Investigating Seed Longevity of Big Sagebrush
(Artemisia tridentata)

Open-File Report 2009–1146

U.S. Department of the Interior
U.S. Geological Survey
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By Upekala C. Wijayratne and David A. Pyke

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Conversion Factors
SI to Inch/Pound

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Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

°F = (1.8 × °C) + 32

Temperature in degrees Fahrenheit (°F) may be converted to degrees Celsius (°C) as follows:

°C = (°F - 32) / 1.8.

Datum
Vertical coordinate information is referenced to the North American Vertical Datum of 1988 (NAVD 88).

Acronyms and Abbreviations

ANOVA  analysis of variance
h      hour
min    minute
NMS    Nonparametric multidimensional scaling
s      second
TZ     tetrazolium
Investigating Seed Longevity of Big Sagebrush 
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**Executive Summary**

The Intermountain West is dominated by big sagebrush communities (*Artemisia tridentata* subspecies) that provide habitat and forage for wildlife, prevent erosion, and are economically important to recreation and livestock industries. The two most prominent subspecies of big sagebrush in this region are Wyoming big sagebrush (*A. t. ssp. wyomingensis*) and mountain big sagebrush (*A. t. ssp. vaseyana*). Increased understanding of seed bank dynamics will assist with sustainable management and persistence of sagebrush communities. For example, mountain big sagebrush may be subjected to shorter fire return intervals and prescribed fire is a tool used often to rejuvenate stands and reduce tree (*Juniperus* sp. or *Pinus* sp.) encroachment into these communities. A persistent seed bank for mountain big sagebrush would be advantageous under these circumstances.

Laboratory germination trials indicate that seed dormancy in big sagebrush may be habitat-specific, with collections from colder sites being more dormant. Our objective was to investigate seed longevity of both subspecies by evaluating viability of seeds in the field with a seed retrieval experiment and sampling for seeds *in situ*. We chose six study sites for each subspecies. These sites were dispersed across eastern Oregon, southern Idaho, northwestern Utah, and eastern Nevada. Ninety-six polyester mesh bags, each containing 100 seeds of a subspecies, were placed at each site during November 2006. Seed bags were placed in three locations: (1) at the soil surface above litter, (2) on the soil surface beneath litter, and (3) 3 cm below the soil surface to determine whether dormancy is affected by continued darkness or environmental conditions. Subsets of seeds were examined in April and November in both 2007 and 2008 to determine seed viability dynamics. Seed bank samples were taken at each site, separated into litter and soil fractions, and assessed for number of germinable seeds in a greenhouse. Community composition data from each site, as well as several environmental variables, were used to evaluate seed viability within the context of habitat variation.

Initial viability of seeds used in the seed retrieval experiment was 81 and 92 percent for mountain and Wyoming big sagebrush, respectively. After remaining in the field for 24 months, buried Wyoming big sagebrush seeds retained 28–58 percent viability, 11–23 percent of seeds under litter remained viable, and no seeds remained viable on the surface (estimates are 95-percent confidence intervals). The odds of remaining viable did not change from 12 to 24 months. However, after 24 months the odds of seeds beneath litter being viable decreased to 75 percent of the odds of viability at 12 months. Similar to Wyoming big sagebrush, buried seeds of mountain big sagebrush were 31–68 percent viable, seeds under litter retained 10–22 percent of their viability, and no surface seeds were viable after 24 months.
Both subspecies of big sagebrush had some portion of seed that remained viable for more than one growing season provided they were buried or under litter. Although seeds beneath litter may remain viable in intact communities, seeds are susceptible to incineration during fires. Nine months after seed dispersal, seed bank estimates for Wyoming big sagebrush ranged from 19 to 49 viable seeds/m² in litter samples and 19–57 viable seeds/m² in soil samples (95-percent confidence interval). For mountain big sagebrush, estimates were 27–75 viable seeds/m² in litter samples and 54–139 viable seeds/m² in soil (95-percent confidence interval). The number of viable seeds present in the seed bank 9 months after seed dispersal was not significantly different from the number present immediately after seed dispersal. Seed viability was highest in mountain big sagebrush sites for seeds on the surface and beneath litter, but decreased after one season. Buried seeds of both subspecies were in equal abundances and may be insulated from the effects of the environment.

Seed longevity of big sagebrush depends on vertical position of seeds in the soil, indicating that seeds have higher odds of persisting in the seed bank if they are buried. Naturally occurring soil disturbances such as frost-heaving or rodent burrowing may enhance seed bank persistence by incorporating a portion of seeds beneath the soil surface. Restoration methods that ensure seed contact with soil and provide a soil surface disturbance that aids in seed burial may increase the probability of having big sagebrush seeds present when conditions for germination are favorable.

