Genetic and environmental effects on seed weight in subspecies of big sagebrush: applications for restoration

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Abstract. The sagebrush steppe is a patchwork of species and subspecies occupying distinct environmental niches across the intermountain regions of western North America. These ecosystems face degradation from disturbances and exotic weeds. Using sagebrush seed that is matched to its appropriate niche is a critical component to successful restoration, improving habitat for the threatened greater sage-grouse and other species. The need for restoration is greatest in basin habitats composed of two subspecies: diploid basin big sagebrush (A. tridentata subsp. tridentata) and tetraploid Wyoming big sagebrush (subsp. wyomingensis). In this study we assess seed weights across five subspecies-cytotype groups of big sagebrush and examine the genetic and environmental components. Our goal is to determine if seed weight can be used as a diagnostic test for subspecies and seed certification. Seed weight was measured from 55 wild collections and from progeny derived from these collections and grown in two common gardens. A linear mixed-effect model showed 91% of the variation in seed weight is explained by genetic, genetic × environment and environmental effects (conditional $R^2 = 0.91$). Moreover, genetic effects alone, subspecies-cytotype groups, explained 39% of the variation (marginal $R^2 = 0.39$). Of the five subspecies-cytotype groups, most had overlapping weights using conservative 99% confidence intervals. However, diploid tridentata and wyomingensis had non-overlapping 99% confidence intervals. To demonstrate the application of seed weighing to assess the subspecies purity of commercial seed lots, we compared confidence intervals of tridentata and wyomingensis developed from the experimental data to seed weights of commercial lots. The results showed that only 17% of the commercial seed lots certified as wyomingensis had mean seed weights that fell within the confidence intervals for this subspecies. The remaining lighter seed lots (83%) matched weights of tridentata. While restoring sagebrush ecosystems is a multifaceted problem, a fundamental component to restoration is ensuring the appropriate seed is used. We found seed weight is principally affected by genetic factors, with limited environmental effects. Seed weighing is an effective application to assess subspecies purity of wyomingensis and tridentata seed and could be used as a certification step for evaluating commercial collections used in restoration.

Key words: Artemisia tridentata; cytotype; environmental effects; genetic effects; linear mixed-effects; polyploidy; seed certification.

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**INTRODUCTION**

Even with due diligence in site preparation and planning, obtaining goals set for ecological restoration can be forfeit if the seed and seedling traits (i.e., genetics) are not appropriate for the site. Restoration of arid lands is fraught with challenges including weed control and infrequent episodes of favorable weather necessary for seedling establishment (Hardegree et al. 2011). These challenges are common in efforts to restore sagebrush ecosystems (Davies et al. 2011). Sagebrushes (*Artemisia* subgenus Tridentatae) are the cornerstone of North American cold deserts, fostering a diverse assemblage of native grasses and forbs, insects, and obligate fauna including the greater sage-grouse (*Centrocercus urophasianus*). Sagebrush ecosystems have been degraded primarily by fire and human disturbances and subsequent displacement by exotic weeds. Degradation of sagebrush ecosystems has been the principal cause of sage-grouse loss (Connelly et al. 2004), leading to its threatened listing status. Sagebrush is composed of several species, but big sagebrush (*Artemisia tridentata*) is by far the most ubiquitous in North American cold deserts. This species can be divided into three widespread subspecies (*tridentata*, *vaseyana* and *wyomingensis*), based on characters including growth, cytotype and chemical compounds (McArthur and Welch 1982, McArthur et al. 1988). In addition, these characteristics reflect genetic and physiological attributes (Kolb and Sperry 1999, Richardson et al. 2012), and most importantly for restoration and resiliency, different adaptive strategies. Subspecies are associated with climatic and / or soil properties (Barker and McKell 1983, McArthur and Sanderson 1999, Still and Richardson 2015). Subspecies *vaseyana* occupies cooler, more mesic mountain environments, whereas *tridentata* and *wyomingensis* typically occupy warmer, drier basins (McArthur and Plummer 1978, Mahalovich and McArthur 2004). Within these basins, *tridentata* occupies drainages, washes and floodplains with deeper soils and lengthened summer retention of soil moisture (Barker and McKell 1983), supporting greater growth and fecundity (McArthur and Welch 1982). In contrast, *wyomingensis* exhibits slower growth and occupies arid uplands, which comprise much of the landscape found in basin habitats. The spatial separation of *tridentata* and *wyomingensis* habitat can be meters depending on the topographic and soil heterogeneity. Polyploidy, hereafter referred to as cytotype, is an important taxonomic and adaptive characteristic. McArthur and Sanderson (1999) have shown *wyomingensis* to be exclusively tetraploid, while *vaseyana* and *tridentata* include both diploid (2x) and tetraploid (4x) populations. It is important to note, especially in the context of this study, that diploids of *tridentata* and *vaseyana* are more frequent comprising of about 75% of the samples collected. Moreover, 4x-*tridentata* occurrences were generally found in Washington State and in the southern periphery (i.e., Arizona, New Mexico and southern California) of this species’ distribution (McArthur and Sanderson, 1999). These areas are generally outside the geographic area of where much of the sagebrush restoration and seed collection is conducted (i.e., the Great Basin).

