Figure 1. Wyoming big sagebrush (*Artemisia tridentata* ssp. *wyomingensis*) growing at the 45 Ranch in southern Idaho. Photo by Matthew Fisk, USDA Forest Service

Banking Wyoming big sagebrush seeds

Robert P Karrfalt and Nancy Shaw

ABSTRACT

Five commercially produced seed lots of Wyoming big sagebrush (*Artemisia tridentata* Nutt. var. *wyomingensis* (Beetle & Young) S.L. Welsh [Asteraceae]) were stored under various conditions for 5 y. Purity, moisture content as measured by equilibrium relative humidity, and storage temperature were all important factors to successful seed storage. Our results indicate that no losses in germination will occur for at least 5 y if seeds are cleaned to 66 to 80% purity, dried to 30% equilibrium relative humidity, sealed in moisture-proof containers, and kept frozen at temperatures ≤ 8 °C. Because they can deteriorate rapidly, Wyoming big sagebrush seeds should be dried, cleaned, and placed in storage soon after harvest.

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KEY WORDS
Artemisia tridentata ssp. *wyomingensis*, equilibrium relative humidity, germination, seed storage requirements, seed viability, Asteraceae

NOMENCLATURE
Plants: USDA NRCS (2012)
Birds: ITIS (2012)

Conversions

°C	–20	–8	2	10	15	20	22	105
°F	–4	18	36	50	59	68	72	221

Big sagebrush (*Artemisia tridentata* Nutt. [Asteraceae]) habitat (Figure 1) has been greatly reduced or degraded during the last 100 y resulting in major ecological impacts. One area of current interest is the effect of habitat loss on Greater Sage-Grouse (*Centrocercus urophasianus* Bonaparte [Phasianidae]). The range of this species has been reduced to 56% of its pre-settlement status (Connelly and others 2004) and in some areas, such as eastern Washington, it currently occupies only 8 to 10% of its former range (Stinson and others 2004). In December 2011 the USDA Forest Service and the USDI Bureau of Land Management announced plans for conservation of Greater Sage-Grouse and its habitat. Many other species are also being impacted by the diminishing sagebrush biome. Consequently, the need for sagebrush seeds and seedlings is great and likely will increase as restoration efforts intensify.

Sagebrush seeds are harvested from wild stands, and good seed crops are produced at only irregular intervals because of drought, browsing by ungulates, insect predation, and other factors (Meyer 2008). Stevens and others (1981) reported that sagebrush seed viability declined after only 2 or 3 y of storage under ambient conditions in a warehouse. Meyer (2008) recommended storage at moisture contents below 10% and temperatures below 10 °C to prolong seed viability. This study was initiated to more clearly define the limits of seed storage parameters on sagebrush seed longevity. Results provide land managers with practical guidelines for managing and banking seeds in good crop years to improve the availability of regionally adapted seeds for restoration.

TABLE 1

Seed lot descriptions: seed source, purity, and initial moisture content (MC), equilibrium relative humidity (ERH), and germination.

State	County	"High" purity (%)	MC (%)	ERH (%)	Germination (%)
California	Lassen	80	6.6	32.5	48
Idaho	Twin Falls	71	6.8	39.0	35
Nevada	Ely	75	7.3	39.4	56
Nevada	Elko and Humboldt	80	7.4	44.7	52
Utah	Sanpete	66	7.5	37.4	61

State	County	"Low" purity (%)	MC (%)	ERH (%)	Germination (seedlings/g)
California	Lassen	18	13.5	67.7	48
Idaho	Twin Falls	27	9.1	39.2	248
Nevada	Ely	27	14.2	70.6	0
Nevada	Elko and Humboldt	15	14.9	72.2	0
Utah	Sanpete	18	14.2	72.6	0

MATERIALS AND METHODS

In 2006, we purchased 5 seed lots of Wyoming big sagebrush (*Artemisia tridentata* Nutt. var. *wyomingensis* (Beetle & Young) S.L. Welsh) harvested from wildland stands in the Great Basin during fall 2005 (Table 1). The purities of these seed lots were typical of commercially available seeds and ranged from 15 to 27% (Table 1). Figure 2 shows 4 levels of sagebrush seeds from unprocessed to "low" purity to "high" purity to 100% pure seeds.