Introduction

Big sagebrush (*Artemisia tridentata*) communities in the Intermountain Region provide habitat and forage for wildlife (Sands and others, 1999), prevent erosion (Goff and others, 1993), and are economically important to recreation and livestock industries (Maher, 2007; Evans and Rollins, 2008). Land managers are responsible for supervising lands for multiple, often competing, uses (Federal Land Policy and Management Act). Sagebrush subspecies are integral to their specific communities, and land managers would benefit from a sound understanding of the population biology of these subspecies, particularly seed bank dynamics.

Several subspecies of big sagebrush occur in this region, but two currently dominate, Wyoming big sagebrush (*A. t. ssp. wyomingensis*) and mountain big sagebrush (*A. t. ssp. vaseyana*). Both communities have been declining due to expansion of invasive weeds such as cheatgrass (*Bromus tectorum*) in the Wyoming big sagebrush community and encroachment of native conifers like juniper (*Juniperus* sp.) in both communities (Miller and Rose, 1999; Miller and Tausch, 2001). Cheatgrass invasion has shortened fire return intervals in lowland habitat (Whisenant, 1990), which provides favorable conditions for further habitat conversion (D’Antonio and Vitousek, 1992), and conifers have shifted fire regimes from relatively low to high intensity fires (Miller and Tausch, 2001). All big sagebrush subspecies lack the ability to sprout after burning and must regenerate from seed (McArthur and Stevens, 2004). A persistent seed bank for both subspecies of sagebrush would be advantageous to reestablishing populations after disturbance.

Thorough knowledge of seed longevity and dormancy for key big sagebrush subspecies in the Intermountain West will aid land managers in restoring and conserving these native landscapes. For example, prescribed burning in montane regions is a tool commonly used to rejuvenate native stands and reduce tree (*Juniperus* sp. or *Pinus* sp.) encroachment. If viable seeds of big sagebrush were available in the soil, then funds spent on reseeding or transplanting could be allocated elsewhere. In contrast, if seeds of big sagebrush do not persist beyond a year, then restoration of sagebrush may be necessary and likely would involve active reseeding or transplanting of this species. Exploring the link between seed location and seed longevity is important for appropriate placement of seeds, which may improve seed survival, germination and seedling establishment.
Big sagebrush seeds exhibit characteristics of nondeep physiological dormancy (Baskin and Baskin, 2001), which is caused by a physiological inhibiting mechanism of the embryo. This kind of dormancy can be broken by relatively short periods of cold stratification. Seeds of both subspecies show increased germination after being cold-stratified (McDonough and Harniss, 1974; Meyer and Monsen, 1991). Seeds also may undergo afterripening in dry storage or come out of dormancy over time. Afterripening decreases the length of cold stratification necessary for breaking dormancy. Seeds in warehouse storage (6–8 percent relative humidity and less than 10 °C) typically hold full viability for 2–3 years (Stevens and others, 1981; Hillary Parkinson, unpub. data, Boise State University, 2004), and germination rates were documented to be higher than rates immediately after harvest. A higher percentage of *A. t.* ssp. *vaseyana* seed collected from cold winter sites with mean January temperatures below -5 °C were nearly 100 percent light-requiring (Meyer and others, 1990), another manifestation of nondeep physiological dormancy. Germination trials indicate that dormancy may differ across subspecies and population (Meyer and Monsen, 1992). Wyoming big sagebrush was largely non-dormant whereas mountain big sagebrush exhibited site-dependent dormancy. All Wyoming big sagebrush collections in the study had seeds that germinated quickly and supported previous field studies that concluded little or no seed carry-over from one year to the next (Hassan and West, 1986; Young and Evans, 1989).

Unexplained Wyoming big sagebrush seedling emergence taking place years after reseeding efforts (Schuman and others, 1998) and mountain big sagebrush recolonization occurring considerable distances from potential maternal plants after a large fire (Ziegenhagen, 2003) indicate the possibility of acquired seed dormancy or seed persistence under a particular set of environmental conditions (Young and Evans, 1989). Although laboratory studies suggest dormancy in big sagebrush is habitat-specific, to our knowledge, there have been no direct field experiments or observations to confirm this hypothesis. There has been no empirical verification whether seeds of either subspecies could experience extended viability in the field beyond one winter season.

**Objectives**

We investigated seed longevity of Wyoming and mountain big sagebrush in the field with a seed retrieval experiment and seed bank sampling. Very little old seed has been found in the soil after initial seed dispersal; thus it is assumed that most big sagebrush seeds either germinate quickly or die, and do not persist in the soil as viable seeds. Meyer and others (1990) argue that the small amount of seed that does carry over is likely buried, receiving inadequate light for germination. Soil disturbances like burrowing by subterranean mammals or frost-heaving could expose the seed to surface light and allow germination. We asked three questions to clarify seed viability and longevity in these subspecies:

1. *Do viable seeds of either Wyoming or mountain big sagebrush survive in the soil for more than one growing season?*
2. *Are seed longevity and persistence similar within a subspecies regardless of the seed’s location in the soil and its age?*
3. *Does seed longevity and persistence change with environmental conditions (or habitats)?*
Methods

