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CONSERVATION ISSUES

Genebanking Seeds from Natural Populations

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ABSTRACT: Conventional storage protocols have been developed to preserve genetic diversity of seeds of crops in genebanks. These same principles have been applied to preserve seeds from wild populations. While most principles for conventional storage protocols are applicable to a broad range of wild species, seeds from wild populations are not amenable to some practices that assume high uniformity within the seed lot. Small sample sizes and high heterogeneity of seeds from wild populations demand greater a priori knowledge of characteristic longevity as well as new tools to monitor viability without germinating seeds. Some of the challenges handling seeds from undomesticated plants are exemplified from an experiment with sagebrush (*Artemisia tridentata*) seeds. Sagebrush seeds deteriorate very quickly at high humidity and moderately fast at room temperature. Rapid drying of seeds and immediate placement in the freezer might boost longevity. As with seeds from most wild species, there is insufficient knowledge of sagebrush seed storage traits to guide viability monitoring in the genebank.

Index terms: Artemisia tridentata, conventional storage, longevity, orthodox seed storage, temperature, wild seed storage

INTRODUCTION

Concerted Efforts to Genebank Wild-collected Seeds

Early efforts for seed genebanking focused mostly on crops that produced orthodox seeds. From these activities, we learned essential concepts about interrelationships between moisture and temperature that affect seed longevity (Justice and Bass 1978; Ellis and Roberts 1981; Priestley 1986; Walters 1998), within-species variation in seed aging rates (Walters et al. 2005; Probert et al. 2009), and exceptional behavior of some species preventing their storage using conventional methods (Ellis et al. 1990; Berjak and Pammenter 2008; Hay and Probert 2013; Walters et al. 2013). These accomplishments demonstrated the feasibility of using seed genebanks as an effective ex situ conservation strategy for many species.

Concerted efforts to sample wild populations for seeds began during the early part of last century with plant explorers such as N.I. Vavilov, F.N. Meyer and J.R. Harlan (Vavilov 1992). Targeted collections were most often land races of crops but also included wild congeners of crop species that provided genetic resources for crop improvement from secondary or tertiary genepools. These collections were the foundation materials used to identify "domestication traits"—traits selected by humans to make plants more suitable for cultivation, harvest, yield, storage or nutrition (Black et al. 2006). More recently, wild relatives of crops are valued for alleles, frequently masked by the wild phenotype, that can be used to enhance yield, stress tolerance, or food quality (Hajar and Hodkin 2007; McCouch et al. 2012). The importance of these genetic resources and their imperilment in natural environments as a result of human pressures has sparked renewed efforts to collect and preserve seeds of crop wild relatives ex situ (GCDT 2013; McCouch et al. 2013).

The onset of the new millennium brought realization of the feasibility and opportunities for developing seed collections of plants that are of ecological interest, but not necessarily agriculturally relevant. Successful seed collections of rare or threatened populations of plants supported the rallying cry for additional efforts to collect seeds of native flora (Guerrent et al. 2004; Merritt and Dixon 2011; Hay and Probert 2013). Seed collections also support research efforts, for example serving as "time capsules" of representative populations for future studies of evolutionary change (Franks et al. 2008). They also support restoration efforts that use native species (Merritt and Dixon 2011; Maschinski and Haskins 2012; BLM 2014). Many countries are developing national genebanks with the goal of making representative collections of their flora.

Collecting seeds from natural populations creates a snap-shot in time to provide the future with a physical sample of germplasm from plants that are adapting (or not) to current conditions. Population traits and associated habitat data provide unique and

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irreplaceable information about historical processes and future prospects for the population. Seed collections of wild plants are intrinsically more complex and expensive to make and manage compared to collections from cultivated plants. Collection sites may be remote and plant phenology and fecundity is as difficult to predict as the weather. Obtaining representative samples may require a priori knowledge of the populations. The smaller size, greater heterogeneity, upfront costs, and uniqueness of wild-collected seeds require careful handling in a genebank to ensure changes are not imposed by the genebanking experience or that the precious sample is not consumed in the process.

The availability of seeds from phylogenetically diverse species and broad ranging geographic distributions provides the opportunity to contrast storage behavior of seeds from cultivated and wild origins, and bring a broader understanding of the types of seed physiologies produced by plants around the world as well as the effects of habitat, growth environment, and climate change on seed traits (Tweddle et al. 2003; Walters et al. 2005; Probert et al. 2009).

