude. Locating the seed store at higher altitude may be applicable in hilly areas. However, since relative humidity also increases with increasing altitude, seed to be stored at lowest possible humidity should be stored in hermetic containers before transfer from a lowland processing unit to an uphill seed storage.

Figure 8.6
Well insulated tree seed store; the Sudan.

8.8.3 Situation and construction of storerooms

Situation and construction of storage room should aim at avoiding high temperatures and large fluctuations. Naturally cool places improve storage environment under ambient conditions and reduce power cost of cooling rooms. Permanently shaded buildings, e.g. under shading trees, are preferred to direct exposure to the sun (FAO 1984). Metal sheet roofing should be avoided since this material tends to increase temperature inside the building. If buildings have more than one storey, seed stores should be in the lower storey or preferably in the basement.

Storerooms should have walls with minimum heat transmission to minimise temperature fluctuations. Concrete walls and floors are preferred to avoid rodents and to ease cleaning.

8.8.4 Cold stores

Where storage is to be conducted below ambient temperature, some artificial cooling system must be applied. For small seed lots and small seeds refrigerators are excellent. A 200-litre refrigerator may easily hold more than 100 litre of seeds, which make up a large quantity of *Eucalyptus*, *Casuarina* or other small size seeds, but is insufficient for larger seeded species. Temperature of refrigerators can usually be adjusted between 5 and 10°C. Deep freezers with capacities from 50 to more than 800 litres are available and are frequently used for sub-zero temperature (-15°C to -20°C) storage of relatively small seeded species or research samples. Refrigerators and freezers should not be placed in closed rooms where seeds are stored at ambient temperature or in cold rooms since their operation generates heat which will warm up the room they are placed in.

Where large quantities of seed are to be stored under sub-ambient temperature, cold rooms are appropriate. Because of the energy costs of operation, cold stores must be designed to make the most efficient use of the space, and insulated to minimise heat transmission through walls and ceiling. Cooling systems range from ordinary air conditioners, where temperature is lowered typically to 20 - 25°C, to powerful freezing systems where the temperature can be lowered to below 0°C (FAO 1984). The latter are, however, very expensive in both establishment and operation and only applicable to large storage units. Some energy calculations of cold storerooms appear in appendix A8.3. It is important for the efficient use of the cooling system that it is placed in the relatively cool side of the building.

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Appendix A8.1 Predicting storage life of seeds

Natural ageing and loss of viability take place in all seed lots over a variable length of time. Ageing appears to be species-typical for seed lots stored under a given set of storage conditions (moisture content and temperature). Survival curves with viability plotted against time over the total life span of the seed typically shows a sigmoid shape. The sigmoid curve becomes a straight line when transferred to probit or plotted on a probability scale (Fig. A8.1). The rate of decline in viability during the storage period varies with storage conditions. On the probit transformation different slopes of the lines represent the different storage conditions: poor storage conditions give a steep slope, good conditions a flat slope. (Compare figure 8.3.).