Study Sites

In August 2006, we chose study sites in Oregon and Idaho, located in the Western Intermountain Sagebrush Steppe, and in Utah and Nevada, in the Great Basin–Colorado Plateau Sagebrush Semi-Desert (West, 1983) (fig. 1). We located three sites in an east-west gradient, and three sites in a north-south gradient. At each site, we established one plot each in a Wyoming big sagebrush and mountain big sagebrush stand. Placement of plots was based on ocular estimation of good sagebrush and bunchgrass cover, and low cover of cheatgrass in the interspaces. Environmental differences between Wyoming big sagebrush plots and mountain big sagebrush plots (table 1) were not a concern because each subspecies was evaluated separately.

Experimental Design

We used a seed bag retrieval experiment to examine fates of viable seeds in three soil positions and four collection times over about a 24-month period. Seed bags were positioned at three soil levels: (1) buried 3 cm below soil surface, (2) at the soil surface but buried beneath 2 cm of sagebrush leaf litter, and (3) above soil and litter. In the collection time treatment, one-fourth of the seed bags from each soil level were collected at each of four time intervals: (1) late spring after normal germination during the first season – April 2007, (2) autumn around the time of seed dispersal and one year after placement – November 2007, (3) after germination during the second field season – April 2008, and (4) autumn at seed dispersal at the end of 2 years – November 2008.

Plots were established at least 20 m from the nearest road or obvious disturbance. Eight transects in a 35 × 40 m area were used as guides to place and locate seed bags and to sample for seeds in the soil (seed bank sampling). Every 4 m along each transect, we located the nearest big sagebrush shrub with a minimum canopy size of 0.25 m². Number of paces and general cardinal direction from the point were recorded, along with shrub canopy area (long axis × short axis). In this manner, we located \( n = 96 \) shrubs (3 soil positions × 4 collection times × 8 replicates) for each plot. Each seed bag was randomly assigned a soil position and collection time, and placed beneath a specified shrub 10 cm from the outer edge of the foliage and directly beneath an inflorescence. In addition, a set of eight replicates was set aside as a pre-treatment control to determine initial viability of the seeds placed in the experiment. These seed control replicates were randomly selected during seed bag construction.

After seed bags were retrieved from the field, seeds were examined and placed into categories: (1) germinated – having evidence of either cotyledons or a radicle protruding from the seed (achene), (2) intact and possibly viable, and (3) not intact and/or obviously decomposing. Viability of intact seeds was tested by a tetrazolium (TZ) test using standard Association of Official Seed Analysts techniques (2000) and conducted by the Utah Department of Agriculture (State Seed Laboratory, Salt Lake City, Utah). We used the number of seeds determined to be viable by the TZ test plus the number of seeds recently germinated as the response variable to indicate total number of seeds that retained viability to that moment.

We also sampled for seeds in the seed bank by taking soil cores beneath a big sagebrush shrub every 8 m along the same series of transects used for placement of seed bags, yielding a total of 48 samples per plot. Samples were taken by pounding a length of sharpened PVC pipe (about 5 cm diameter) to a depth of 3 cm. We sampled the seed bank twice during the growing season for two seasons (2006–07 and 2007–08): once immediately after seed dispersal (November) and again 9 months later (August), before the next season’s seed dispersal. Location of the first season’s soil samples could
easily be detected, thereby avoiding the problem of resampling during the second season. Soil cores were divided into the litter fraction and soil fraction. Litter fractions were mixed with about 300 g of sterile sand. Both fractions then were wetted to field capacity before being stored in a cooler for 3–4 months at 4 °C for cold stratification. After stratification, samples were placed in a greenhouse in the spring (February–April) and monitored for big sagebrush seedling emergence. Each big sagebrush seedling was counted and removed. Daytime temperatures ranged from 20.8 to 41.3 °C and night temperatures ranged from -1.2 to 11.8 °C. Samples received ambient light and were kept continuously wetted until emergence of new seedlings stopped.

Plant community data were recorded at each site by the line-point intercept method (similar to Herrick and others, 2005) with points every 1 m along four transects in May 2008. Individual species foliar cover, overall foliar cover, bare ground, and moss/lichen crust cover calculated from these data were used as variables for statistical analyses.

Seed Bag Construction

To keep the experiment manageable, we did not collect seeds from study sites, but purchased seeds of both subspecies (from Granite Seed Co., Lehi, Utah) certified by the Association of Seed Certifying Agencies. The purchased seed was collected in autumn 2005 and was source-identified. The mountain big sagebrush seed was collected from Wyoming, and the Wyoming big sagebrush seed was collected from Utah. Although this experiment does not allow comparisons of genetic differences (genotypic differences) among populations, it does allow for comparisons among sites (environmental differences) for a site-identified population because all seeds come from a single population. The Wyoming big sagebrush was 17.45 percent pure, and mountain big sagebrush was 26.98 percent pure.