The purpose of this paper is to provide a current and critical assessment of our knowledge of seed banking natural populations. We provide some general observations of limitations and pitfalls that have been encountered in our work preserving seeds from plants growing in the wild. There are large gaps in the general knowledge of the reproductive biology of specific species. Frequently, there is little existing documentation on major life history traits and pollination requirements, let alone information on seed traits such as maturation period, germination requirements, or storage behavior. In addition, there are general genebanking questions about how to optimize handling seeds collected from the wild, which are frequently characterized by having small sample size, heterogeneous quality, and unknown genetic composition or population boundaries. Until this information is available, we will need to draw upon generalizations gleaned from plants under cultivation or from case studies of wild species.

SEED STORAGE BEHAVIOR

Predictions of Seed Longevity and Seed Aging Kinetics in a Genebank

Underpinning the effectiveness of seed genebanks is the assumption that seeds can stay alive for a prolonged period so that a high quality sample will be available when needed. Storage environment (temperature and moisture) and species strongly affect longevity (Ellis and Roberts 1981; Walters et al. 2011; Hay and Probert 2013). "Conventional" storage uses stringent control of moisture conditions (20% RH) and temperature (-20 ºC, i.e., a conventional freezer) to maintain seed viability (FAO 2013). Under these conditions, genebank operators estimate that seed longevity may range from 50 to 400 years depending on species, but also assuming high quality seed at the outset (Ellis and Roberts 1981; Walters et al. 2004, 2005). This contrasts with 4 to 50 years predicted for seed survival in refrigerated storage at about 5 ºC and a RH of <50% (Justice and Bass 1978; Priestley 1986; Walters et al. 2004).

Extensive observation of seed aging kinetics (mostly of agronomic species stored under ambient or refrigerated conditions) illustrates why predicting seed lifespans in storage is so difficult (Figure 1). Freshly harvested seeds initially show no symptoms of aging until a threshold time, and then viability is lost quickly (e.g., Walters et al. 2010). The duration of that initial asymptomatic period is what we consider longevity. Currently we have no good tools that detect changes during early phases of seed aging; hence, the only way to measure longevity is retrospectively—that is, when viability of a seed lot declines, we learn how long it survived.

Incorporating data of measured seed longevity into models is currently the only way to give seed genebank or warehouse operators an "expiration date" for genebanked samples. "Ballpark" estimates of how long a species will survive in conventional storage should guide decisions on how to optimize physical and human resources during genebanking and to determine whether cryogenic storage, which presumably increases seed longevity (Dickie et al. 1990; Walters et al. 2004), is an economically viable alternative storage platform. Expected longevity modeled from species characteristics, pre- and

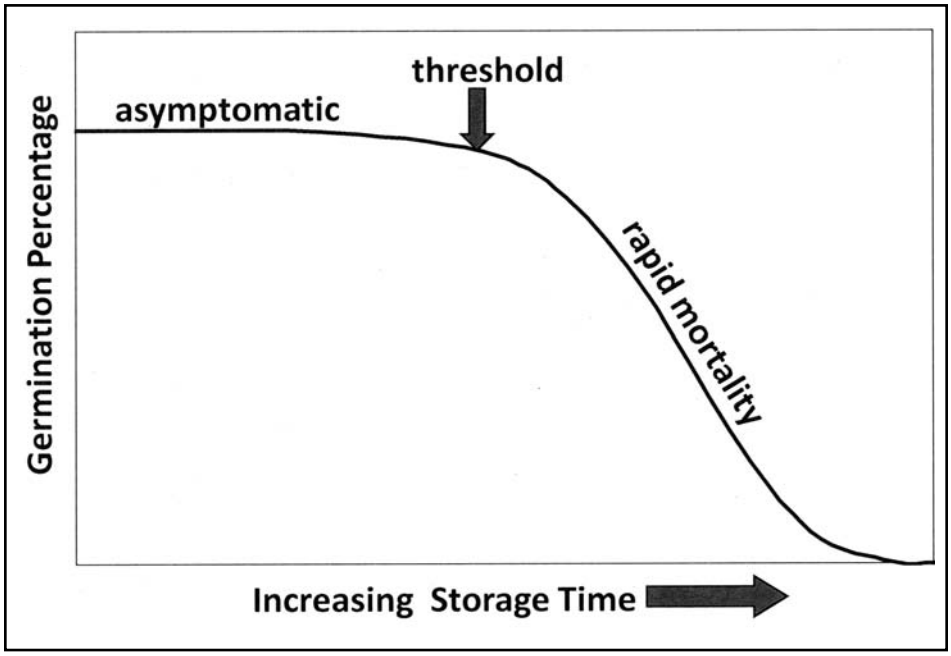

Figure 1. Schematic diagram of seed deterioration with time showing a period where aging is initially asymptomatic, but after a threshold, viability is lost rapidly. The duration of the asymptomatic period is defined as the longevity. Longevity is influenced by moisture and temperature of storage as well as traits of the seed that are regulated by growth environment and genetics.

