

Chapter **36**

## Optimising Seed Banking Procedures



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### Summary

This chapter outlines factors important for gene banking. Optimising gene banking procedures is often interpreted as storing seeds under environmental conditions that give them maximum longevity. This paper argues that even this narrow view of optimised gene banking is difficult to define or predict and that the interacting factors that contribute to seed lifespans are not completely understood. The concept of optimum procedures to bank seeds should be broadened and based on the intended use of the germplasm as well as other *ex situ* conservation steps, particularly collection and regeneration schemes. Considerations of the costs of alternative strategies and changes in the genetic heterogeneity of the seed sample with different storage conditions are critical to the development of optimised protocols.

### Introduction

Gene banking seeds to preserve genetic diversity has received widespread attention in the last two decades. Though criticised as a moderately effective conservation strategy (Soulé, 1991; Tuxill, 1999; Schoen and Brown, 2001), gene banking is also regarded as a powerful tool if it is part of a larger conservation effort (e.g., Falk and Holsinger, 1991; Stuessy and Sohmner, 1996; FAO, 1998; Guerrant *et al.*, 2003). Agriculturalists, environmentalists and conservationists agree that gene banking has the utilitarian role of ensuring that genetic resources are documented and readily available, and of “buying time” when *in situ* conservation strategies are hampered. The establishment of seed banks all over the world attests to the feasibility of preserving genetic resources in gene banks. The question now becomes how best to design and operate gene banks so that they effectively accomplish the goal of conserving genetic variation within and among populations for future use in breeding or restoration programmes.

Efficient management of genetic resources in *ex situ* collections is critical in these times of limited resources for seed banks (see, Science, 2002, vol. 297 p. 1625), restricted exchange of germplasm through the Convention on Biological Diversity (<http://www.biodiv.org>), and increasing erosion of habitat and plant diversity (Walter and Gillett, 1997; Stork, 1997). The first step is a clear idea of the *raison d'être* of the gene bank which considers the conservation target (populations, genotypes, or genes), the intended use of the germplasm (usually breeding or restoration) and the time when stored germplasm will be retrieved. Based on the goals of the gene bank, an integrated approach is needed to optimise collection, preservation and

regeneration efforts. This chapter focuses on preservation strategies for seeds exhibiting orthodox storage behaviour (i.e., those that survive desiccation and sub-freezing temperatures). Strategies should consider the interactions among various factors in different gene banking scenarios. Factors include the required shelf life of stored germplasm, the volume of samples that are processed and stored, and the types of resources that are available. An understanding of the strengths and limitations of different gene banking scenarios can be used to develop cost-effective strategies to accomplish the gene bank's mission. For example, gene banks with access to human resources may invoke strategies that are labour-intensive. This chapter examines some of the criteria used in the USDA National Plant Germplasm System (NPGS) to design and operate gene banks that store germplasm for short and long-term uses.

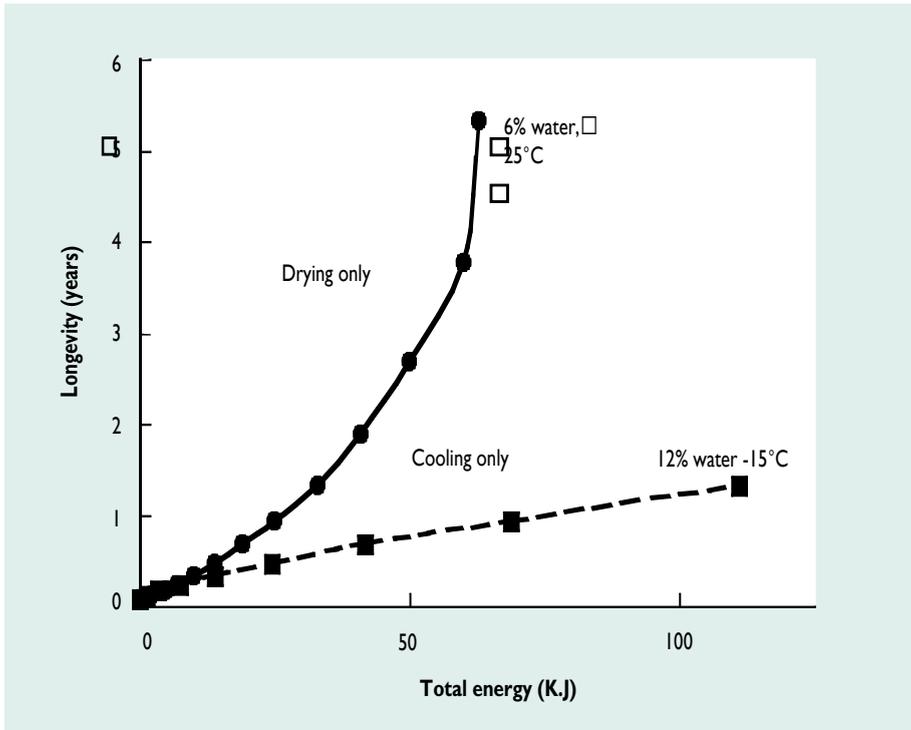
In gene banks, germplasm is placed under conditions that limit deteriorative reactions – usually a dry and/or cold environment. Because they tolerate complete desiccation (at least initially) (Roberts, 1973; Walters *et al.*, 2002a), orthodox seeds can be placed in a variety of conditions and retain longevity for several years. The flexibility in storage conditions have provided gene bank operators with options, and ‘rules of thumb’ (Justice and Bass, 1978), viability equations (Ellis and Roberts, 1980), and ultra-dry technology (FAO/IPGRI, 1994; Walters, 1998a) are guidelines for choosing how to balance moisture conditions and temperature to achieve the required longevity. The required longevity should, but rarely does, drive decisions about preservation strategies. Required longevity is a function of what and why germplasm is preserved and the capacity of the gene bank (size of collections and resources available). Germplasm used for re-seeding or cultivation should remain vigorous for up to 5 years; seeds stored as breeders’ stock may be needed for 10–20 years; and seeds stored as genetic resources for future but unidentified uses must be kept alive until needed. The unspecified expectations of gene banks used to store genetic resources is a source of confusion that has led some gene banks and research programs into a quixotic quest for immortality. More concrete expectations and estimates of life spans will help gene bank operators to set more realistic goals and use better gene banking practices to achieve them.