Each seed lot was divided and one-half was cleaned at the USDA Forest Service Lucky Peak Nursery near Boise, Idaho, to "high purity," which ranged from 66 to 80% (Table 1). The resulting 10 sub-lots (2 from each seed lot) were sent to the USDA Forest Service National Seed Laboratory, Dry Branch, Georgia. Each sub-lot was mixed and divided into 16 equal samples following the International Seed Testing Association (ISTA 2013a) rules for mixing and dividing seed lots (see Figure 3). The 16 samples were then randomly divided into 4 groups of 4 fractions each. Seeds of each fraction were spread into a thin layer in an open container. Each group of 4 fractions was equilibrated at one of 4 relative humidities (30, 40, 50, and 70%) by placing the group in ambient conditions in and around the laboratory that approximated the target humidity. Once seeds were believed to have equilibrated with the ambient relative humidity, that is, reached equilibrium relative humidity (ERH), ERH was verified using a hygrometer (Colas and others 2010; Karrfalt 2010) (Figure 4). The 4 samples for each species, purity sub-lot, and ERH level were then sealed in 6 mil polyethylene bags (polybags) to maintain the specified ERH and stored at 20, 2, –8, or –20 °C, with one exception: the treatment combination of ERH 70% and temperature –20 °C was not tested as seeds at this high moisture content were not expected to survive. Therefore, we had 15 treatment combinations of moisture (30, 40, 50, and 70% ERH) and temperature (20, 2, –8, or –20 °C) in each purity level (low, <15 to 27%; high, 66 to 80%) for each seed lot.

Germination was tested at 15 °C following the Association of Official Seed Analysts rule for big sagebrush (AOSA 2011). High purity seed lots were tested using 4 samples of 100 seeds each, and low purity seed lots were tested using 4 weighed samples of approximately 1 g (0.04 oz) each. We used weighed samples because weighing is much faster and follows the International