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post-harvest treatments, and storage conditions is needed to develop biology-based intervals for monitoring, regeneration, and recollection as well as the timeframe to use stored seeds.

Existing models consider the effects of storage environment on known species tendencies. Seeds from many agronomic species have been characterized (Ellis and Roberts 1981; Walters et al. 2005); but, there is a dearth of information about seeds from wild species and an even greater lack of understanding about variation from average performance (Hay and Probert 2013). Some models are based on short-term observations of seed deterioration under poor storage conditions, and longevity under conventional storage conditions is predicted through extrapolation (Ellis and Roberts 1981). Another approach applies biophysical principles used in materials sciences to predict the kinetics of deterioration (Walters 1998; Walters et al. 2004, 2010; Ballesteros and Walters 2011). The two approaches give similar predictions of longevity under moderate storage conditions (i.e., near room temperature and ambient relative humidity); however, they diverge significantly as the storage conditions become colder or drier. Several experiments on freezer storage were begun around the world in the 1960s (e.g., Walters et al. 2004, 2005) and conventional freezer storage for seeds began in the late 1970s or early 1980s; hence, another 20 to 80 years are needed to validate longevity predictions. Even still, it is reassuring to know that survival of freezer-stored seeds remains high after 50 years (Walters, unpubl. data).

The actual shelf life obtained using current storage standards (FAO 2013) is hard to predict for any particular seed lot, even with a priori knowledge of seed longevity characteristics of the species. Seed longevity is considered a "complex trait," meaning that the phenotype is determined by interactions among many factors including genetics, growth environment, post-harvest processing, and storage conditions. Even when storage temperature and moisture are carefully controlled, high within-species variation in longevity underscores the uncertainty of predicting shelf life (Walters et al. 2005).

Because survival duration within species is highly variable, seed genebanks must monitor germination of stored seeds in prescribed intervals to determine whether viability has changed (FAO 2013). If the genebank operator does not have a priori knowledge of potential longevity of the seed lot, the monitoring intervals will be fairly arbitrary. If, for example, monitoring intervals are set at 20 years, a seed lot with longevity of 50 years might lose significant quality between monitoring intervals 2 and 3. If, on the other hand, the seed lot can survive for 200 years, a monitoring interval of 20 years will require 10 germination tests. Over-testing like this unnecessarily increases genebanking costs and depletes the sample. The consequences of consuming a sample by monitoring are disastrous if the sample is irreplaceable or difficult to regenerate because the value of the sample is lost and the cost of collection and maintenance can never be recouped.

Longevity Tendencies among Species Related to Life History Traits and Geographic Origin

One can expect to observe diverse responses to standardized storage conditions when genebanking genetic diversity. Hence, variation in seed longevity within and among species should be expected. Plants adapted to harsh environments have likely evolved survival strategies to the seeds they produce—especially if the sustainability of the population depends on it, as is the case for many annual plants. Plants that fruit in the spring or experience mild winters or short dry seasons may have lower tolerance to freezing and desiccation, or fleeting viability (Dussert et al. 2000; Farnsworth 2000; Dickie and Pritchard 2002). Even if a seed does not strongly express genes for stress tolerance, remnants of the traits may persist if the seed fill or maturation period is adequately long to allow cellular protectants to accumulate (Daws et al. 2004, 2006; Daws and Jensen 2011).