## Cost-benefit for Different Storage Conditions

The concept of optimum seed storage is often misinterpreted to mean maximum seed longevity. This unfortunate interpretation sparked debates on what conditions will keep seeds alive the longest (Ellis *et al.*, 1991; Vertucci and Roos, 1991; Smith, 1992; Vertucci and Roos, 1993a; Zheng *et al.*, 1998; Walters *et al.*, 1998b; Walters, 1998a), and masked the more relevant questions of what is a realistic goal and how can the goal be accomplished without squandering resources. Optimised storage is situational and depends on the interacting factors of required shelf life, storage and processing capacity, and resources. Maximum seed longevity is a separate topic (discussed below) that may or may not drive gene banking practices.

The shelf life of seeds required to accomplish gene bank goals should be the predominant consideration for gene bank design and operation. Preservation strategies can be further refined by focusing on the cost of achieving the desired shelf life and the risk if seeds die earlier than expected. The consequences if gene banks fail to achieve goals must also figure into the equation of costs. If the germplasm is not valuable or can be easily replaced, then poor shelf life is acceptable (but then one wonders why it is worth gene banking at all!). Valuable or irreplaceable germplasm must be monitored to ensure adequate viability and contingency plans to regenerate germplasm are required if viability slips below an acceptable value. The dollar costs for monitoring and regeneration are a function of the frequency that these operations are performed, which in turn, is determined by the achieved shelf life. In general, it costs the USDA National Plant Germplasm System about \$4 and \$50 each time an accession is tested for viability or regenerated, respectively, though costs can vary widely depending on species and life history. Mortality during storage or frequent regeneration also compounds the risk of genetic erosion (Breese, 1989; Parzies *et al.*, 2000; Schoen and Brown, 2001), leading to diminished value of germplasm collected to preserve genetic diversity. Value of germplasm collections and accountability in maintaining the genetic integrity of samples during *ex situ* conservation have not been adequately addressed by the gene banking community (but see Smale *et al.*, 2002), making a complete cost-benefit analysis impossible.

Costs of preserving seeds involve a myriad of parameters that affect operational (energy and labour) and capital (facility and equipment) investments. Some operational costs, such as accession documentation, seed cleaning, and information management, are independent of the gene banking scenario. Other costs, such as the energy required to dry and store seeds, are dependent on climate, construction specifications, seed harvest dates and volume of accessions processed in a yearly cycle and scale (cost per sample decreases with increasing room dimensions). The energy consumed by drying seeds to different water contents and storing them at different

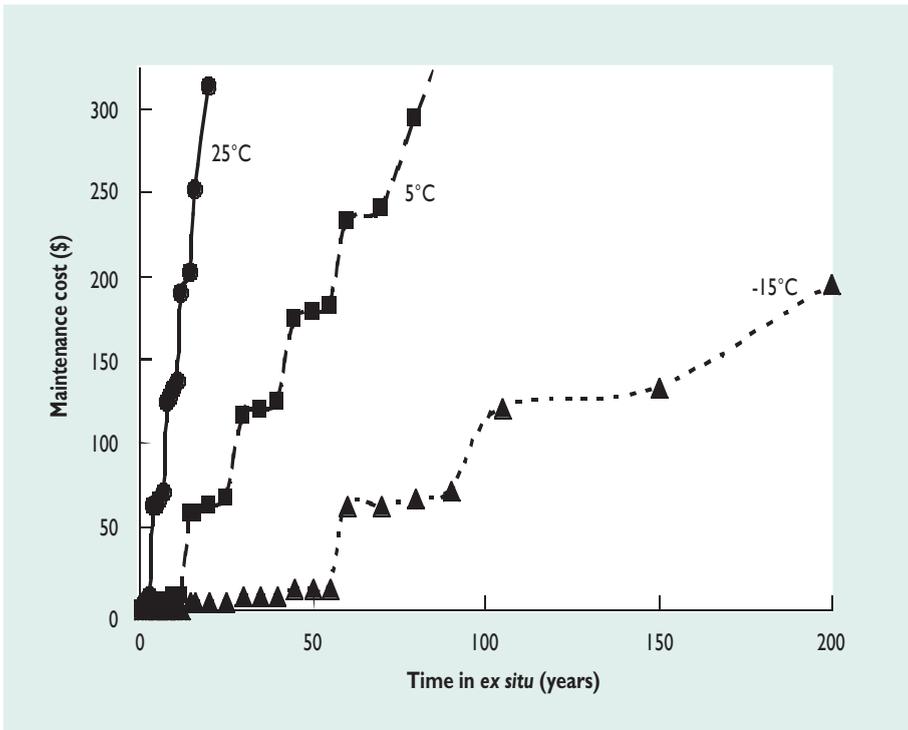


**Figure 36.1** Energy consumed during drying or cooling versus longevity of soybean seeds. Longevity is calculated using Harrington's Thumb Rules and assuming storage at 12% water (75% RH) and 25°C allows survival for 1 month. Energy for drying assumes a one-time drying procedure and airtight packaging. Energy for cold storage is calculated for the total lifetime of the sample (i.e., integrated by longevity). Points are in 0.5% or 5°C intervals.