From the research described above, a clear picture has emerged that *A. tridentata* subspecies occupy distinct environmental niches. Among these niches, there is a disproportionate need for restoration in *wyomingensis* habitat. The vast majority of area that is degraded by disturbances and weed invasion has occurred at the driest end of the spectrum of sagebrush habitats (Chambers et al. 2007). These areas are largely comprised of *wyomingensis* habitat (Rowland et al. 2010). Therefore, it is critical that seed be identified to subspecies prior to use in restoration (Shaw et al. 2005). Sagebrush seed is wild collected and purchased by commercial seed brokers who pay seed collectors based on bulk weight. From there, seed is cleaned and subsequently purchased by private landowners (e.g., mining companies) and by U.S. state and federal land management agencies, principally the Bureau of Land Management (BLM). Subspecies purity is verified by site inspection, typically by the Utah Crop Improvement Association (http://www.utahcrop.org/). However, assessing purity of *wyomingensis* seed collections based on site inspection is particularity challenging because (1) the frequent co-occurrence of *wyomingensis* with 2x-*tridentata* and (2) 2x-*tridentata* produces ca. four times more seed per plant than *wyomingensis* (Richardson, unpublished data from com-
mon gardens). This difference in seed yield is likely amplified in natural stands given the resource disparities in the niches occupied by the two subspecies. Therefore, harvesting 2x-*tridentata* in an intended *wyomingensis* collection potentially has a major influence on subspecies purity.

Intraspecific variation in seed size can be an important strategy for environmental adaptation. There is now broad support among studies that increased seed size can have a positive effect on emergence and survival (Bonfil 1998, Søber and Ramula 2013), and could be especially important in arid ecosystems (Larson et al. 2014). Other studies have noted a positive relationship between seed size, increasing DNA content (Chung et al. 1998) and cytotype (Bretagnolle et al. 1995). Here, we investigate seed weight differences among subspecies and cytotype of big sagebrush. Our aims are to: (1) determine if significant seed weight differences exist between subspecies-cytotype groups, (2) assess the extent to which differences among subspecies-cytotype groups are attributable to genetic, genetic × environmental (G × E) or environmental effects based on a linear mixed-effects model, (3) use seed weight means and confidence intervals derived from *tridentata* and *wyomingensis* to evaluate commercially collected seed purchased by the BLM for restoration and (4) discuss the potential applications to assess *wyomingensis* seed purity.

**Materials and Methods**

**Seed collections**

Experimental collections of big sagebrush seed were obtained from three collection sources: from wild collected, hereafter referred to as original seed, and from two common gardens, Majors Flat, Utah and Orchard, Idaho, USA. Majors Flat is a relatively cool and mesic climate, and Orchard is a relatively warm and dry climate. The original seed was collected from 55 natural populations located throughout the distribution of the species in fall 2009 (Fig. 1). The original seed was used to generate seedlings for the common gardens, and seedlings derived from a single wild-collected plant are considered to be maternal half-sibs, hereafter referred to as a family. Families from each population were arranged so that at least one family member was represented at both gardens. Common garden locations were chosen based on logistics and contrasting ranges in climate for big sagebrush (Appendix: Table A1). Seedlings were grown in a greenhouse for 3 months, hardened outside, and planted in the spring of 2010 into the two common gardens. Seed was collected from a total of 44 populations from the Orchard garden and 55 from the Majors Flat garden in 2012 and 2013. The discrepancy in number of collected populations was due to poor seed yield of some populations at Orchard. Of the 55 populations of original seed, only 39 could be included in this study because the seed supply had been exhausted in generating plants for the common gardens (Table 1).