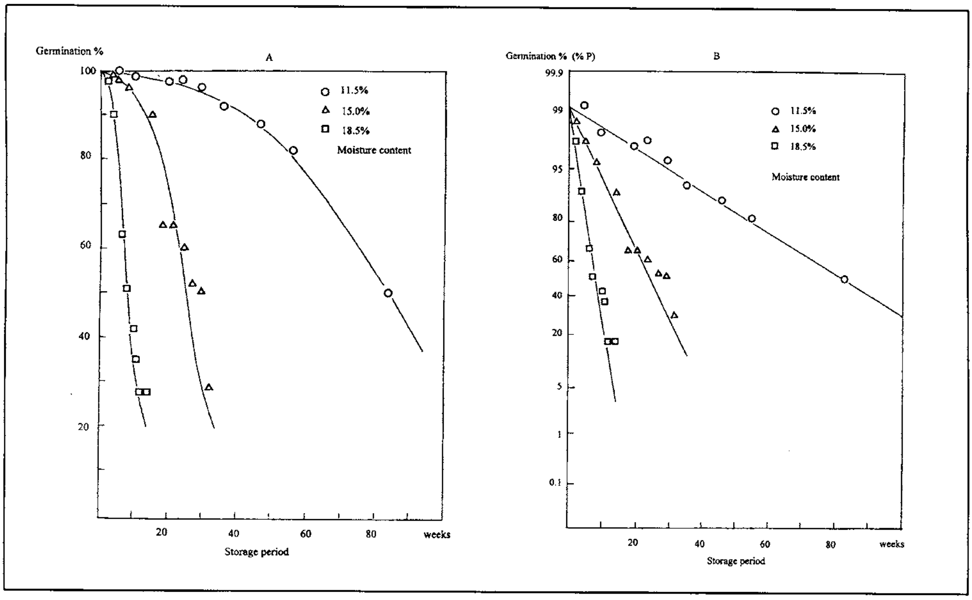


Figure A8.1. Viability curve on ordinary scale (A) transformed to probit scale (B)

Since the slope of the viability curve is independent of initial viability, a seed lot of a particular species, stored under conditions for which probit curves have been established, is likely to behave in the way predicted by the probit curves. When the initial viability is known, the expected germination percentage after a given storage period can be read directly from the curve or line.

The viability can be calculated more accurately using the parameters of the probit curve. An estimate of the expected viability (v) of a seed lot with an initial viability (K_i), stored under a constant storage condition for a given period (p days) with a given standard deviation (σ) of the distribution of death in time is:

$v = K_i - p/\sigma$ The slope of the linear relation is $1/\sigma$

Example: The expected viability of a seed lot with an initial viability of 90% stored for 60 days under storage conditions where σ = 10 gives:

$$v = 90 - 60/10 = 84\%$$

After 600 days the viability has declined:

$$v = 90 - 600/10 = 30\%$$

Under the same conditions, the period the seed lot can be stored before its viability declines to 60% is calculated:

$$60\% = 90 - p/10, \Rightarrow p = 300 \text{ days}$$

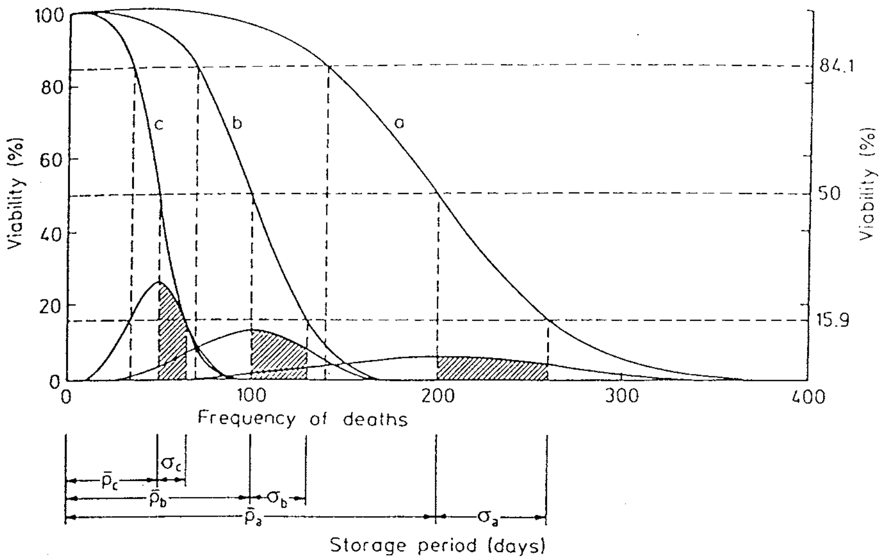


Figure A8.1
The relationship between the mean viability period (p) and the standard deviation (σ) of the distribution of the viability periods of individual seeds of a seed lot.