temperatures can be calculated using existing data from psychrometric charts, seed isotherms, thermal conductivity of insulators, room size, etc. A comparison of these energy costs with longevity achieved provide a rational basis for selecting storage environments. In the subsequent comparisons using soybean seeds, longevity is approximated using a modified set of Harrington's Thumb Rules, which state that seed lifespan is doubled for each 10°C drop in temperature and 1% drop in water content (Justice and Bass, 1978), and assuming that seeds stored at 12% water and 25°C have a life span of 1 month. Calculations of energy costs for drying and storage assume 500 g of seeds at ambient conditions of 25°C and 75% RH, room dimensions of 1m<sup>3</sup> (drying) and 3 m<sup>3</sup> (storage) and 50% of the storage room volume is filled with seeds (Figure 36.1).

According to the scenario in Figure 36.1, drying to 6% water (25% RH) gives a 64 fold ( $2^6$ ) increase in longevity and consumes only 67 KJ (assuming seeds are packaged in airtight bags following drying). In contrast, storage at 5°C or -15°C gives a 4 ( $2^2$ ) and 16 ( $2^4$ ) fold increase in longevity, respectively, but consumes 42 and 83 KJ/year, respectively, times the number of years the seeds remain alive. These calculations may lead to the conclusion that drying is the more cost-effective method of preserving seeds. Indeed, this is true if relatively short lifespans are acceptable – why consume energy on refrigeration when a simple drying procedure and adequate packaging will achieve about 5 years storage? However, if gene banks require prolonged lifespans, the above analyses present an oversimplification of the costs of preserving samples. Prolonged life spans require a combination of drying and refrigeration, since both temperature and water content affect seed longevity. Most equations that predict seed lifespans handle the effects of water content and temperature separately so that one may predict about a 250 or 1,000 fold increase in seed longevity if the soybean seeds originally at 12% water and 25°C are dried to 6% water and cooled to 5°C or -15°C. In other words, seeds are predicted to survive for 21 years or 85 years, respectively. The energy costs per year of longevity for seeds dried to 6% and stored at 25°C, 5°C and -15°C is about 13, 45 and 84 KJ/year. Clearly, refrigerated storage is expensive. According to these calculations, optimum storage for accessions that are only needed for 4 years can be achieved by adequate drying and no refrigeration; optimum storage for accessions that are needed for about 12 years may require some refrigeration (5°C), but not the expense of a freezer.

As the time the accession is kept *ex situ* increases, the cost of maintenance increases and provisions to rejuvenate deteriorated samples are needed. In this case, the high cost of refrigeration must be considered in the context of the higher costs of monitoring and regeneration. In the next analysis, the preservation costs (energy for drying 500 g of soybeans to 6% water content and storage at 25°C, 5°C, or -15°C times the approximate dollar cost of energy in the US ( $\$10^{-4}/\text{KJ}$ )) is added to the monitoring ( $\$4/\text{assay}$ ) and regeneration costs ( $\$50/\text{accession}$ ) (Figure 36.2). According to longevity predictions, monitoring is recommended every 1, 5 and 15 years and regeneration is needed about every 4, 14 and 56 years for seeds stored at 25°C, 5°C and -15°C, respectively. When the additional costs of gene banking are considered, storage at 5°C and -15°C becomes cost-effective after the first monitoring assay and increasingly inexpensive with each regeneration performed on counterparts stored at 25°C. In other words, preservation is inexpensive compared to other aspects of gene banking. Procedures that prolong life spans become increasingly cost-effective with time. The analyses also suggest that monitoring viability can account for as much as 20–25% of the total preservation costs.



**Figure 36.2** The cost of maintaining accessions in *ex situ* gene banks over the lifetime of the accession for different storage temperatures. Initial conditions assume soybean seeds (as in Figure 36.1) dried to 6% water and stored without temperature controls (25°C), under refrigeration (5°C) and in the freezer (-15°C). Costs are calculated for energy of drying and storage and for periodic monitoring and regeneration. Monitoring and regeneration frequencies are determined from ageing rates of seeds calculated from Harrington's Thumb Rules (as in Figure 36.1).

## Allowable Deterioration Before Regeneration

The above analyses showed that viability decreased with time in seeds, no matter what the storage environment. Deteriorated accessions in gene banks must be regenerated, and the cost of regeneration is prohibitively high compared to other aspects of seed preservation. Frequent regeneration also increases risks of genetic shifts in populations (Breese, 1989; Parzies *et al.*, 2000; Schoen and Brown, 2001). To prevent genetic bottlenecks associated with preservation-regeneration cycles and to provide more cost-effective storage strategies, regenerations must be optimally timed. To do this, we need to know how much deterioration should occur in a seed lot before regeneration is necessary.