Based on environmental challenges and adaptive strategies, one might postulate that post-shedding behaviors in seeds vary considerably. The poles of this range are typically classified as "orthodox" and "recalcitrant" to convey the tendencies of seeds to tolerate or succumb, respectively, to desiccation and low temperatures (Berjak and Pammenter 2008). These innate differences in stress tolerance also dictate whether seeds can survive conventional freezer conditions, or if cryogenic conditions are required (Walters et al. 2013). In agricultural species, grains are the usual exemplars of orthodox seeds (e.g., corn and soybean), and tropical fruits provide examples of recalcitrant seeded species (e.g., citrus, mango, avocado, and cacao). The incidence of recalcitrance is dispersed among angiosperm families, with some families (i.e., Lauraceae and Fagaceae) having high incidence and other families (i.e., Asteraceae and Solanaceae) with no genera reported to have the recalcitrant trait (Dickie and Pritchard 2002). Several hardwood species of North America produce seeds that are considered recalcitrant, (i.e., oaks (*Quercus* spp.), silver maple (*Acer saccharinum* L.), and horsechestnut (*Aesculus glabra* Willd.)), and a few annual species from riparian habitat (i.e., wildrice (*Zizania* spp.) and water howellia (*Howellia aquatilis* A. Gray)) also share this trait.

The term "recalcitrant" is also commonly used in a horticultural context and has provided misleading information about whether a seed is difficult to store (current context) or difficult to germinate (USFWS 1991; Pomper et al. 2000; Tabak and von Wettberg 2008; Han and Long 2010). In our hands, "recalcitrant" germination is often associated with a rudimentary embryo that grows *in planta* post-shedding and/or a seed with fastidious germination cues (Walters and colleagues, unpubl. data). These seeds may also have limited desiccation tolerance (perhaps seeds from *Torreya* sp.) or fleeting longevity (e.g., seeds in Apiaceae); however, their physiology is probably better understood in the context of the seed dormancy literature (Baskin and Baskin 1998).

Seeds of several tree species of North America are classically categorized as recalcitrant (i.e., *Juglans*, *Carya*, *Corylus*, *Taxus*, *Torreya*, some palms), and this has impeded efforts to genebank seeds for ex situ conservation of genetic diversity. In

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our hands, seeds of these species exhibit sufficient desiccation tolerance for relatively easy genebanking, though cryogenic storage is recommended to protect against damage in the lipid fraction of the seed and increase cost-effectiveness. Potentially longer lifespans in cryopreserved seeds may also increase cost-efficiency of genebanking plants that are expensive to regenerate, such as those that are large and have long juvenile periods (e.g., trees) (Walters, unpubl. data).

Seed physiologies that do not quite fit the orthodox or recalcitrant paradigm were recognized in the early 1990s in a "catch-all" category of "intermediate" storage physiology (Ellis et al. 1990), with exemplar genera of *Citrus* (citrus), *Coffea* (coffee), and *Carica* (papaya). Further exploration of diverse species suggests that the intermediate category represents a number of syndromes of damage to seeds that are stored conventionally (Crane et al. 2006; Mondoni et al. 2011; Popova et al. 2013). At the National Center for Genetic Resources Preservation (NCGRP), we recognize several responses of seeds to low temperature and moisture that require adjustments to conventional storage protocols:

1. Recalcitrant seeds that cannot be dried sufficiently to avoid freezing damage (ice formation) when stored at -20 ºC.
2. Seeds that survive sufficient drying to avoid ice formation during -20 ºC storage, but crystallization of lipids tends to reduce viability.
3. Seeds that survive some drying, but do not survive the extreme drying of orthodox seeds; drying to less than 50% RH tends to increase mortality.
4. Seeds that survive drying and cooling but age rapidly regardless of the storage conditions.
5. Orthodox seeds that can be genebanked using conventional storage conditions and survive for long periods. Nonetheless, all seeds deteriorate in storage and even orthodox seeds will eventually succumb with time.
6. Seeds exhibiting different storage behaviors appear to be broadly distributed geographically.

For example, response #2 involving crystallization of lipids was discovered in species from the US Southwest that deposited oils with medium and long chain saturated fatty acids as food reserves into seeds (Crane et al. 2006). We now expect seeds from tropical origin to show this type of response as a result of accumulation of tropical oils. Intermediate tolerance to desiccation (response #3) appears regularly among congeners of tropical species (e.g., *Citrus* and *Coffea*) (Ellis et al. 1990; Dussert et al. 2000) and North American tree species (Walters, unpubl. data). Short-lived seeds (response #4) have been noted in alpine forbs and riparian trees (Mondoni et al. 2011; Popova et al. 2013). As mentioned previously, longevity varies considerably among seeds within the orthodox seed category (response #5). Species originating from drier climates tend to produce longer-lived seeds (Walters et al. 2005; Probert et al. 2009). As the science develops and we become familiar with the range of post-shedding seed physiologies beyond the orthodox-recalcitrant dichotomy, it is likely that the "one-size-fits-all" protocols of conventional storage for orthodox seeds will evolve into several protocols that minimize damage and maximize longevity (Hay and Probert 2013).