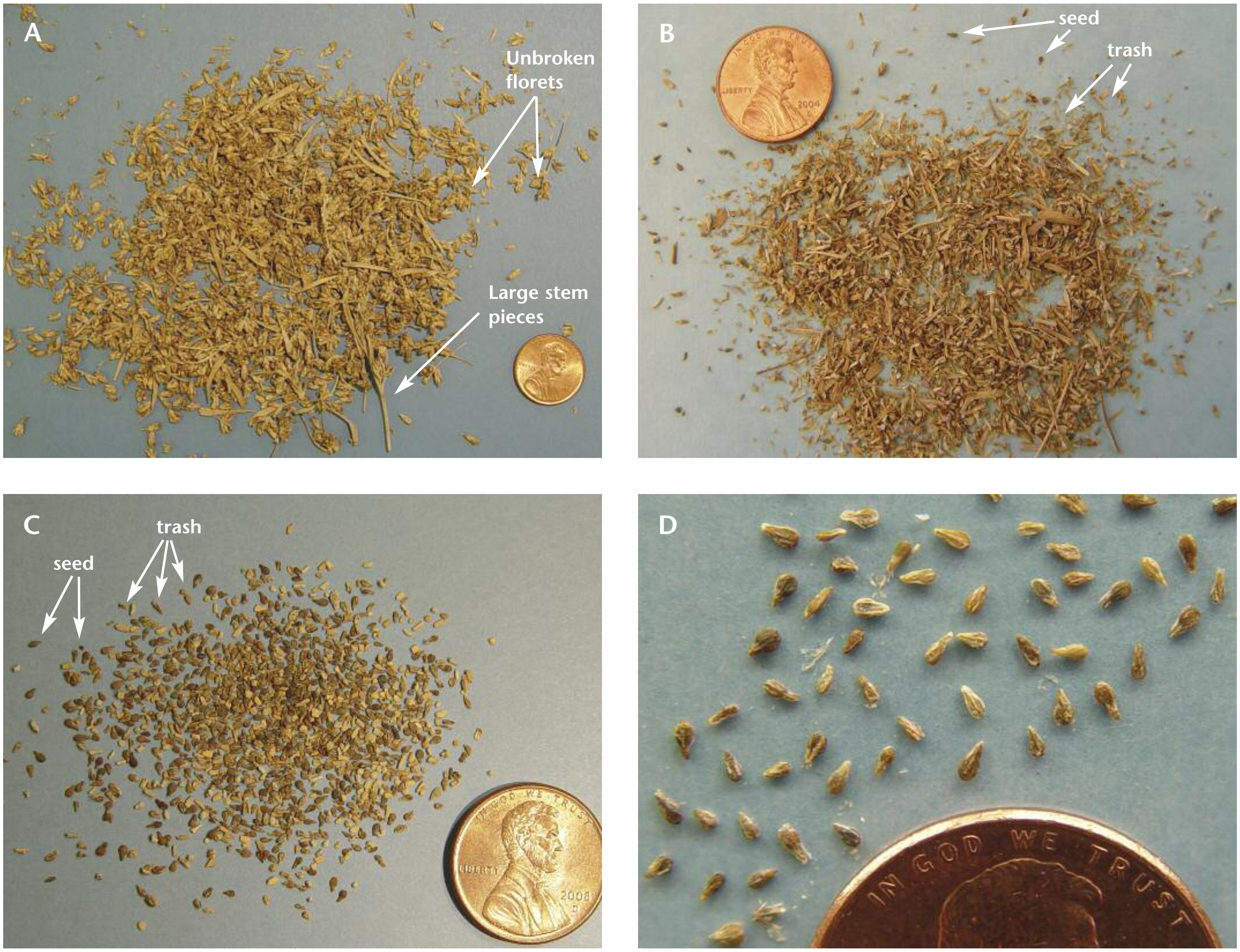

Figure 2. Various stages of cleaning a seed lot of Wyoming big sagebrush, from unprocessed (A) to "low" purity (B) to "high" purity (C) to 100% pure seeds (D). Photos by Matthew Fisk, USDA Forest Service

Rules for Seed Testing (ISTA 2013a) for other low purity, small-seeded genera, such as *Eucalyptus* L'Hér (Myrtaceae).

Germination of high purity seeds was retested after 3, 6, 15, 29, 41, and 60 mo; retesting began on low purity seeds at 6 mo. ERH of each fraction was tested before each germination test to ensure ERH had been maintained. At all storage periods (except 60 mo), germination tests on high purity seeds were conducted using 50 seeds per replicate, which we felt was adequate to observe trends in germination. At 60 mo, 100-seed replications were used to make a best estimate of viability.

Germination after 60 mo was compared to initial germination using the International Seed Testing Association Germination Tool Box (ISTA 2013b). The Tool Box was specifically designed for this type of comparison, and following Miles (1963) it uses the standard deviation of a binomial distribution and the Studentized range distribution to construct a 95% confidence interval for test results.

To compare this study with previous prescriptions for sagebrush seed storage, a comparison of ERH was made to moisture content. We used saturated salt solutions in 8 small chambers to obtain 11, 22, 33, 43, 53, 76, 84, and 100% relative humidity (Greenspan 1977). A 1-g (0.04-oz) sample from each of the 5 high purity sub-lots was placed into each chamber for 24 h at 22 °C to equilibrate to the chamber's relative humidity. At the end of the equilibration period, ERH of each sample was measured using a Rotronic Hygropalm water activity meter (Rotronic Instrument, Hauppauge, New York), and its moisture content was determined by oven drying at 105 °C (ISTA 2013a).