The process of seed deterioration is usually measured from periodic germination assays (described above as monitoring). Allowable changes from initial germination tests are established by each gene bank to flag accessions for regeneration. The USDA NPGS regenerates seeds when percent germination slips below 80% in laboratory tests. All criteria are subject to Type I (i.e., statistical test incorrectly declares in favour of the hypothesis) or Type II (i.e., failure of a statistical test to accept a true hypothesis) errors when compared with field establishment. Some seed lots may be more deteriorated than indicated by the laboratory results, leading to a failed crop and subsequent procedures to “rescue” the germplasm. Alternatively, some seed lots with poor laboratory germination establish easily in the field by simply planting more densely. Regenerating seeds either too late or too soon causes an unnecessary expenditure of funds and a greater risk of genetic erosion. “Rules” that relate laboratory assessments of seed quality with stand establishment have been developed by organisations such as ISTA and AOSA for commercial uses and need to be modified for the specific needs of the gene banking community.

Guidelines to optimise the frequency of seed regenerations should take the life history of accessions into account. Many gene banks use the same criterion to signal the need to regenerate for all accessions (e.g., 80% germination is used by the USDA NPGS). This practice may have originated from the idea that seed deaths are normally distributed in time, a basic assumption of the viability equations that model seed ageing rates (Roberts, 1973; Ellis and Roberts, 1980). The viability equations were developed using crop seeds that were fairly homogenous, and individuals within a seed lot are expected to die at random with a characteristic peak frequency (but see Moore and Roos, 1982). Accessions collected from the wild are heterogenous, and there is an assumption that seed deaths in these lots are also randomly distributed. Differences in flowering time, dry matter accumulation, dormancy, and maturation date are commonly observed among individuals in non-domesticated populations, and these genetically-regulated traits are

contributing factors to seed longevity (reviewed by Walters, 1998b). Ageing rates of seeds from non-crop species sometimes do not conform to the viability equations, suggesting a greater complexity than previously assumed (reviewed by Walters, 1998b). Non-normal distribution of seed deaths in heterogenous samples may indicate potential selection against genotypes that are predisposed to rapid deterioration and for genotypes that are long-lived. Selection of “domestication” traits is one of the greatest risks to populations held in *ex situ* collections (Breese, 1989; Parzies *et al.*, 2000; Schoen and Brown, 2001; Hayward and Sackville Hamilton, 1997), and it is important that the preservation procedures do not impose this mechanism of genetic change.

Ideally, guidelines that suggest when an accession should be regenerated will consider heterogeneity. Accessions that represent cultivated varieties are, by definition, more homogenous than accessions from landraces or wild populations. Deterioration in these samples will not impose a genetic change through selection or drift, and allowing greater deterioration before regeneration may be a money-saving alternative as long as field establishment remains adequate. On the other hand, the risk of genetic changes in heterogenous samples is largely unknown. Here, the question is not how long the accession maintains germination above a set percentage; but rather, whether mortality during the early stages of deterioration of the accession imposes a significant bottleneck.

A final concern about the timing of regeneration specifically applies to seeds harvested from wild populations. As noted earlier, these seeds are genetically diverse. It is also likely that seeds from wild populations have variable quality resulting from stressful growing conditions and one-time harvesting opportunities in remote areas. Though there are reports of extreme longevity for some seeds harvested in the wild (Toole and Brown, 1946; Went, 1969; Kivilaan and Bandurski, 1981), we usually find that seeds under cultivation have longer life-spans, even if the parent plant was wild (Walters, unpublished). Many wild-collected accessions are small (<< 3,000 to 5,000 seeds recommended for gene banking) making it difficult to monitor viability without depleting the sample. Yet, the rapid deterioration likely in these samples can lead to selection (described above) or inadequate sample sizes for regeneration. Current practice is to regenerate an accession once germination percentage has dropped; this could lead to large bottlenecks for wild-collected accessions. We hypothesise that the risk of genetic erosion in samples collected from wild populations can be reduced by regenerating (and increasing) the accession immediately and storing the seeds (F1 generation) in gene banks. Obviously, early regeneration of seeds is only practical for annuals and some perennials. Additional schemes, such as pollen collection or clonal propagation may help preserve genetic diversity of large perennials with prolonged juvenile stages (Volk and Walters, 2003).

## “Maximum” Seed Longevity

Problems associated with genetic shifts during storage and regeneration and the cost of monitoring and regeneration could be solved if gene banks could guarantee eternal life for stored seeds. Even using the best available science, gene bank operators have been able to prolong life spans, but not stop seed ageing altogether (Walters *et al.*, 2002b). The questions arise as to whether seed life-spans are finite, and how gene bank conditions can maximise the intrinsic potential for long life in dried seeds. These questions form the basis of a decade-long debate that focused on an empirical understanding of seed ageing under poor storage conditions and a theoretical understanding of moisture-temperature interactions on reaction kinetics (Ellis *et al.*, 1988, 1989, 1990; Vertucci and Roos, 1990, 1993b; Vertucci *et al.*, 1994; Walters, 1998a). Though the debate is now discussed less than resolved, review of specific elements can bring a broader understanding of how temperature and water content affect the relative rate of seed deterioration (Walters, 1998b, 2003).