Subspecies and cytotype was determined by a combination of morphology, UV fluorescence, flow cytometry and genetic markers for the experimental collections. As described in Richardson et al., (2012), tissue samples from the original seed collection were used to evaluate morphology and UV fluorescence (Stevens and McArthur 1974). Flow cytometry was performed on at least one plant per family in each population. These data were used to create five subspecies-cytotype groups: subspecies *tridentata* and *vaseyana* included both diploid (2x) and tetraploid (4x) populations, while subspecies *wyomingensis* was exclusively tetraploid. Similar findings were reported by (McArthur and Sanderson 1999).

The seed weight data from experimental collections were used to evaluate weights of subspecies-certified commercial collections. Commercial seed lots, provided courtesy of the BLM, were harvested from geographically defined sites in the Great Basin in 2013 and 2014 (Table 1; Appendix: Table A2). These collections are bulked on a site basis (ca. 5 to 10 hectares) and used for restoration plantings. Seed was certified for location and subspecies based on site inspection and morphological examination by the Utah Crop Improvement Association. A total of 30 seed lots were examined (weight and UV test) from 5 *tridentata*, 18 *wyomingensis* and 7 *vaseyana*.

**Seed cleaning and measurements**

For the common garden and original collections, seed harvesting was conducted in November to ensure all seed had ripened. Flowering
stalks were clipped, bagged and then dried at room temperature for a few days to aid in cleaning. Seed was cleaned of chaff using soil sieves of decreasing size from 1, 0.5 and 0.4 mm screen size. Any remaining chaff was removed by hand. Cleaned seed was placed in plastic bags and stored in a −20°C freezer until weighing. An analytical scale (0.1 mg readability and repeatability) was used to weigh 10 randomly selected seeds per sample. For the original seed collections, typically five families per population were selected for weighing and seed from individual plants were subsampled 3 times. In the common gardens, typically two plants from different families were collected from each population. Individual plants were subsampled 6 times. This process was replicated for two years, 2012 and 2013, for both common garden collections (Table 1). For the commercial collections, seed was cleaned as described above, and then each seed lot was subsampled 10 times. Increased subsampling was conducted because the collections were from bulked seed.

To better confirm the relationship between seed weight and composition of 2x-tridentata and wyomingensis in commercial collections, two high and two low weight, wyomingensis-labeled seed lots were chosen for flow cytometry analysis. Seeds were germinated and grown for two weeks, and then the leaves were harvested for flow cytometry. Flow cytometry was conducted on 25 to 30 seedlings of each seed lot. Subspecies
Tridentata and wyomingensis were putatively assigned 2x and 4x cytotypes, respectively.

**Statistical analysis**

To assess the variance components affecting seed weight, the experimental collections (i.e., original and common gardens) were fitted to linear mixed-effect models (LMM) using restricted maximum likelihood. A LMM was conducted in R v3.1.2 (R Core Team 2014) using the packages lme4 v1.7 (Bates et al. 2012) and lmerTest v2.2 (Kuznetsova and Brockhoff 2015). The study design described above was used to develop the LMM response function. Subspecies-cytotype groupings were specified as fixed effects and random effects. Random effects were arranged in a nested hierarchy with the following structure: collection + year × collection + population × (year × collection) + family × (population × (year × collection)). The response function can also be segregated into genetic, environmental and G × E components. Subspecies-cytotype groups are considered genetic components, collection and year as environmental and population and family as G × E. For the LMM, P values were generated using F tests with Satterthwaite approximation for fixed effects and likelihood-ratio chi-squared tests for random effects. Variances and P values were used as a guide to evaluate the importance of model predictors. In addition, variability explained by fixed and random effects and fixed effects alone were evaluated using conditional and marginal $R^2$, respectively (Johnson 2014). Seed weight confidence intervals were calculated for each subspecies-cytotype group. These calculations were completed with the ‘profile’ method at 99% confidence limits using the confint function in the lme4 package.