Within-sample and Within-species Variation

The implicit promise of seed genebanks is to maintain the genetic diversity of the sampled population ex situ. Accessions needing regeneration should be flagged when viability—measured as germination percentage plus viable, dormant seeds—falls below a critical value of 85% of original value or 65% viability, depending on genebank. This germination threshold is intended to minimize genetic changes in aging samples as well as ensure that sufficient "vigor" remains for stand establishment and reproduction. If the accession is highly homogeneous—a single genotype, such as in hybrids or inbred lines—the chances of genetic erosion during storage are negligible and efficient regeneration becomes the predominant issue. If the accession is highly heterogeneous, there is greater risk of genetic shifts in the sample during storage and regeneration through a range of mechanisms that include selection and drift (Richards et al. 2010). For example, phenological traits can affect seed maturity at time of harvest, which, in turn, can create a population segregating for shorter (immature seeds) and longer (mature seeds) life spans (Hay et al. 1997). For wild species, seed genebanking can result in genetic bottlenecks that narrow the range of germination, phenology, and growth habit traits in an accession, so it is very important to prolong viability and minimize mortality within the sample.

Longevity models seek to track progress of an aging seed sample along the asymptomatic phase as well as make comparisons among samples of the same species. If factors such as differences in seed maturity are accounted for, models may consider the asymptomatic phase of seed aging a period of slow and subtle decline as a result of random processes (Walters et al. 2010). Given enough statistical power (i.e., number of seeds in a viability test), a slight reduction in viability can be detected. In wild-collected samples that often have a finite number of seeds and were expensive to collect, using large subsamples to detect small changes in germination seems counter-productive, especially since germination results in wild-collected seeds can exhibit high levels of unexplained variation from assay to assay, perhaps due to subtle differences in germination treatments or requirements or variation in the percentage of empty or damaged seeds used in the assay. Complementary longevity models, based on biophysical considerations, consider the asymptomatic phase in terms of molecular movement and accumulation of damage within the seed (Walters et al. 2010; Ballesteros and Walters 2011). This approach looks for symptoms of aging using assays that do not require seeds to be germinated and may prove to give promising techniques that detect position along the asymptomatic time course with high sensitivity.

Identifying seed samples that tend to age rapidly would be a boon to the seed genebank operator who could then monitor aging progress in this smaller subset of samples with less concern for longer-lived counterparts. Some models,

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following from the idea that subtle losses in viability occur during the asymptomatic phase, predict seed samples with higher initial germination will survive longer than samples with lower initial germination. This led to the assumption that initial viability correlated with seed longevity (Ellis and Roberts 1981). The poor correlation between initial germination and germination after 40 years of storage for NCGRP's collection (Walters et al. 2005) suggests that the assumption is flawed, leading us to believe that the factors contributing to high initial germination are not the same as those that contribute to maintenance of germination capacity through time. We have speculated that maintenance is related to the accumulation of aging protectants (e.g., antioxidants) and low mobility of molecules under the particular storage conditions studied (Walters et al. 2010). Several labs have also demonstrated poor correlation between aging rates in seeds stored under humid and dry conditions (Niedzielski et al. 2009; Schwember and Bradford 2010; Ballesteros and Walters 2011), suggesting an interaction between seed lot and storage environment consistent with a complex trait.

New standards for storing seeds from domesticated plants contain many ambiguities such as "critical" moisture level, risks of overdrying, optimum drying protocols, and viability monitoring frequency (FAO 2013). In addition, most of the quality assessments (such as germination assays) currently used in genebanks around the world rely on uniformity and use models with some flawed assumptions. Most genebanks are unaccustomed to working with the inherently small sample sizes that are an occupational hazard of wild-collected seeds. Even with obvious contrasts between wild and domesticated plants, the principles and methods developed for preserving seeds from agronomic species are directly, and sometimes quite rigidly, applied to wild-collected seeds. With the growing knowledge that the post-shedding physiology in seeds from wild species is more diverse than seeds originating from domesticated plants, it behooves genebank operators to confirm that conventional seed storage methods are applicable to seeds that have not been exposed to domestication pressures (Hay and Probert 2013). New approaches are needed to predict longevity and monitor the progress of aging to ensure that seeds are used before their utility expires through lost viability or genetic erosion. These methods must accommodate the inherent heterogeneity, small size, and intrinsic value of samples that are likely irreplaceable.