The rate of seed ageing, like all reactions, is a function of the driving force of the reaction and barriers that prevent it from occurring according to the equation:

$$J = \Delta G \times R \quad (1)$$

where  $J$  is the rate,  $\Delta G$  is the free energy change of reactions (driving force) and  $R$  is the resistance (barriers) to the reaction (Walters, 2003). Barriers slow reactions, usually by reducing molecular motions (Buitink *et al.*, 1998b, 2000), but also by creating physical boundaries through which molecules can not penetrate. Viscous states, such as glasses (Leopold *et al.*, 1994), slow reactions but do not change the thermodynamic equilibrium. Driving forces are the thermodynamic parameters that determine whether reactions can occur. Specific reactions that cause seed ageing are not known, making it difficult to define the equilibrium coefficient of reactants and products, let alone how the coefficient is affected by storage environment.

Most preservation procedures are based on the premise that drying and cooling slow deterioration by creating kinetic barriers, and that drier or colder conditions extend life spans indefinitely. For temperature, this idea probably originated with an assumption that ageing reactions follow Arrhenius kinetics. Arrhenius kinetics describe the relationship between the molecular motion in a system (a function of temperature) and how fast reactions occur (Atkins, 1982; Franks, 1985). Slower molecular motion at lower temperatures cause slower reactions. The amount of thermal energy required to activate a reaction is calculated from the slope of the relationship between the natural logarithm of the reaction rate and the reciprocal of temperature (in Kelvin) in an Arrhenius plot. For simple reactions, this relationship is linear, indicating that the activation energy is constant with temperature. When a suite of reactions are involved, the activation energy usually changes with

temperature because of the different response of each reaction to temperature (changes in the driving forces), resulting in a curvilinear Arrhenius plot (Franks, 1985; Walters, 1998b). Structural changes in the reactants, products or catalysts (denaturation or state-phase transition) also give non-linear Arrhenius plots (e.g., Buitink *et al.* (1998a) report non-linear Arrhenius behaviour to pollen ageing and attributed “breaks” to a state change, though the non-linear response may also be explained by temperature interactions among several reactions). The temperature coefficient used in Harrington’s Thumb Rules described above (a doubling of life span for each 10°C drop in temperature) is an application of Arrhenius theory. Because other chemical or physical reactions were not considered, Harrington’s Thumb Rules predict a continuous benefit of cooling. In contrast, the viability equations (Ellis *et al.*, 1980), its modifications (Dickie *et al.*, 1990), and pollen ageing data (Buitink *et al.*, 1998a) show a declining benefit of low temperatures with progressive cooling.

Most models of seed ageing also predict a continuous benefit of drying seeds. The theory behind this prediction is not as rich as the theory governing temperature effects. Concepts of molecular mobility were adapted from temperature theory (Vertucci and Roos, 1990) and then measured directly (Buitink *et al.*, 1998b; 2000). These analyses showed that drying decreased molecular mobility to a point. Coincidentally, empirical studies of seed ageing also showed a limit to the beneficial effects of drying (Ellis *et al.*, 1988, 1989, 1990). Existence of a critical water content that marks a change in relationships among drying, intracellular viscosity, and seed ageing rates is analogous to curvilinear Arrhenius plots described above for temperature when several reactions or structural changes are involved.

Temperature and water content have been typically regarded as parameters that affect barriers to reactions ( $R$  in equation 1). However, the variable effects of these parameters on seed longevity suggest that they also contribute to changes in the driving forces ( $\Delta G$  in equation 1) of the myriad reactions that cause seeds to age (Walters, 2003). Changes in water content affect the concentration of reactants and products. Temperature affects the likelihood of these reactions, with exothermic reactions favoured by lowering the temperature and endothermic reactions favoured by raising the temperature. The implications of increased contribution of exothermic reactions when temperature or water content is reduced has not been considered even though exothermic reactions, such as lipid phase changes, have been implicated in seed ageing (e.g., van Bilsen *et al.*, 1994)

To summarise, there are a number of theoretical considerations and sets of empirical evidence that suggest that both drying and cooling may have diminishing benefits on extending seed life spans. The limiting benefit portends a maximum longevity if seeds are dried alone or cooled only, and hence the curves in Figure 36.1 eventually bend concavely. Seed bank operators need to know when energy consumption does not substantially increase seed longevity.

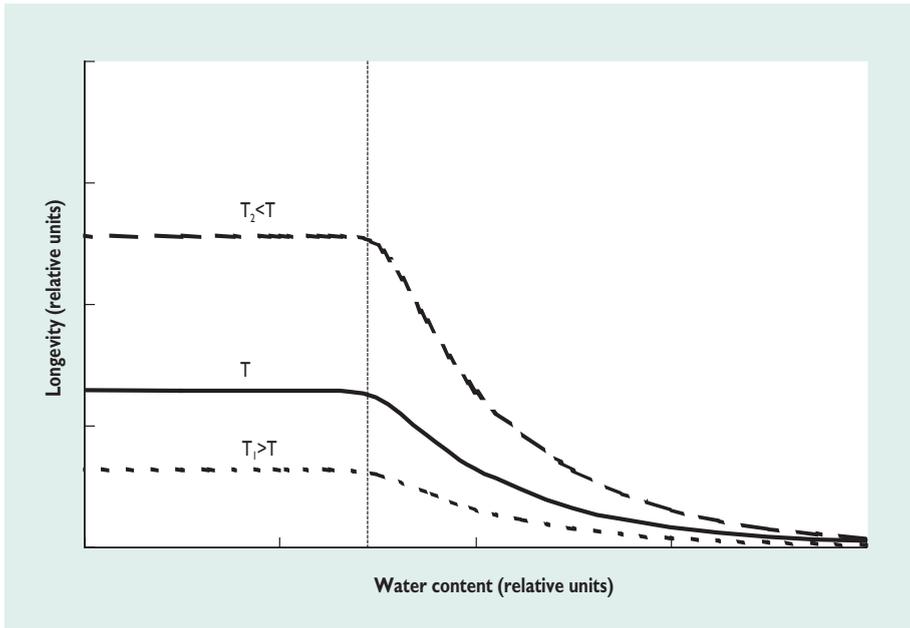
The existence of critical water contents is undisputed in the literature (Ellis *et al.*, 1988, 1989, 1990; Vertucci and Roos, 1999, 1993; Vertucci *et al.*, 1994; Walters, 1998a). These values define when drying no longer extends longevity. Future research may show that the concept is also applicable to temperature.