**RESULTS**

A box plot illustrates the distribution of observed data and notable patterns (Fig. 2). First,
mean seed weights of subspecies-cytotype groups from the experimental data ranged from 1.8 mg, 2x-tridentata from the original collection, to 3.5 mg, 4x-vaseyana from Majors Flat. Second, subspecies-cytotype groups had similar seed weights among collection sources; however, the Majors Flat collection had slightly higher seed weights compared to other collections. Third, 2x-tridentata had consistently the lowest weight and 4x-vaseyana and wyomingensis had the highest seed weight across collections (Fig. 2). The statistical significance of these observations is explored below.

Results from the LMM indicate that seed weight is largely determined by genetic factors. Fixed effects, genetic groupings of subspecies and ploidy, explained 39\% of the variation (marginal $R^2 = 0.39$), and combined fixed and random effects explained 91\% of the variation (conditional $R^2 = 0.91$). Based on the experimental design, the random effects can be segregated into two factors: (1) environmental, collection and collection × year, and (2) G × E, population and family, nested within the environmental factors. Collection alone was not significant. However, collection × year, had a significant variance component (0.025, $p = 0.004$). This environmental variance was relatively small compared to G × E variances of population (0.096, $p < 0.0001$) and family (0.127, $p < 0.0001$; Table 2).

Substantial differences in slopes were estimated between the intercept (2x-tridentata) and other subspecies-cytotype groups. Tetraploid tridentata and vaseyana are predicted to weigh 41\% (0.72 mg) and 20\% (0.47 mg) heavier, respectively, compared to their 2x counterparts (Table 2). The largest differences among subspecies-cytotype groups were approximately 1 mg. These differences were found between the 2x-tridentata and wyomingensis and between 2x-tridentata and 4x-vaseyana (Table 2). Confidence intervals at the 99\% level support distinct weight difference between 2x-tridentata and wyomingensis and 2x-tridentata and 4x-vaseyana. Most importantly, geographically co-occurring 2x-tridentata and wyomingensis had the widest margin between upper and lower confidence intervals (Fig. 3; Appendix: Table A3).

Environmental effects, collection × year, had varying degrees of positive and negative effects on the seed weight intercept. These effects ranged from −0.19 mg for the original collection to 0.27 mg at the Majors Flat garden in 2012 (Fig. 4). Consistent collection × year effects are estimated for the Orchard garden that appear to not be substantially different than zero. However, the year effect was considerably different at Majors Flat. Following the 0.27 mg effect in 2012, the 2013 effect was not different from zero (Fig. 4).

Significant differences in LMM slopes among subspecies-cytotype groups (Table 2) translated into non-overlapping confidence intervals for two group comparisons. Diploid tridentata (1.77 mg) and wyomingensis (2.76 mg), and 2x-tridentata and 4x-vaseyana (2.81 mg) were distinct. Other group comparisons had overlap in confidence intervals (Fig. 3; Appendix: Table A3). Confidence intervals from 2x-tridentata and wyomingensis were used to evaluate seed weights of commercial seed lots used in restoration. This comparison focused on seed lots certified for either subspecies wyomingensis or 2x-tridentata for
two reasons: (1) these subspecies are by far the most abundant in basin ecosystems and (2) differentiation of *vaseyana* from other subspecies can be accomplished with a UV fluorescence test (Stevens and McArthur, 1974). Of the 18 seed lots labeled as subspecies *wyomingensis*, only three (17%) had mean weights that fell within the confidence intervals of *wyomingensis*. The 15 seed lots with lower weights fell within the confidence intervals of 2x-*tridentata*. Of the five seed lots labeled as subspecies *tridentata*, four (80%) fell within the confidence intervals of 2x-*tridentata*.