Case study – sagebrush seed (Artemisia tridentata Nutt.*)*

An example of many of the principles discussed above can be illustrated with some simple storage experiments conducted using big sagebrush seeds, a species native to the western US. Sagebrush seeds are reputed to be short-lived, losing full viability within two to three years if stored under ambient conditions (Stevens et al. 1981; Meyer 2008; Karrfalt and Shaw 2013). Seed lots of sagebrush are frequently contaminated with inert material (80 to 90% of the volume is leaf, stem and floral parts), and considerable cleaning is required to maintain a sample suitable for genebanking and to allow comparisons of seed germination tests with time (Karrfalt and Shaw 2013). Even with extensive seed cleaning, large variation among tests is expected because of the high incidence of empty seeds, as is common in plants from Asteraceae (Figure 2, photo on right). In addition, sagebrush seeds show variable levels of dormancy, which can be relieved by cool stratification for different periods or after-ripening (Stevens et al. 1981; Meyer 2008). The high heterogeneity of the seed lot means that germination tests must use large sample sizes.

Sagebrush seeds are believed to contain high amounts of storage lipid. Lipid reserves are not correlated with short seed lifespans, as once suggested (Walters et al. 2005). However, lipids strongly influence water sorption characteristics, which ultimately affect optimum moisture level for seed storage in orthodox seeds as well as deterioration kinetics at nonoptimum water contents (Walters 1998). Water sorption isotherms of sagebrush seeds were constructed at 5 and 25 ºC using three seed lots and sample sizes of 15–25 mg after 95% of the inert material was removed (i.e., further cleaning of the sample in Figure 2 photo (left) to remove most leaf and floral parts) and using methods reported earlier in our lab (Figure 3). Isotherms of lettuce and sunflower are also provided for comparison (Walters 1998). At 25 ºC and RH < 50%, the water content of sagebrush seeds is similar to sunflower seeds, which contain 48% lipid, and lower than lettuce seeds, which contain 35% lipid, suggesting lipid content of sagebrush and sunflower are comparable. An interesting feature of sagebrush seed is the steep slope of the RH versus water content relationship at RH > 60% compared to the other seed samples. The rapid upswing in water content at high RH may portend samples that are particularly prone to deterioration when held at high RH. A sagebrush seed that contains 0.14 g H_2O/g dry mass (14%

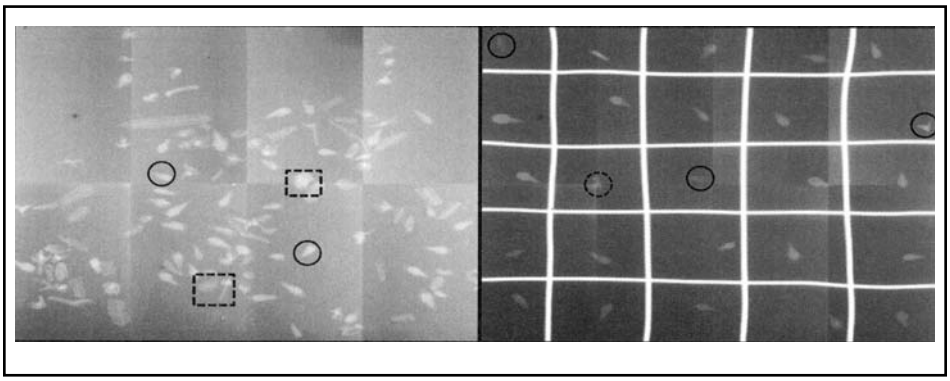

Figure 2. X-rays of a sagebrush seed lot at various stages of cleaning. The x-ray to the left is a seed lot that has been partially cleaned from the received sample containing >88% inert material. Encircled items are seeds, and items within the dashed square are leaves and flower parts. The x-ray in the right photograph shows cleaned seeds that were judged to be filled. The encircled seeds likely contain no embryo and are referred to as "empty." The seed within the dashed circle may be filled.