Temperature and water content affect both  $R$  and  $\Delta G$  in Equation 1. Isopleths, state-phase diagrams and isoviscosity curves of water and polar lipids in seeds show that the effects of temperature and water content are inextricably linked (Walters, 1998b, 2003). In all circumstances, there is a trend towards increasing water content with decreasing temperature to achieve the same thermodynamic status. Thus, water content and temperature have interacting effects on ageing kinetics despite the assumption of Harrington's Thumb Rules and the viability equations that they behave independently. Consideration of these biophysical realities leads to the hypothesis that critical water contents are affected by temperature (Vertucci and Roos, 1993; Walters, 1998a, b, 2003). There are few experiments that test this hypothesis, as it requires long-term storage data where both water content and temperature are manipulated independently (Vertucci *et al.*, 1994; Buitink *et al.*, 1998a; Walters *et al.*, 1998b). However, a thought experiment can describe the consequences of the possible interactions of critical water content with temperature.

In the first scenario of the thought experiment (Figure 36.3), a critical water content exists (vertical line) and Arrhenius kinetics are assumed following Harrington's Thumb Rules. Seed lifespans are doubled when storage temperature is reduced by 10°C, but there is a maximum longevity at each temperature. If critical water content is independent of temperature, cooling provides additional and unlimited benefit. This scenario provides great news to gene bank operators, if true. Gene bank operators would know that excessively drying seeds wastes energy and that lowering temperature could pay for itself if accessions were kept for many years. This is the simplest of the scenarios and is antithetical to the concepts used by proponents of ultra-dry technology (Zheng *et al.*, 1998; Walters *et al.*, 1998a).

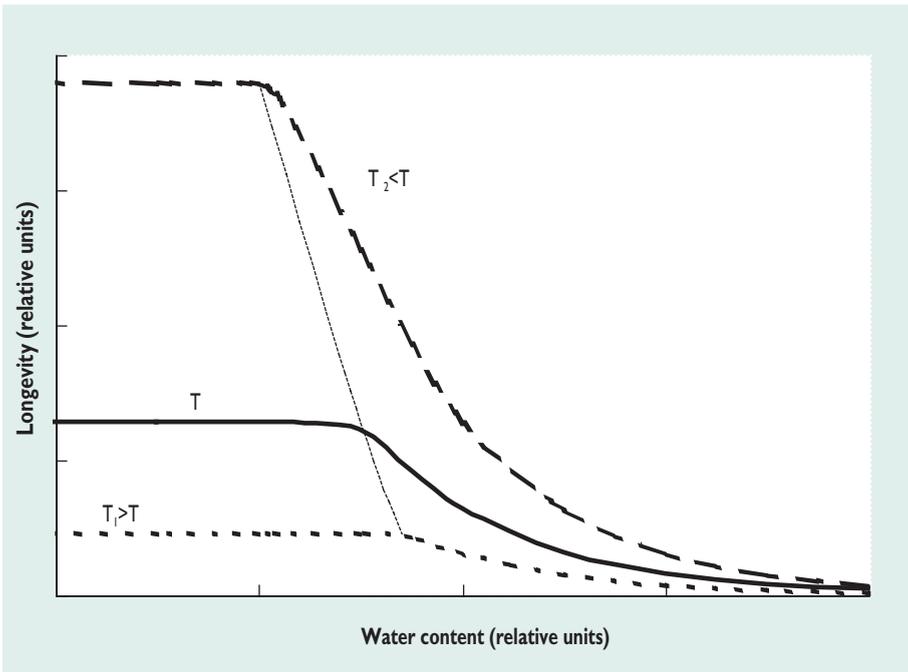
In a second scenario (Figure 36.4) critical water content decreases with decreasing temperature (line slanting to left). This scenario provides fantastic news to gene bank operators, if true. Though there is a maximum benefit to drying at each temperature, the benefit increases with decreasing temperature so that longevity more than doubles by lowering temperature by 10°C and drying below the critical water content. This scenario does not obviate the need for temperature reductions, but suggests that efficiency improves when seeds are stored at progressively lower temperatures because of the additional benefit of extreme drying. This scenario does not conform to theoretical considerations or existing data.

In the final scenario (Figure 36.5), critical water content increases with decreasing temperature (line slanting to right). As the critical water content increases, the temperature coefficient for longevity (related to activation energy in Arrhenius plots described above) decreases and Arrhenius plots



**Figure 36.3** A schematic drawing of the effect of water content and temperature on seed longevity. The diagram shows that drying below a critical water content (vertical line) at temperature  $T$  (solid curve) does not increase seed longevity. Dashed curves show longevity changes for  $T_2 + 10 = T = T_1 - 10$ , assuming Harrington's Thumb Rules. If critical water content is constant with temperature, maximum longevity at  $T_2$  is twice the level at  $T$ .