The diagram shows that an estimate of the mean viability period (p) may be made by noting the point on the time scale at which the survival curve intersects the 50% level of viability (i.e. 50% germination). The standard deviation (σ) may be estimated by noting the point at which the survival curve intersects the 15.9% (or 84.1%) level and measuring the distance from this point of the time scale to the point representing the mean viability period. This is based on the fact that the area under the normal curve between the mean and one standard deviation contains 34.1% of the area under the whole curve (see shaded areas). It is assumed that three seed samples from the same seed lot have been stored under different conditions so that the mean viability period of sample a (p_a) is twice that of sample b (p_b) and four times that of sample c (p_c). The value p can be estimated quite accurately graphically when the data for percentage viability are transformed to probit values or when they are plotted on a probability scale, since survival curves then become straight lines (see appendix A8.1). After Roberts 1973a.

More sophisticated equations have been developed for predicting the storage life of seed under different combinations of temperature and moisture contents. The solution to these equations is somewhat more complicated and requires multiple regression analysis. The equations are therefore not presented here; interested readers are referred to Roberts (1973a), Ellis (1986 and 1988).

Accelerated ageing

Accelerated ageing (AA) is a process by which orthodox seeds are exposed to poor storage conditions, which in turn leads to rapid decline in viability. AA is based on an inversion of the principle of seed storage for orthodox seeds: if seed longevity can be extended by reducing moisture content and temperature, then viability would be reduced in an accelerated manner by increasing temperature and moisture content. Without quantification of ageing, AA can be used as a simple comparison between quality and storability of different seed lots, e.g. in relation to maturity or effect of processing. Seed lots that rapidly lose viability under AA are believed to have a generally shorter viability under normal storage conditions.
The use of AA for predicting storage life of seeds is only valid and applicable where the processes that cause ageing under AA are the same as those that cause loss of viability during storage. There are some indications that this may not always entirely be the case. For example, AA takes place under conditions favourable to fungal development, and unless the seeds are thoroughly surface sterilized before AA, fungi may cause deterioration. Also the high moisture content during AA may activate the repair mechanism of cells which are not active under natural ageing of dry seed, and this would counteract the ageing process (see e.g. Bewley and Black 1982, Roberts 1983, Wilson and McDonald 1986 for discussion of AA applicability).

Appendix A8.2 Storage containers

This appendix lists some types of storage container used for seed storage by different seed stores around the world. More examples are given in Stubsgaard (1992). Many alternatives not listed here may be available from local manufacturers and dealers. Containers should be:

1. Airtight
2. Easy to fill, empty and clean
3. Of suitable volume
4. Reasonably strong and made of material that will not perforate or break during normal handling

Re.1. Airtight fittings are usually gaskets of rubber or other material. It is important that the fitting remains completely airtight when closed. Rubber tends to wear when old and dry, and is then no longer airtight. Greasing can extend the lifetime of rubber. When the fitting is no longer tight it should be replaced. Standard size, locally available container types in which the rubber gaskets can be replaced are therefore preferred.

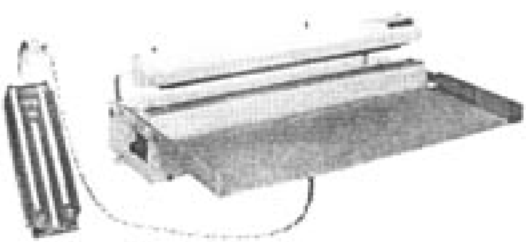
Re. 2. Narrow openings make filling, cleaning and sometimes also emptying more difficult. It is practical for the opening to be at least large enough for the easy passage of a hand, in case anything gets stuck inside.

Re. 3. The selection of container volume is primarily determined by the seed size but is also influenced by the likely size of portions to be taken out during storage. The best seed environment and most efficient use of container space is obtained when the containers are filled. If small portions of seeds are likely to be taken out regularly, e.g. for trials, it is appropriate to split up the seed lot before storage and store them in smaller portions in the containers e.g. 10 bags of 50 g rather than 1 bag of 500 g.