We used the facilities at the USDA Forage and Seed Research Center (Corvallis, OR) to purify seed lots further to 95.16 and 95.37 percent purity for Wyoming and mountain big sagebrush, respectively. Seed lots of both species were sieved through 20 × 20 (0.94 mm² opening) and 40 × 40 (0.43 mm² opening) mesh screens on a custom built autoshaker (Vibration Systems, Inc., Aurora, OH) at 10 Hz and 10 mm amplitude. This separates the seed lot into chaff, seed, and fine dust fractions. The chaff fraction was sieved again to separate out any residual seed. The seed fraction was run through an indent cylinder with a pocket diameter of 2.25 mm (Westrup Inc., Plano, TX) to divide heavy and light fractions of seed. The heavy seed fraction then was passed through a round-hole sieve (1.22 mm circumference) that segregates fine vegetative matter from seeds. The seeds were then placed in a seed blower (E. L. Erickson Products, Brookings, SD) with air velocity set to about 1.4 m/s for 45 seconds to blow away any remaining pieces of chaff. The fraction used for this experiment contained the heaviest seeds because they have the greatest germination potential (Busso and others, 2005). We used a seed counter to count out sets of 100 seeds for Wyoming (± 0.52) and mountain (± 0.90) big sagebrush to put into the seed bags. Seed bags, measuring 5 × 5 cm, were constructed from fine mesh (less than 0.5-mm opening), white polyester (chiffon) that allowed 85 percent ambient light penetration.

Bag Effect

We investigated how seed bags would affect seed viability by replicating the field seed retrieval experiment in the greenhouse in June 2008. Topsoil and litter from each field site were collected and bulked by subspecies. Soil was sieved to remove large debris and rocks and to homogenize across sites. An equal amount of sterile sand was mixed with the soil and used as a potting medium. The experiment was a factorial design with three levels of the bag treatment (bag, free seeds, control) and three levels of the depth treatment (surface, litter, buried). Seed bags were constructed in the same manner as for the field experiment. Seeds in seed bags and free seeds were subjected to a 21-day, cold stratification (4 °C)
before being placed in treatment pots. Each 5 × 5 cm pot was randomly assigned to one of nine treatment combinations, which were replicated six times. Seed bags and sets of 100 free seeds were placed 3 cm below the soil surface, on the soil surface, or atop 2 cm of litter. Controls were filled with soil and topped with litter but contained no added seeds. After 1 month we assessed viability of intact seeds. Free seeds from control and treatment pots were extracted from the soil medium using a modification of the method by Malone (1967). Soil was first passed through sieves (2 mm, 1 mm, 425 μm opening) to obtain the seed-containing soil fraction. The fraction then was mixed with a high-density 1 M sugar solution and centrifuged for 10 minutes at 2,600 rpm. Seeds from all treatments were sent to the Utah Department of Agriculture (State Seed Laboratory, Salt Lake City, UT) to test for viability.

**Statistical Analysis**

Data were analyzed separately using SAS 9.13 statistical software (SAS Institute Inc., Cary, NC) and PC-ORD 5.19 (MjM Software Design). A mixed-model analysis of variance (ANOVA) was carried out on seed bag data (number of viable seeds) with site as a random effect. Modeling the response as a proportion was appropriate in this case because seed bags initially contained 100 seeds and after log-transformation the data approached a normal distribution (Manuela Huso, oral commun., Oregon State University, 2008). We averaged replicates of each treatment combination (extraction time and seed position) within each site and removed any treatment combinations for which seed viability responses yielded zero values (no variance). Autumn 2008 surface treatments were removed from the Wyoming big sagebrush dataset, and spring 2008 and autumn 2008 surface treatments were removed for the mountain big sagebrush dataset. This technique, along with log-transformation, enabled responses to remaining treatment combinations to better meet assumptions of the statistical test (McEvoy and others, 1993). Analysis of the greenhouse data was carried out with a mixed-model ANOVA after logit-transformation. Bonferroni adjustments for multiple comparisons were applied where appropriate.

Seed bank data (number of germinable seeds) were modeled using a generalized linear model with a Poisson distribution. Overdispersion was included in the model with site as a fixed effect. As with the seed bag data, we averaged replicates of seed bank samples for each treatment combination within each site (sampling month by site) and removed those that had all zero values before analysis.