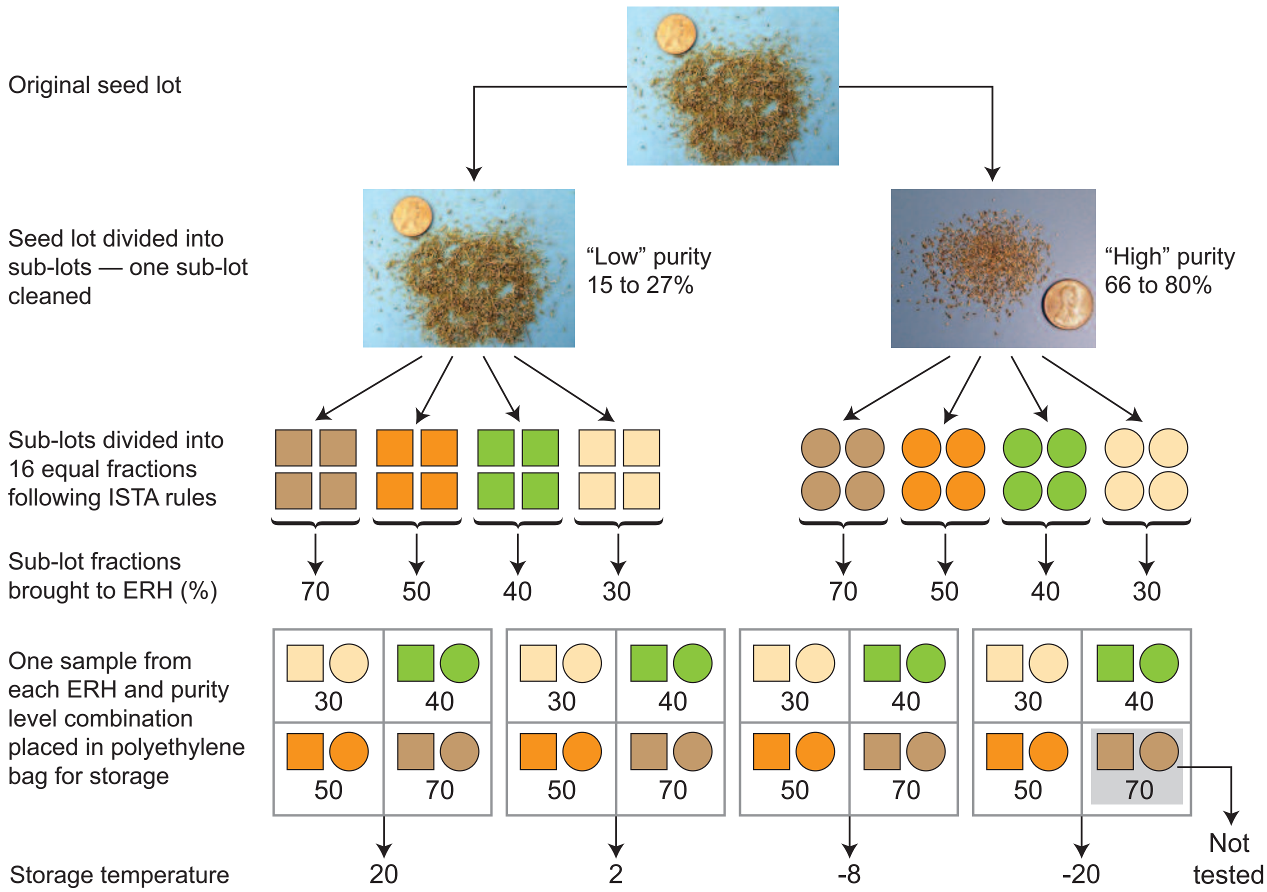

Figure 3. Schematic of experimental design used to test each of 5 seed lots of Wyoming big sagebrush seeds at 2 levels of purity (low, <15 to 27%; high, about 66 to 80%), dried to 4 equilibrium relative humidity (ERH) levels (30, 40, 50, or 70%), and stored at 4 temperatures (20, 2, –8, or –20 °C). Photos by Matthew Fisk, USDA Forest Service; figure design by Jim Marin Graphics

Figure 4. Equilibrium relative humidity (ERH) of seeds is measured with a hygrometer sealed in a test chamber with the seeds. Photo by Robert P Karrfalt, USDA Forest Service

Storage Bag Comparison

Foil bags provide a better physical barrier to moisture than polybags, and are, therefore, often used for marketing high-priced vegetable and flower seeds and for storing samples of valuable germplasm. To determine whether the foil laminate bags provided an advantage over the 6 mil polybags, we equilibrated samples from each of the 5 high purity sub-lots at 30% ERH and 40% ERH, placed half of each sample in a polybag and half in a foil laminate bag, and stored them at –20 °C for 60 mo.