Re. 4. Storage containers can be re-used many times if reasonably strong. Metal tins must be internally protected from rust by an anticorrosive covering. Glass material must be fairly thick to withstand handling. Polyethylene bags are normally used inside stronger containers.

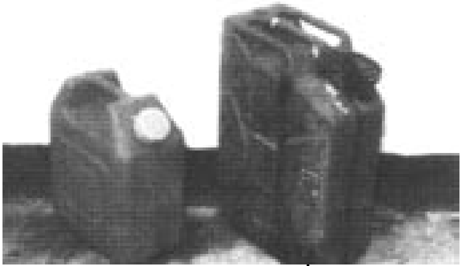
Transparent plastic bags. These are versatile containers for seed storage and suitable for many species and storage conditions. Their main advantages are:

1. The material is reasonably airtight, at least thick material
2. Transparency permits inspection of the contents without opening
3. The material is cheap
4. They are available or can be made in almost any size
5. They need no cleaning since the bags are discarded after use
6. Bags can be closed with minimal air around the seeds



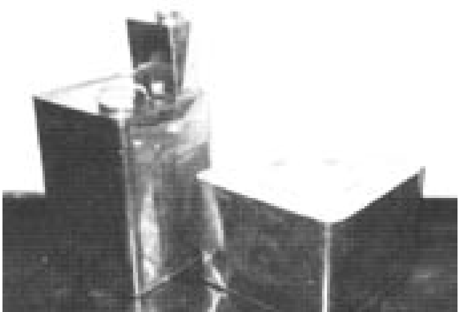
A thickness of 0.07-0.1 mm is applicable; thinner material may permit some moisture to pass and is easily punctured during handling. Very thick material makes closing of the bags more difficult. Standard size plastic bags are readily available in most general purpose stores, but are often of thin material. Alternatively, bags may be home made from rolls of polythene sheets, using a heat-sealing machine. Heat sealing is used for airtight sealing of the opening after filling. Such sealing is also necessary if the seeds are stored under vacuum or in CO_2.

Glass jars and bottles. Several models are available. Volumes of 0.5-3 litres are suitable. Jars and bottles should preferably have wide openings to facilitate efficient filling, emptying and cleaning. There are types with a screw lid and types with a closing clamp. All types have a fitted rubber gasket between the glass and the cover. Glass jars are convenient because they are transparent and the content hence directly visible. Their main disadvantage is their fragility. Particularly suitable for relatively small volumes of small to medium size seeds, e.g. *Eucalyptus* spp.



Jerrycans. Metal and plastic models are available. Volumes normally 10-20 litres. Metal cans close with a clamp, plastic cans with a screw cap. Both types have an airtight rubber gasket. Relatively large quantities of seed are easy to handle in jerrycans. However, the containers are very difficult to clean and metal cans easily rust from inside. Suitable for e.g. pine seeds.

Plastic drums and barrels with cover. Volumes often 60-150 litres. A suitable type of drum is used for transport by chemical industries. It has a cover that can be closed airtight with a rubber gasket and clamp ring. Suitable for large seeds e.g. *Terminalia*, *Khaya*, *Swietenia*



Metal tins. Volumes of 2-10 litres are available. Screw lids or fitted light lid with rubber gasket. The square types are easy to store with efficient use of space. Types with large lids are easier to empty and clean. The tins must be protected from rust by an anti-corrosive coating. Suitable for small volume seed lots of most species, especially when the lid is relatively large. Large-lid type suitable for storing small samples of seeds stored in plastic bags.

Appendix A8.3 Energy balance in cold rooms

Cold rooms must be continuously cooled down because heat is transmitted from the outside through the walls of the room.