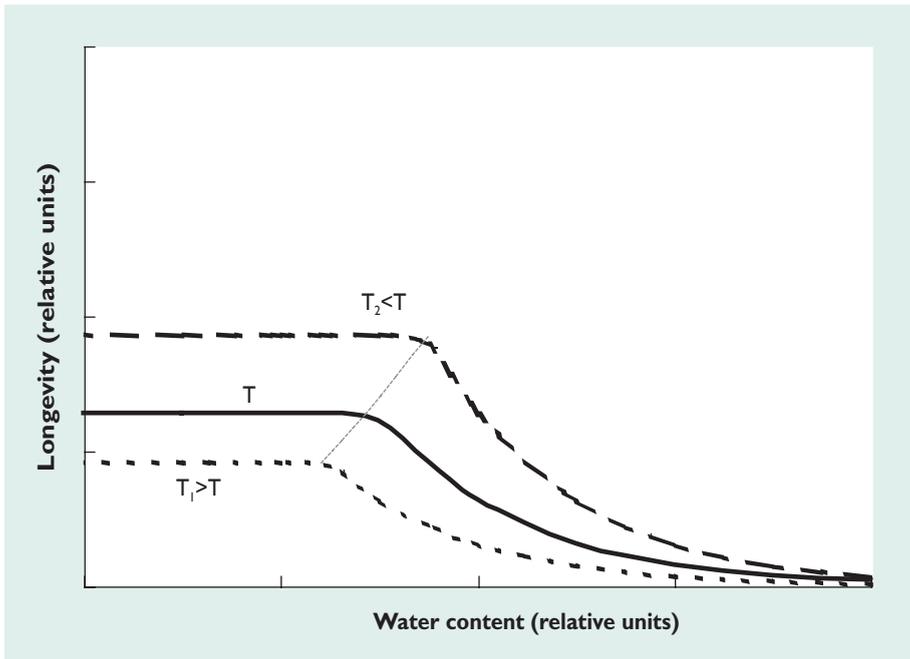
become curvilinear as seen for pollen ageing (Buitink 1998a). This scenario is most consistent with theoretical considerations and published data. It shows that the limited benefit of drying causes a limited benefit of cooling. Based on these considerations, it is conceivable that maximum longevity at two different temperatures is the same. This conclusion may sound similar to the basic tenet of ultra-dry technology (Zheng *et al.*, 1998; Walters, 1998a), but it is not. Ultra-dry technology, exemplified by data in Figure 36.1, argues that progressively drying seeds will give comparable longevity under non-refrigerated conditions as longevity achieved under refrigerated storage (and presumes that gene bank operators didn't dry their seeds prior to refrigeration). Ultra-dry technology does not invoke limitations in the benefits of temperature or interactions between critical water content and temperature, and proponents of the technology generally dispute the latter point altogether. The scenario in Figure 36.5 provides gene bank operators with some bad news. It may be impossible to extend seed longevity beyond a finite level. Further, the combination of drying and cooling may waste energy and provide no benefit,



**Figure 36.4** A schematic drawing of the effect of water content and temperature on seed longevity. The kinetics and critical water content for temperature  $T$  are identical to those given in Figure 36.3 and  $T_1$  and  $T_2$  are  $10^\circ\text{C}$  greater and less than  $T$  (as in Figure 36.3). In this scenario, the critical water content decreases with decreasing temperature, allowing longevity to more than double when temperature is reduced  $10^\circ\text{C}$ .

depending on the temperature range of storage. To maximise seed longevity and minimise energy consumption, gene bank operators need to know how steeply critical water content changes with temperature, and whether the change is constant among temperature ranges.

The relationship between critical water content and temperature is critical for optimising seed preservation protocols. However, gene bank operators should also be aware that critical water contents may actually be optimum water contents for a given temperature (Vertucci and Roos, 1990; reviewed by Walters, 1998b). Literally, this means that drying increases longevity to a point and then decreases longevity. Decreases in cellular viscosity with extreme drying provide a mechanistic explanation for damage by overdrying (Buitink *et al.*, 1998b). Data to support this finding have been collected at moderate storage temperatures (5 to  $45^\circ\text{C}$ ) (reviewed by Walters, 1998b; see also Buitink *et al.*, 1998a). There was sparse evidence of abbreviated life spans in extremely dry seeds stored at high temperatures ( $>50^\circ\text{C}$ ), perhaps because the dry conditions protected against protein denaturation occurring at high



**Figure 36.5**

A schematic drawing of the effect of water content and temperature on seed longevity. The kinetics and critical water content for temperature  $T$  are identical to those given in Figure 36.3 and  $T_1$  and  $T_2$  are  $10^\circ\text{C}$  greater and less than  $T$  (as in Figure 36.3). In this scenario, the critical water content increases with decreasing temperature, leading to a potential diminishing effect of temperature on seed longevity.