RESULTS AND DISCUSSION

Initial Seed Lot Conditions

Initial germination tests conducted at the National Seed Laboratory revealed that 3 of the low purity seed lots were no longer viable (see Table 1), most likely because their moisture content exceeded 14%, which is high and deleterious to seed viability in storage. Therefore, these treatments were not used in the storage study. No attempt was made to remove immature or aborted seeds from high purity seed lots beyond what might have occurred during the cleaning process. Therefore, baseline viabilities of these seed lots ranged from 35 to 61%.

Adjusting the seed moisture levels to the desired ERH values for storage required less than 2 h, indicating that Wyoming big sagebrush seeds absorb moisture rapidly and that they must be managed carefully to prevent deterioration. This also indicates that Wyoming big sagebrush seeds can likely be dried quickly to preserve viability following harvest.

ERH and Moisture Content

ERH at 22 °C was highly correlated ($R^2 = 0.99$) with moisture content and followed an exponential curve (Figure 5). The 3 driest ERH levels in this study (30, 40, and 50%) corresponded, respectively, to moisture contents of 7, 7.5, and about 8%, well within the existing moisture content recommendations for storing seeds of big sagebrush (Meyer 2008) and other orthodox woody plants in sealed containers (Bonner 2008).

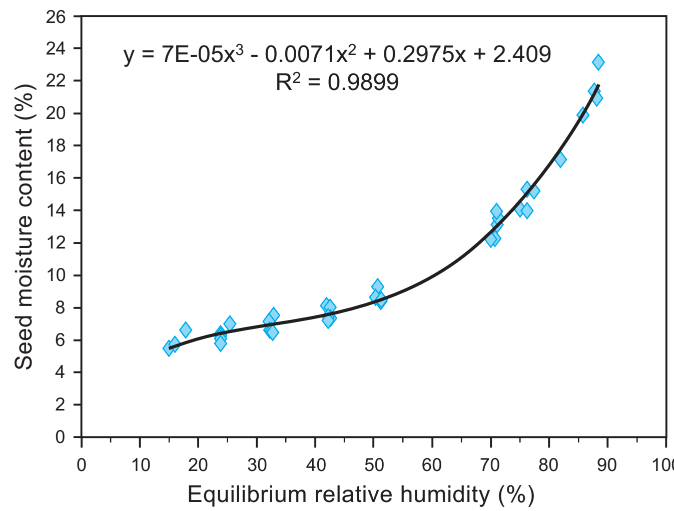

Figure 5. Equilibrium relative humidity (ERH) at 22 °C plotted against Wyoming big sagebrush seed moisture content. Each diamond represents a high purity seed lot (5) x relative humidity (11, 22, 33, 43, 53, 76, 84, 100%) combination ($n = 40$).

TABLE 2

Germination of low purity seed lots of Artemisia tridentata *ssp.* wyomingensis.

		California				Idaho			
		Storage temperature (°C)							
ERH (%)	Storage (mo)	20	2	–8	–20	20	2	–8	–20
	0	**48**	**48**	**48**	**48**	**248**	**248**	**248**	**248**
30	6	5	69	57	73	330	247	290	297
	15	6	45	65	46	95	282	294	235
	29	NT	35	81	75	NT	166	99	86
40	6	0	10	6	4	0	42	27	44
	15	0	4	6	2	0	5	29	35
	29	NT	1	11	8	NT	0	39	32
50	6	0	2	2	0	0	13	5	16
	15	0	1	2	1	0	1	8	4
	29	NT	0	3	4	NT	0	8	10
70	6	0	0	1	NT	0	126	105	NT
	15	0	0	1	NT	0	73	121	NT
	29	NT	0	0	NT	NT	2	2	NT

Notes: Initial (0 mo storage) germination (expressed as numbers of seedlings per gram of seeds presented in **bold**) of low purity seed lots from California and Idaho and following storage at 4 levels of equilibrium relative humidity (ERH) for 6, 15, and 29 mo. NT= germination not tested.