The temperature inside a cold room tends to be in equilibrium with the outside temperature because heat is transmitted through the walls of the room and lost by ventilation. Therefore, cold rooms must be continuously cooled down to compensate for the energy transmission. Major heat transmission is through the walls and is proportional to the total wall area (ceiling, floor and walls), and temperature difference between the inside and outside of the room. A large wall area and large temperature difference increases the loss of energy per unit time. Heat transfer through the wall depends on its insulation, which in turn is determined by the material and wall thickness: transmission is slow through a thick wall of insulating material like polyurethane, and fast through a thin wall of wood. The exact heat transmission through a wall is calculated as:

$$P = S \times \lambda \times \Delta t$$

where P = heat transmission in kilo Joule per hour (kJ/h)
S = wall area in m^2
Δt = temperature difference between inside and outside the wall
λ = specific heat transmission value of the wall material measured in kJ/h x m^2 x °C

If heat transmission is likely to be different through different sections of the walls (e.g. floor, ceiling and walls), the individual sides should be calculated separately.

Heat transmission also occurs through ventilation, e.g. through the doors during handling. A value of 5 times the volume of the empty chamber is used in this calculation (which is half of that suggested by FAO (1984) for general cold stores, but justified here because there is less frequent opening of seed stores than e.g. food stores).

The quantity of heat per m^3 of air exchanged may be taken as 2 kJ/m^3 °C (FAO 1984). Heat transmission through ventilation is then calculated as:

$$R = 5 \times V \times 2 \times (\theta_e - \theta_i)$$

where R = heat transmission in kJ/24h
V = volume of empty store room in m^3
θ_e = external temperature
θ_i = internal temperature

Example of use of the formula for calculating heat transmission:

Total energy transmission for a cold store of 16 m^3 with dimensions 2 x 2 x 4 m, and cooled down from 30°C to 10°C is calculated. The walls consist of 80mm cold store panels with heat transmission values of $\lambda = 0.93$ kJ/h m^2 °C.

Wall area is 40m^2; $\Delta t = 20$°C
Heat loss through the walls during 24 hours is:
24h x 40m^2 x 20°C x 0.93 kJ/h m^2 °C = 17856 kJ
Heat loss by ventilation is:
5 x 16m^3 x 2 kJ / m^3 °C x 20°C = 3200 kJ
Total heat transmission: 17856 + 3200 kJ per day = 21056 kJ

Several other calculations are listed in the table below. The efficiency of the cooling system varies but because of energy transfer considerably more electric energy must be used than actually disappears through the walls and ventilation. In the table an efficiency of 20% is assumed.

Store room capacity, m^3	Wall area, m^2	Heat transmission, λ (kJ/h m^2 °C)	Temperature difference (θ_e - θ_i), °C	Wall transmission per 24h, kJ/m^2 °C	Ventilation transmission per 24h, kJ/m^2 °C	Total heat transmission per 24h, kJ/m^2 °C	Energy loss, kWh per day	Energy consumption at 20% efficiency of source, kWh
12	32	0.93	10	7142	1200	8342	2.3	10.6
12	32	0.58	10	4454	1200	5654	1.6	8.0
12	32	0.58	20	8909	2400	11309	3.1	15.1
16	40	0.93	10	8928	1600	10528	2.9	14.5
16	40	0.58	10	5568	1600	7168	2.0	10.0
16	40	0.58	20	11136	3200	14336	4.0	20.0
20	48	0.93	10	10714	2000	12714	3.5	17.5
20	48	0.93	20	21427	4000	25427	7.1	35.5
20	48	0.58	10	6682	2000	8682	2.4	12.0
20	48	0.58	20	13363	4000	17363	4.8	24.0
24	56	0.93	10	12499	2400	14899	4.1	20.5
24	56	0.93	20	24998	4800	29798	8.3	41.5
24	56	0.58	10	7795	2400	10195	2.8	14.0
24	56	0.58	20	15590	4800	20300	5.6	28.0