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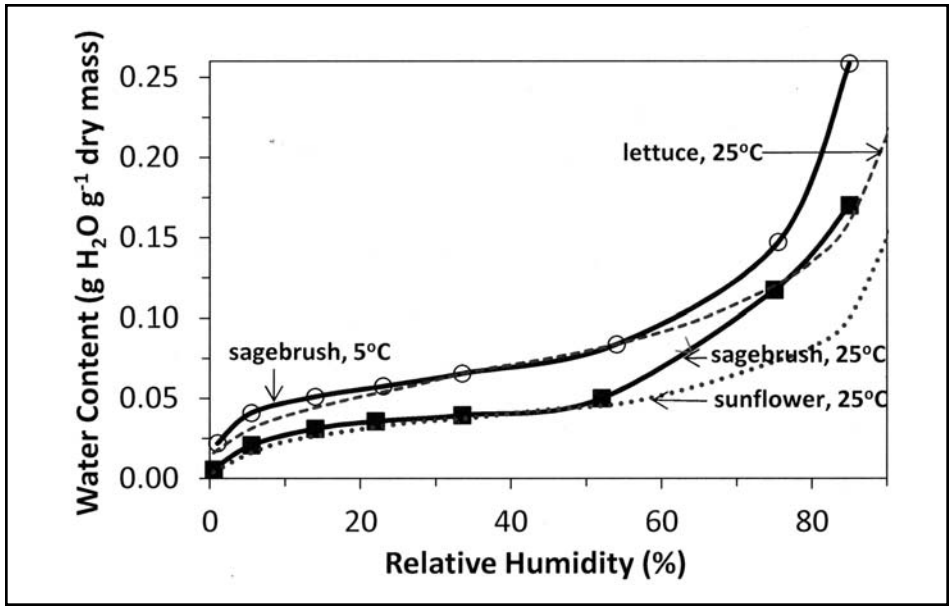

Figure 3. Isotherms of fully cleaned sagebrush seeds measured at 25 and 5 °C, showing the relationship between storage relative humidity and water content. Isotherms of lettuce and sunflower are provided for the purposes of comparison and were taken from Walters (1998).

water) will be in equilibrium with 80% RH, a humidity that supports microbial growth and degrading reactions within seed cells (Walters 1998). Changes in water content are oblique at RH between 15 and 55%, suggesting precise control of storage RH will be difficult in sagebrush seed.

We conducted storage experiments on two seed lots of Wyoming big sagebrush (*A. tridentata* ssp. *wyomingenis* Beetle & A. L. Young) (lots 130 and 161) and one seed lot of mountain big sagebrush (*A. tridentata* ssp. *vaseyana* (Rydb.) Beetle) (lot 164). Seeds were harvested in fall 2000, and viability was assessed in February 2001 at the Idaho State Seed Laboratory using tetrazolium staining. Viability was 95% (lot 130), 70% (lot 161), and 80% (lot 164). These seeds were stored under ambient conditions in Provo, Utah, until shipped to NCGRP a year later. In our hands, germination percent of these approximately 15-month-old seeds was 44, 38 and 55%, respectively, when samples of over 200 seeds were incubated at 20 °C with and without a stratification period until all seeds germinated or lost physical integrity (evidence that it is dead). Two additional seed lots of Wyoming big sagebrush had 59 and 33% viability according to February 2001 tetrazolium tests and 10 and 0% germination in February 2002. These deteriorated seeds were not used in further studies. Sagebrush seeds were stored at five temperatures (45, 25, 5, -20 (freezer), and -160 °C (vapor above liquid nitrogen)) and eight relative humidities (RH) ranging from 0.5 to 85%. RH was controlled using saturated salt solutions for 45, 25 and 5 °C treatments and RH was adjusted at 25 °C before placing seeds in foil laminate bags (Barrier Foils, UK) or cryovials for storage at -20 °C and -160 °C, respectively. Germination percentage was monitored periodically over the next 12 years using samples of 50 seeds. Representative deterioration curves are provided in Figure 4, showing substantial variation among individual assays (as predicted) and seed lots. The deterioration data are fit to an Avrami kinetic equation (Walters et al. 2004, 2005) so that we could calculate the time for deterioration to decrease to half the maximum (P50) (Table 1). Though seed lot 130 had the highest initial viability, seed lot 164 had the greatest shelf life.