Effects of Purity

For low purity seeds equilibrated at 30% ERH, the California source maintained germination at –8 and –20 °C after 29 mo while the Idaho seeds maintained germination at 2, –8, and –20 °C through 15 mo (Table 2). After only 6 mo of storage, germination of low purity seeds equilibrated at all higher ERH levels had declined by 49 to 100%, across all temperatures (Table 2). Consequently, germination testing was discontinued on low purity seed lots after 29 mo.

The limited longevity of low purity Wyoming big sagebrush in storage may be explained 2 ways. First, the impurities are moisture sinks. In the 40% ERH treatments, high purity seed lots had moisture contents close to 7%, whereas those with low purity had moisture contents of 9% (see Table 1). Further, when the initial ERH of low purity seed lots from California, Nevada (Ely, Elko, and Humboldt), and Utah is compared to the predicted curve of ERH and seed moisture content (Figure 5), the actual moisture content is 2 to 3 percentage points higher than expected (14 or 15% rather than 12%) demonstrating that impurities hold more moisture at the same ERH than do the seeds. Therefore, impurities provide a high-moisture environment for the seeds, contributing to their deterioration. Second, volatile compounds in the impurities (mainly flower, leaf, and stem parts) can inhibit big sagebrush seed germination (Groves and Anderson 1981). This may be particularly true when seeds are stored in the sealed polybags.

In addition to improving germination, cleaning seeds to higher purity levels can reduce storage volume by at least 10-fold, thus requiring less cold storage space. Therefore, cleaning seeds to a relatively high purity (about 80%) appears to be a requisite first step to banking Wyoming big sagebrush seeds. Fortunately, cleaning to a high purity is easily accomplished (Fleege 2010).

Effects of Temperature and Moisture on Seed Lots with High Purity

Storage at 70% ERH

Seeds at this moisture level did not store well. Deterioration was slower as storage temperature decreased. Seeds stored at 20 °C lost viability within 3 mo, while seeds stored at 2 or –8 °C were mostly nonviable within 29 mo (Figure 6A).

Storage at 50% ERH

Declines in germination were less at this moisture level than at 70% ERH, but loss of germination still occurred at most temperatures (Figure 6B). At 2 °C, germination was maintained for about 1 y, but by 60 mo most germination was lost. Even at –8 °C, germination significantly decreased for all seed lots except Nevada (Elko and Humboldt) (Table 3). Compared with initial germination test results, storage at –20 °C reduced germination in 2 seed lots, increased it in one lot, and maintained it within tolerance levels for 2 seed lots (Table 3).

Storage at 40% ERH

Although maintenance of germination was stronger at this level than at the first 2 ERH levels, germination loss after storage at 20 °C was similar to that observed at 50% ERH. At 2 °C, the decline in germination occurred more slowly but was ultimately too high for seed banking. Germination was maintained well at –8 °C through 41 mo of storage, but by 60 mo, 3 of the 5 seed lots showed losses of 10 or more percentage points amounting to the loss of one-fifth to one-half of the viable seeds. At this ERH, storage at –20 °C yielded the best germination after 60 mo (Figure 6C; Table 3).

Storage at 30% ERH

Of the 4 ERHs tested, this level yielded the best results. Similar to the other ERH levels, loss of germination was rapid at 20 °C, but loss of viability was slower at 2 °C than at the other levels. Both –8 and –20 °C maintained germination within tolerance of the initial test values, with one exception each (Figure 6D; Table 3).