Nonparametric multidimensional scaling (NMS) with the Sørensen distance measure was used to analyze plant community data at the site level. NMS is an iterative ordination technique that seeks a stable solution by minimizing stress or departure from monotonicity in the reduced ordination space (Kruskal, 1964; Mather 1976). NMS avoids assumptions of linearity and normality that are prevalent in community ecological data (McCune and Grace, 2002). The ‘slow and thorough’ setting of the autopilot mode in PC-ORD version 5 was used with random starting configurations and 250 runs of real data. Monte Carlo tests were used to test for significance of the k-dimensional solution against 250 runs of randomized data.
Individual species’ foliar cover was used to ordinate study sites. Environmental variables and seed viability responses (number of viable seeds retrieved in bags and sampled in seed bank) associated with each site were added to the resulting ordination to create a joint plot. Environmental variables included total precipitation for study duration, elevation, heat load, potential incident radiation, soil texture, overall foliar cover, percent of bare ground, and moss/lichen crust cover. Heat load and percentage of incident radiation were estimated using latitude, slope, and aspect of each site in HyperNiche (version, 2.60 Beta), after the method described in McCune (2007). Overall foliar cover, moss/lichen crust cover, and percentage of cover of bare ground were calculated from line-point intercept data by dividing the number of intercepts for each cover response and dividing by the number of total intercepts (180) for each plot (Herrick and others, 2005). Seed viability responses were averaged over each treatment combination. This method describes the variation in plant community among sites (habitats) and allows the evaluation of the seed viability responses within that variation.

Results

Seed Retrieval Experiment

The median proportion of seeds remaining viable was significantly affected by length of exposure in the field for Wyoming \(p < 0.01, F_{3,50} = 39.41\) and mountain \(p < 0.01, F_{3,45} = 23.16\) big sagebrush. In autumn 2007, after one growing season, we determined that some proportion of Wyoming and mountain big sagebrush seeds were still viable across all depth treatments (fig. 2). Both subspecies experienced a reduction in median seed viability over time, but magnitude of the reductions depended on the location of seeds in soil (Wyoming big sagebrush \(p < 0.01, F_{5,50} = 6.14\); mountain big sagebrush \(p < 0.01, F_{4,45} = 5.29\)). After 12 months, there was a significant difference in median seed viability between buried seeds and seeds beneath litter or on the surface for Wyoming big sagebrush \(p < 0.01\) (both differences) and mountain big sagebrush \(p < 0.01\) litter difference; surface value is zero).

Initial viability of seeds used in the seed retrieval experiment was 81 and 92 percent for mountain and Wyoming big sagebrush, respectively. At 12 months, buried Wyoming big sagebrush seeds were 27–57 percent viable, seeds under litter were 14–29 percent viable, and surface seeds were 9–19 percent viable. After remaining in the field for 24 months, buried seeds and seeds beneath litter retained their viability at 28–58 and 11–23 percent, respectively (estimates are 95-percent confidence intervals). However, no seeds remained viable on the surface at the end of 2 years in the field. After one year, buried seeds of mountain big sagebrush were 22–49 percent viable, seeds beneath litter were 12–26 percent viable, and surface seeds were 11–23 percent viable. Buried mountain big sagebrush seeds retained 31–68 percent viability, whereas seeds beneath the litter retained 10–22 percent of their viability, and no surface seeds retained viability after 24 months. Both subspecies of big sagebrush had some portion of seed that stayed viable for more than one growing season provided they were buried or under litter.

Proportions of seeds remaining viable in different seed location treatments are similar between subspecies during the first year of the study. Mountain big sagebrush seeds in the surface treatment did not remain viable beyond 12 months, but one Wyoming big sagebrush site had seeds that survived for 18 months at the surface. The proportion of seeds remaining viable at the study’s conclusion was lower than when the study began. Wyoming big sagebrush seeds at the beginning of the study were about 13 times more likely to be viable than seeds in the field after 6 months and 40 times more likely to be viable than seeds after 12 months. After one growing season, the location of the seed above or below the soil or litter affected the odds of the seed remaining viable. Buried seeds of Wyoming big sagebrush
were 2.5 times more likely to be viable than seeds beneath litter after one growing season, and were 3.5 times more likely after two growing seasons. Mountain big sagebrush also experienced a loss of viable seeds over time. At the beginning of the experiment, mountain big sagebrush seeds were 4.5 times more likely to be viable than seeds in the field after 6 months and 16 times more likely to be viable after 12 months. Buried seeds were twice as likely to be viable than seeds beneath litter after one growing season and 5 times more likely to be viable after 12 months.

**Bag Effect**

Seeds in bags on the surface had higher viability than seeds without bags for both subspecies (Wyoming $p < 0.01, t = 5.87$ and mountain $p = 0.04, t = 2.13$; fig. 3). Wyoming and mountain big sagebrush seeds beneath litter in bags were more viable than free seeds ($p = 0.02, t = 2.39$ and $p = 0.02, t = 2.55$, respectively). However, there was no effect of bags on seed viability of buried seeds for either Wyoming or mountain big sagebrush ($p = 0.17, t = -1.39$ and $p = 0.89, t = -0.14$, respectively). There were no viable seeds found in mountain big sagebrush control treatments, while a few were found in the Wyoming big sagebrush controls.