The results presented here are roughly similar to those presented previously (Karrfalt and Shaw 2013), although we have covered a broader range of storage RH and temperatures over a longer period of time and have quantified a deterioration rate. Deterioration was observed in all treatments over the 12 years of the experiment, but the general trend towards increasing longevity with decreasing temperature and decreasing RH is observable (Table 1). Disappointing observations include lifespans of only 3–4 years (1100–1700 days) for sagebrush seeds stored in the

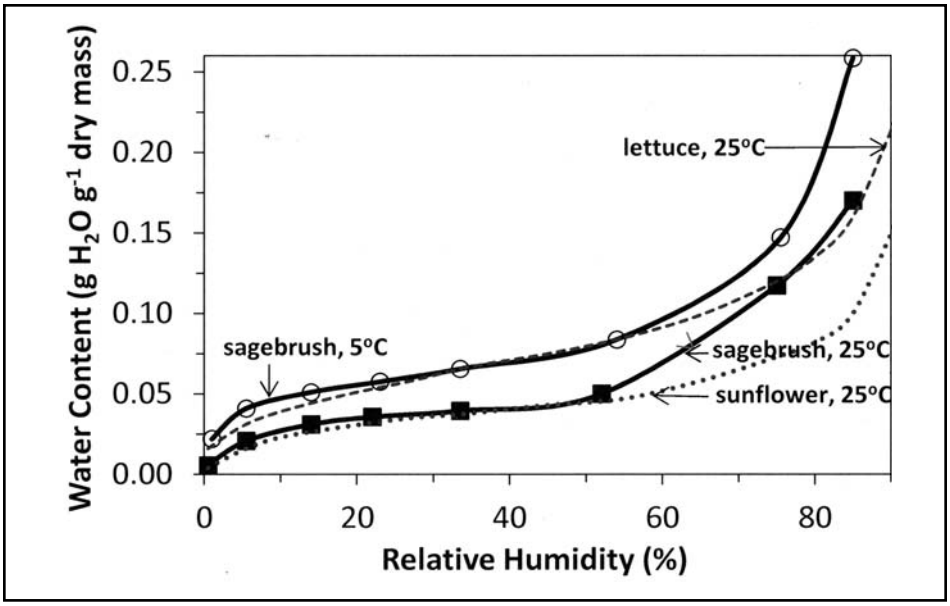

Figure 4. Deterioration time courses of three seed lots of sagebrush seeds that were stored at 5 °C and 5% RH (4% water content). Percent germination among sampling times is highly variable, likely a result of both fastidious germination requirements and uncertainty about seed fill. The seeds were received 15 months after harvest and so had already begun to deteriorate. Solid curves represent an Avrami kinetic model fit to the germination data. Dashed lines connect points to illustrate variation.

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Table 1. Rate of seed deterioration in sagebrush seed stored at a range of RH and temperatures. Deterioration is measured by the time for germination to decline to 50% of maximum, and rate (P50) is quantified as the time, in days, over which the decline occurs. Listed P50 values are averaged from experiments using three sagebrush seed lots.

	Relative Humidity Range								
Temp (°C)	ambient (15–50%)	0.5–1%	5–5.5%	11–14%	19–25%	33–34%	46–54%	74–75%	84–86%
45		122	196	153	111	50	48	9	2
25		274	607	211	279	224	241	10	3
5	575	605	783	508	714	1130	259	81	21
-18		772	882	774	1141	1719	986	25	19
-160		1112	2530	1688	1763	1519	1193	15	7

freezer and less than 7 years (2530 days) for seeds stored in liquid nitrogen. The non-sigmoidal kinetic and limited effect of temperature on aging rate is symptomatic of physiology #4 described earlier (i.e., rapidly aging seeds regardless of storage temperature). We also see this effect in deteriorating seeds that are subsequently placed in low temperature storage (Walters et al. 2005)—that is, once deterioration has started, it cannot be stopped, even at liquid nitrogen temperatures. Our tentative conclusions from this study are that sagebrush seeds survive longer if dried and stored at lower RH than prescribed by conventional storage and that seeds must be placed in long-term storage immediately after harvest in order to achieve longevities comparable to other species. With sagebrush, standard harvest and post-harvest practices may not provide the seed quality required for genebanking.

CONCLUSIONS

Genebanking seeds from wild species or natural populations is an effective tool for preserving genetic diversity and ensuring that these resources are available for research and successful reintroduction. Seeds from wild populations are more difficult to collect and samples are usually smaller and more heterogeneous than seeds collected from domesticated plants. In addition, there is a broader range of responses to storage conditions encountered among species from undomesticated plants. Managing the collections of wild seeds requires greater knowledge about structure of the plant populations, biology of the seed, and handling methods that accommodate heterogeneous samples. Right now, collecting seeds and characterizing plant traits is receiving focus. To successfully preserve these valuable accessions, we need better tools to predict longevity and monitor changes in quality on very small samples. Investment in genebanking procedures is critical to realize the promise of ex situ conservation for US native species.

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