Seed Storage Bag

We measured ERH each time germination was tested during the 60-mo period and we detected no changes in ERH. At 60 mo, germination was significantly different in only 3 of 10 comparisons, and for all 3 the germination was lower for seeds held in foil bags (Table 4). Therefore, in our trials, polybags performed as well as the foil laminate bags, and we see no benefit to using the more expensive foil bag.

MANAGEMENT IMPLICATIONS

Wyoming big sagebrush seeds are small and generally do not exhibit dormancy (Meyer 2008). Therefore, managers must be careful to preserve seed quality, especially as we observed that seed moisture content can fluctuate rapidly, and that increases in seed moisture content during storage will lead to some loss of germination. Our results show that purity, storage ERH, and storage temperature were important factors in preserving germination and that all 3 factors interacted. Low purity was detrimental to germination but could be partially offset by low ERH and storage below freezing temperatures. Our data clearly show that cleaning seed lots promptly after harvest should contribute to seed longevity, and clean seed lots require less storage space.

Both the ERH levels and the temperatures we used were within acceptable storage ranges, but ERH of 40 and 50% still required the coldest temperature (–20 °C) to prevent significant germination losses (see Table 3). This requirement for the lowest storage temperature demonstrates the need to carefully

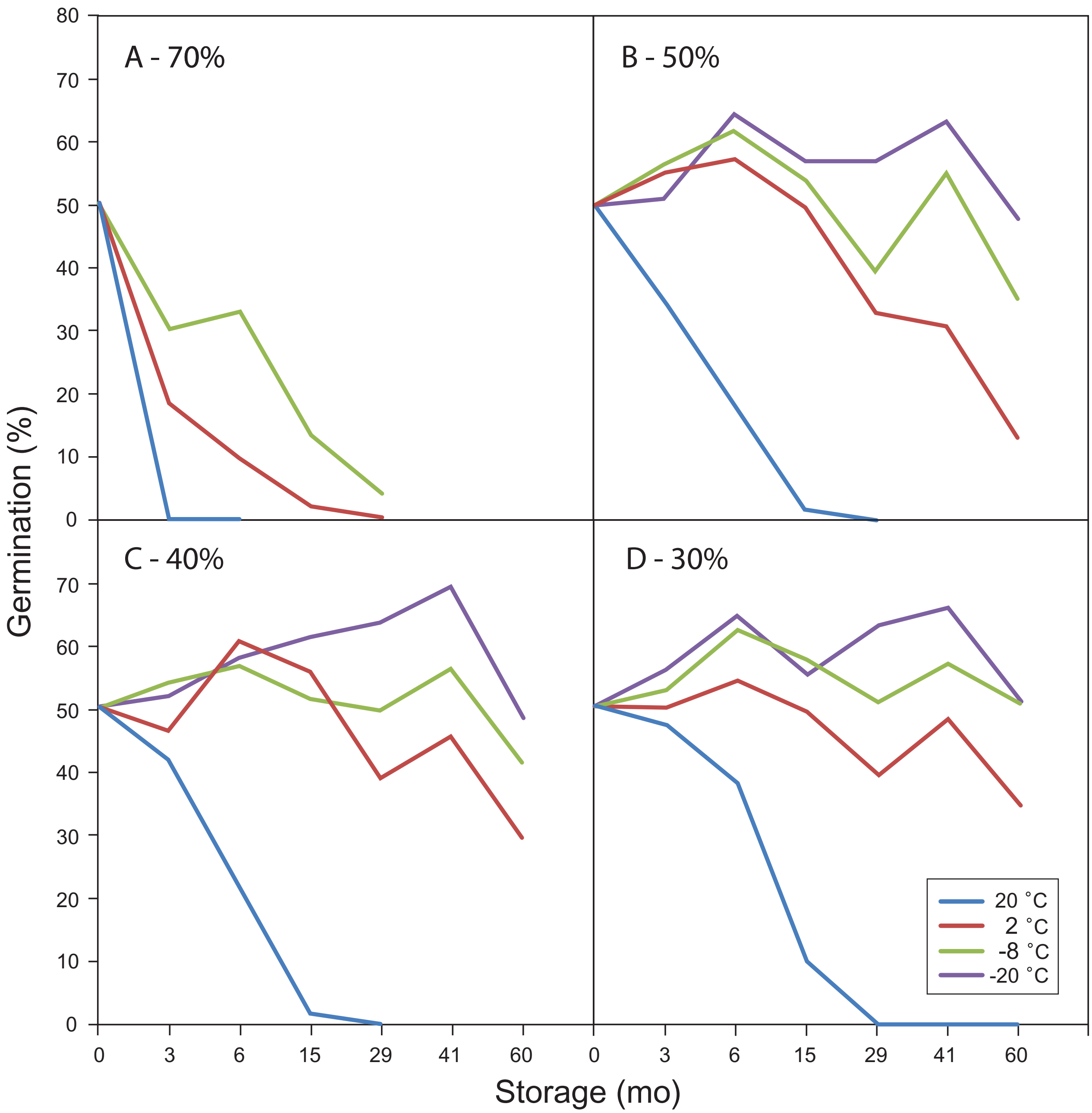

Figure 6. Average germination of 5 high purity seed lots of Wyoming big sagebrush stored at 4 storage temperatures (20, 2, –8, –20 °C) and 4 equilibrium relative humidity (ERH) levels: 70% (A), 50% (B), 40% (C), and 30% (D). Seeds in the –20 °C and 70% ERH level were not tested.

TABLE 3

Change in germination for 5 seed lots of Artemisia tridentata *ssp.* wyomingensis.

Seed source	Initial germination (%)	Storage temperature (°C) 2, ERH 50	2, ERH 40	2, ERH 30	–8, ERH 50	–8, ERH 40	–8, ERH 30	–20, ERH 50	–20, ERH 40	–20, ERH 30