**Seed Bank Sampling**

The median number of viable seeds in the seed bank for Wyoming big sagebrush was highly variable among sites and within sites (fig. 4) for the first year of sampling. Sampling time had a marginal effect on the median number of seeds in the litter ($p = 0.07, X_{1,470} = 3.36$) and no effect on the median number of seeds in the soil ($p = 0.21, X_{1,376} = 1.55$). For mountain big sagebrush, the median number of viable seeds in the seed bank was not as variable, with only one or two sites increasing the overall median (fig. 5). Sampling time had no effect on the median number of seeds in litter ($p = 0.11, X_{1,470} = 2.59$) or in soil ($p = 0.31, X_{1,470} = 1.03$). After 9 months in the field, estimates for Wyoming big sagebrush ranged from 19 to 49 viable seeds/m² in litter samples and 19–57 viable seeds/m² in soil samples (95-percent confidence interval). For mountain big sagebrush, estimates were 27–75 viable seeds/m² in litter samples and 54–139 viable seeds/m² in soil (95-percent confidence interval).

The second year of sampling the seed bank yielded no germinable seeds in litter or soil samples immediately following seed dispersal for Wyoming big sagebrush. Litter samples from one mountain big sagebrush site had 3-36 viable seeds/m² (95-percent confidence interval) following seed dispersal. After 9 months, only a couple Wyoming big sagebrush sites had viable seed, ranging from 5 to 24 viable seeds/m² in litter and soil samples (95-percent confidence interval). The two mountain big sagebrush sites that had viable seeds after 9 months varied considerably. One site had 3-36 viable seeds/m², whereas the other site had 31–91 viable seeds/m² (95-percent confidence interval).
Plant Community

The NMS ordination of plots in species space (fig. 6) showed incomplete grouping of plots by sagebrush subspecies (3-dimensional solution: final stress = 5.04, final instability = 0.00, cumulative $R^2$ = 0.92). Environmental variables and seed viability responses were overlaid on the ordination to examine trends (table 2). Axis 1 was most highly correlated with elevation ($r = 0.74$, Fig 6a), heat load ($r = 0.45$, Fig. 6b), and the percentage of bare ground ($r = 0.50$, Fig. 6b) in the direction of sites located geographically farthest east and south. The other end of Axis 1 was strongly correlated with overall canopy cover ($r = -0.72$, Fig 6a), moss and lichen crust cover ($r = -0.44$, Fig. 6a), and associated primarily with low-elevation Wyoming big sagebrush sites. The environmental variable that had the highest correlation with Axis 2 was elevation ($r = 0.66$, fig. 6b). Axis 3 (fig. 6a) was equally correlated with elevation ($r = 0.33$) and overall canopy ($r = 0.35$).

Seed viability vectors indicate treatments (seed position and collection time) with the highest number of viable seeds and strongest correlation with plots (fig. 6). Viable seeds in surface and litter (not shown) treatments sampled in April 2007 were more abundant in sites with high canopy cover and plots higher in elevation, respectively. In November 2007, the preponderance of viable seeds for both surface and litter (not shown) treatments occurred at plots higher in elevation. By April 2008, seeds beneath litter are still abundant at plots higher in elevation; however, there were more viable surface seeds in plots that were high in moss and lichen crust cover. By November 2008, no seeds on the surface remain and the only seeds viable beneath litter are primarily at one Wyoming big sagebrush plot. However, seed viability of buried seeds is similar across plots of both subspecies over the duration of the study.

The ordination also was overlaid with the second environmental matrix containing seed bank responses (fig. 7; table 2). Axis 1 (fig. 7a) strongly correlated with elevation ($r = 0.48$) and precipitation ($r = 0.30$). Axis 2 was highly correlated with canopy cover ($r = 0.54$, fig. 7a) and moss and lichen cover ($r = 0.43$, fig. 7a), and negatively associated with elevation ($r = -0.64$, fig. 7a). Overall seed bank counts were highest in two mountain big sagebrush plots so all the seed bank vectors were correlated with Axis 2 (fig. 7b).