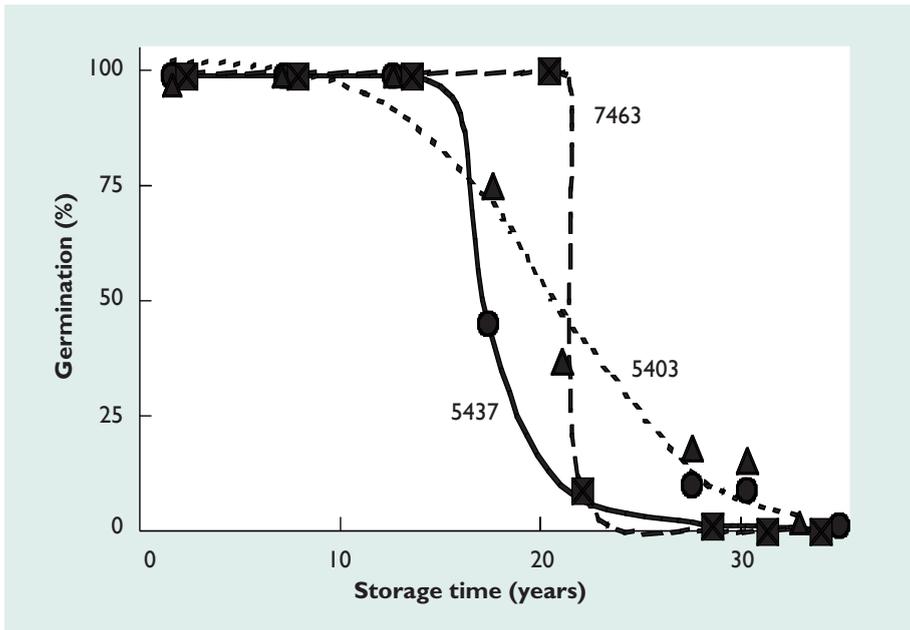
temperatures (Ellis *et al.*, 1988, 1989, 1990). We have also argued that optimum water contents interact with temperature analogously to the way critical water contents interact with water (Figure 36.5). Evidence for this hypothesis is also sparse since damaging effects of drying can only be detected in experiments that include treatments of sufficiently dried seeds (water contents much less than that achieved by equilibrating seeds at  $20^\circ\text{C}$  and 10% RH – see Vertucci and Roos, 1993; Ellis *et al.*, 1989, 1990). The presence of an optimum water content is an interesting phenomenon that may potentially compound the limited benefit of low temperature storage of extremely dry seeds (regardless of how water content and temperature interact).

## Seed Quality Revisited

This chapter argues that preserving seeds in gene banks is among the least expensive operations of *ex situ* conservation. However, there is a limit to gene bank operators' abilities to extend seed life spans by simply adjusting temperature or water content to lower and lower values. Huge expense and the risk of genetic change are incurred when seeds deteriorate and are subsequently regenerated.

The models used in this chapter rely on characteristic ageing rates for seeds. Mean longevities for domesticated species are well-documented (e.g., Priestley, 1986), and species coefficients have been empirically determined so that lifespans can be predicted for a range of storage conditions (Ellis *et al.*, 1988, 1989, 1990). However, there is wide variation in longevity among seed lots of a single species as a result of unknown seed "quality" factors (e.g., Kraak and Vos, 1987; Wiesner unpublished). Uncertainty in how long a specific seed lot will survive in storage makes it necessary to monitor seed viability during storage so that deterioration is detected before it is too late. One problem is defining the monitoring frequency. Loss of percent germination is a sigmoidal function with time and deterioration may be undetectable initially and then decline precipitously within the monitoring interval. This risk is illustrated by data of lettuce seed stored at  $-18^{\circ}\text{C}$  and monitored at 5 year intervals (Figure 36.6). All the accessions had germination  $< 25\%$  after 25 years of storage. However, germination of accession #5437 and #7463 dropped so abruptly after 16 and 21 years, respectively, that deterioration was undetected within the 5 year monitoring protocol. These data illustrate that average longevities for a species are helpful, but the point when signs of deterioration are obvious and the rate that deterioration proceeds after that are elements of seed quality that have yet to be addressed by gene bank operators.

Seed quality is the third parameter (after water content and temperature) that gene bank operators must contend with in order to optimise seed banking procedures. This nebulously-defined factor is under genetic and environmental control. Genetic aspects cannot be manipulated, as that would defeat the gene banks' purpose of preserving genetic integrity of samples and populations. However, genetic markers that indicate relative longevity of an individual or accession would provide better guidelines for setting monitoring and regeneration frequencies. Growing, harvest and post-harvest conditions can dramatically affect seed longevity (reviewed by Walters, 1998b). A better understanding of how seed provenance affects lifespans will lead to higher quality seeds and fewer regenerations, and ultimately optimised gene banking.



**Figure 36.6** Germination of 3 representative lettuce accessions (5403, 5437, and 7463) during 30 years of storage at 5°C (until year 9) and then -18°C.

## Conclusions

Developments from the 1960's to now have demonstrated that genetic diversity can be preserved in gene banks. In this millennium, the question has changed from “if” to “how.” Preservation of seeds in gene banks is one of a series of tasks that need to be optimised to ensure that valuable genetic resources are conserved in *ex situ* collections. Preservation schemes must be considered in the context of what and why germplasm is collected and options for regeneration in the event that stored germplasm ages before it is used.

Preservation of germplasm in gene banks usually involves manipulating the temperature and water content of seeds through environmental controls. The costs of various storage conditions and the benefit to seed longevity can be estimated. Risks of genetic changes with different storage conditions are less known, though these must be considered if gene banking practices are to be

optimised. Though provenance of collected seed is known to be an important factor in seed lifespans, gene bank operators have little control over the condition of the seeds they receive. An integrated approach to preservation protocols that considers seed quality as well and environmental conditions will lead to best gene banking practices.

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