California	**48**	–37*	–20*	–25*	–26*	–11*	0	0	+10*	–4
Idaho	**36**	–30*	–10*	–3	–18*	–5	+5	–8*	–10*	–8*
Nevada (Ely)	**56**	–45*	–16*	–19*	–16*	–12*	–9*	–4	–3	+10
Nevada (Elko and Humboldt)	**52**	–25*	–24*	–16*	+12*	–2	+8	+9*	–8	+10*
Utah	**61**	–50*	–35*	–15*	–28*	–15*	–2	–9*	+3	–4

Notes: Change in germination (+ or – and expressed as percentage points) compared with initial germination (0 mo storage in **bold**) for 5 seed lots stored for 60 mo under 9 combinations of equilibrium relative humidity (ERH) and temperature. Asterisks indicate significant ($p \leq 0.05$) differences in germination between germination at 0 and 60 mo within each ERH, temperature, and seed lot combination.

control storage conditions for Wyoming big sagebrush seeds. Ambient or refrigerated storage, even when humidity is controlled, will not provide long-term preservation of viability.

Therefore, for long-term storage (up to 5 y) of Wyoming big sagebrush seeds, we currently recommend prompt cleaning to a high purity (≥80%), drying to 30% ERH, sealing in a moisture-proof container (for example, 6-mil polyethylene bag) to maintain the low moisture content, and storing at temperatures ≤ –8 °C. This experiment continues and future germination results might improve this recommendation.

TABLE 4

Germination of 5 seed lots of Artemisia tridentata *ssp.* wyomingensis *in 6-mil polyethylene bags (polybag) compared with foil laminate bags.*

Seed lot	30% ERH Polybag	30% ERH Foil bag	40% ERH Polybag	40% ERH Foil bag
California	44	41	58	38*
Idaho	28	35	26	30
Nevada (Ely)	66	59	53	51
Nevada (Elko and Humboldt)	62	43*	44	44
Utah	57	52	64	43*

Notes: Germination of 5 seed lots at either 30 or 40% equilibrium relative humidity (ERH) when stored in 6-mil polyethylene bags (polybag) or foil bags at –20 °C for 60 mo. Asterisks indicates significant ($p \leq 0.05$) differences in percentage germination between bag types for a single seed lot and ERH level.

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