Discussion

Seed Retrieval Experiment

The results of our study indicate that, after 2 seasons, the median proportion of buried viable, non-germinated seeds ranged from 40 to 60 percent for both subspecies. Our findings are similar to those of Bouwmeester and Karssen (1992) who demonstrated that buried seeds of *Polygonum persicaria* are secondarily induced into dormancy by lack of light. Seeds also have the potential to persist in soil seed banks in a non-dormant state for many years (Ooi and others, 2007); therefore, buried big sagebrush seeds of these two subspecies may only be inhibited from germinating as opposed to being dormant. The mechanism for prolonged persistence of big sagebrush seeds in the seed bank may either be a function of induced secondary dormancy or lack of proper germination cues.
Big sagebrush seeds exhibit characteristics of physiological dormancy that can be overcome by cold stratification. Meyer and Monsen (1991, 1992) established that a 20-week, cold stratification at 1°C was effective in removing dormancy in mountain big sagebrush, and cold stratification at 15°C was enough to break dormancy in most Wyoming big sagebrush seeds. Buried seeds of Wyoming and mountain big sagebrush in our study experienced two seasons of winter cold, where soil temperatures 3 cm below the surface can range from 0 to 10 °C in autumn (Evans and Young, 1972). Though conditions were favorable for breaking dormancy, neither subspecies showed a decrease in the quantity of buried seeds that remained viable, indicating that seeds were potentially inhibited from germinating. Dark germination does occur in both subspecies but varies greatly by collection site (Meyer and others, 1990), signifying that a portion of seeds require light to cue full germination. If suitable conditions are not present for germination when physiological dormancy is broken, and germination does not occur, secondary dormancy may be induced (Crocker, 1916; Vleeshouwers and others, 1995; Baskin and Baskin, 2001). In April and November 2007, the highest abundance of surface seeds occurred on sites with high overall canopy cover and with high elevation. These were principally mountain big sagebrush sites that likely would have reduced light due to higher cover than Wyoming big sagebrush sites, potentially inhibiting germination and increasing the portion of viable, intact seeds in the seed bank.

Crist and Friese (1993) determined that decomposition and attack by fungi were responsible for the greatest decrease in seed viability among shrub-steppe species during winter, and that seed viability of big sagebrush decreased most rapidly. Mountain big sagebrush seeds had much lower seed viability overall than Wyoming big sagebrush. We observed that mountain big sagebrush seeds experienced a higher proportion of moldy seeds than Wyoming big sagebrush after cold-stratification. By April 2008, the greatest quantities of viable seeds on the surface were primarily at Wyoming big sagebrush sites. This indicates that mountain big sagebrush seeds on the surface experienced a greater decrease in viability than seeds of Wyoming big sagebrush. We also determined that more than 75 percent of surface and litter samples had evidence of fungal infection whereas less than 50 percent of buried seeds showed signs of infection. Mountain big sagebrush seeds on the surface and beneath litter in wetter, colder sites may have succumbed to fungal infection and lost viability after one growing season. Wyoming big sagebrush surface seeds in drier sites may have experienced less or no fungal infection, and therefore maintained a lower, but still viable, portion of seeds after 24 months in the field.

Seed bags used in our study reduced ambient light by about 15 percent and potentially inhibited seed germination, and thus increased the proportion of viable seed. Seeds in bags for the surface and litter treatments tended to have higher estimates of viability than seeds without bags, which indicate that our estimates of seed viability for these positions in the seed bank are high for both subspecies. However, buried seeds were unaffected by this design artifact and may represent the potential maximum viability of seeds buried beneath the soil surface. There is the possibility that the mesh bags maintained a higher level of moisture and potentially increased the risk of fungal infection. If seed bags increase moisture levels and rate of fungal infection compared to conditions outside seed bags, then estimates of seed viability in the seed retrieval experiment likely are conservative.
Seed Bank Sampling

Estimates of germinable seeds in the seed bank for the 2006–07 growing season were variable among and within Wyoming and mountain big sagebrush sites. Wyoming big sagebrush estimates were an order of magnitude lower than viable seed estimates by Young and Evans (1989) and an order of magnitude higher than estimates by Hassan and West (1986). Each sample was about 48 cm$^3$ in volume and the total surface area sampled per plot amounted to less than one-tenth of a square meter. Despite taking samples directly beneath the inflorescence of a sagebrush shrub, less than 25 percent of the samples at all sites yielded germinable seeds. Young and Evans (1989) determined that the dispersal pattern of big sagebrush seeds is skewed to the east due to prevailing winds from the west. We did not take dispersal direction into account when taking seed bank samples. Future studies of the seed bank in this species may warrant sampling at a finer scale than was used in this study and using a more stratified random approach. The low number of viable seeds found in seed bank samples from the second season may be a result of lower precipitation. On average, precipitation during the 2005-06 growing season was 65 percent of that received during the 2006-07 growing season (climate data from PRISM Climate Group, Oregon State University).

Implications

Ours is the first study to document that seed longevity of big sagebrush depends on vertical position of seeds in the soil, and clarifies observations of seedling emergence years after a dispersal or seeding event (Schuman and others, 1998; Booth, 2002; Schuman and Belden, 2002; Ziegenhagen, 2003). Results of our study indicate that seeds have higher odds of persisting in the seed bank if they are buried. Naturally occurring soil disturbances such as frost-heaving or rodent burrowing would enhance seed bank persistence by incorporating a portion of seeds beneath the soil surface. Burial of seeds may ameliorate effects of the environment (i.e., fluctuating temperature and moisture levels) and increase seed longevity (Facelli and Pickett, 1991; Rotundo and Aguiar, 2005). The same processes that promote burial of big sagebrush seeds may operate to bring seeds to the surface when conditions are favorable for successful germination (Harper, 1977). It is important to note however that although seeds buried in litter remain viable in intact communities they are susceptible to incineration during fires.

Restoration of sagebrush steppe is often conducted by aerial seeding, which would leave seeds on the surface. Aerial seeding of big sagebrush can result in poor establishment of shrubs (Dalzell, 2004). However, seedling emergence of big sagebrush through four post-seeding growing seasons when seeds were broadcast onto the surface of a mulch layer (Booth, 2002) indicates that some portion of the seeds may have worked into the soil over time. Providing a soil surface disturbance or mulch that aids in seed burial may increase restoration success. Restoration methods that ensure seed contact with soil such as pressing seeds into the soil surface (Pyke, 1994), creating microdepressions in the soil (Haferkamp and others, 1987), and harrowing or raking can greatly increase seedling establishment (Shaw and others, 2005). An unintended consequence of some of these methods may be burial of a small percentage of seed, which had long been thought to be undesirable. Indeed, keeping seeds near the surface is critical for germination but there may be a middle ground between surface exposure and deep burial that maximizes restoration potential. We argue that techniques to help big sagebrush seeds to remain viable in the soil as a hedge against unfavorable germination conditions may be one of the keys to successful restoration. A one-time seeding effort with high densities of seed may be sufficient to provide a seed source for years and in the long-term may be more economically feasible.
Conclusions

The results of our study indicate that dynamics of seed viability over time are similar in Wyoming and mountain big sagebrush, and depend on the vertical location of the seed in the seed bank. After the initial loss of seeds, most likely due to germination of the physiologically non-dormant fraction, both subspecies exhibit patterns of a steadily decreasing abundance of viable seeds on the surface and beneath litter. However, 40–60 percent of buried seeds remain viable across all sites for at least 24 months. Our study suggests reseeding techniques that promote ‘caching’ of some seeds in the soil seed bank may increase restoration potential.

Acknowledgments

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References Cited


Role of Fire in the Control and Spread of Invasive Species: Tall Timbers Research Station, Tallahassee, FL, p. 15-30.


Figure 1. Location of study sites. Within each study site, we located one Wyoming and one mountain big sagebrush plot.
Figure 2. Median proportion of seeds remaining viable decreased over a period of 24 months for Wyoming and mountain big sagebrush, primarily in seeds beneath litter and on the surface. Seed were buried 3 cm below the surface (buried), placed on the soil surface beneath 2 cm of litter (litter), and placed on the surface (surface). Bars indicate 95-percent confidence intervals and data points are jittered on the x-axis for display.
Figure 3. Viability of seeds in bags for Wyoming and mountain big sagebrush was higher than viability of free seeds, but only in the surface and litter treatments. Buried seeds were not affected by seed bags. Bars indicate 95-percent confidence intervals.
Figure 4. Median number of viable seeds/m² in litter and soil samples of the Wyoming big sagebrush seed bank. There was no significant effect of sampling time on median seed viability in either litter or soil samples across all sites. Sites are located west-east (JUWY, MHWY, LLWY) and north-south (SAWY, TOWY, HUWY), see table 1 for location abbreviation definitions. Bars indicate 95-percent confidence intervals and data points are jittered on the x-axis for display.
Figure 5. Median number of viable seeds/m² in litter and soil samples of the mountain big sagebrush seed bank. There was no significant effect of sampling time on median seed viability in either litter or soil samples across all sites. Sites are located west-east (JUVA, MHVA, LLVA) and north-south (SAVA, TOVA, HUVA), see table 1 for location abbreviation definitions. Bars indicate 95-percent confidence intervals and data points are jittered on the x-axis for display.
Figure 6. Nonmetric multidimensional scaling (NMS) ordination of 12 Wyoming and mountain big sagebrush plots analyzed for plant species composition for a) Axis 1 and Axis 3, and b) Axis 1 and Axis 2. Vectors represent the direction and magnitude of correlation between sites and five environmental gradients, as well as eight seed viability gradients. Ordination is a 3-dimensional solution.
Figure 7. NMS ordination of twelve Wyoming and mountain big sagebrush plots analyzed for plant species composition for a) Axis 1 and Axis 2, and b) Axis 2 and Axis 3. Vectors represent the direction and magnitude of correlation between sample units and four environmental gradients, as well as four seed bank responses. Ordination is a 3-dimensional solution.

### Table 1. Study site information.

<table>
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<tr>
<th>Site Name</th>
<th>Plot Code</th>
<th>Subspecies of <em>Artemisia tridentata</em></th>
<th>State</th>
<th>Slope in percent</th>
<th>Aspect in degrees</th>
<th>Elevation in meters above NAVD</th>
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Table 2. Description of environmental and seed viability vectors in nonmetric multidimensional scaling (NMS) graphs.

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¹McCune and Keon (2002).