To illustrate the comparisons between seed lot and experimental collection weights, smoothed histograms of subsampled weights from each seed lot are shown in conjunction with confi-

| Effects                                      | df  | Variance or estimate | SD  | P value |
|----------------------------------------------|-----|----------------------|-----|---------|
| Random effects                               |     |                      |     |         |
| Collection                                   | 3   | 0.0116               | 0.1080 | 0.748 |
| Year × collection                            | 5   | 0.0247               | 0.1573 | 0.004 |
| Population × (year × garden)                 | 118  | 0.0963               | 0.3104 | $<0.0001$ |
| Family × (population × (year × collection)   | 443  | 0.1272               | 0.3566 | $<0.0001$ |
| Residual                                     |     | 0.0466               |     |         |
| Fixed effects                                |     |                      |     |         |
| 2x-*tridentata* (intercept)                  |     | 1.7655               | 0.1044 | 0.0032 |
| 4x-*tridentata*                              |     | 0.7150               | 0.1096 | $<0.0001$ |
| 2x-*vaseyana*                                |     | 0.5682               | 0.0943 | $<0.0001$ |
| 4x-*vaseyana*                                |     | 1.0412               | 0.1286 | $<0.0001$ |
| *wyomingensis*                               |     | 0.9926               | 0.0751 | $<0.0001$ |

Fig. 3. Ten-seed weight parameter values (dots) and 99% confidence intervals (whiskers) for subspecies-cytotype groups of *Artemisia tridentata*. Data values can be found in Appendix: Table A3.
idence intervals from the experimental collections (Fig. 5). Upon examination of UV fluorescence in commercial seed lots, it was found that six out of seven (86\%) of the *vaseyana* seed lots had elevated UV fluorescence at the expected level (rating of 3 or greater; Appendix: Table A2).

Examination of cytotype by flow cytometry in *wyomingensis*-labeled seed lots further confirmed the correspondence between weights and the proportions of 2x-*tridentata* and *wyomingensis* seed. Flow cytometry was conducted on leaves of germinated seeds from two seed lots with high weights (ca 2.3 mg), exceeding the lower confidence limit for *wyomingensis* (lot A and C), and two seed lots with low weights (ca 1.7 mg), below the confidence limit for *wyomingensis* (lot B and D; Fig. 5). Higher-weight seed lots were largely comprised of *wyomingensis* (i.e., tetraploids) at 97\% and 57\% for lots A and C, respectively. However, lower weight seed lots were largely 2x-*tridentata*. Seed lots B and D consisted of 11\% and 0\% *wyomingensis* seedlings, respectively. This confirms *wyomingensis* seed present in seed lots A and C raised the seed weights compared to seed lots B and D that were principally comprised of 2x-*tridentata*. Therefore, seed weight can be used as a surrogate to assess the relative proportions or purity of commercial seed lots of 2x-*tridentata* and *wyomingensis*.

**DISCUSSION**

**Genetic and environmental effects**

The feasibility of developing a seed-weight-based application for evaluating big sagebrush subspecies is contingent on the degree of environmental influence. Large environmental effects on seed weight would negate precision needed to determine subspecies. In big sagebrush, variation in seed weight can be largely assigned to genetic effects, mainly from polyplidization, while purely environmental effects were small. These results are supported by other studies reporting a positive relationship between increasing DNA content and seed size (Caceres et al. 1998, Chung et al. 1998). The causal mechanism for this increase is unknown, but Beaulieu et al. (2006) proposed increasing DNA content and concomitant enlargement of cell size could cause a seed size increase.

While environmental effects accounted for a small amount of the variation, patterns appear to be largely predictable among gardens and in years. At Majors Flat, a relatively cool and wet...
positive seed weight effects were observed in 2012, a 0.27 mg effect. However in the following year, 2013, essentially no effect was found (Fig. 4). The yearly effect appears to be inexplicable based on weather. Precipitation and temperature were relatively similar between years at this garden (e.g., ca. 415 mm precipitation). One possible explanation could be competition. The rapid growth at Majors Flat led to interspace closure between most plants by 2013, resulting in a greater degree of competition. Concomitant to a reduction in seed weight in 2013, per plant seed yield was dramatically reduced by 82% between 2012 and 2013 at Majors Flat (Richardson, unpublished data). Reduced allocation of resources to reproduction has been well documented in competition studies (Weiner 2004). In big sagebrush, competition may have a large effect on seed yield, but also a small effect on seed weight. Competition may also explain the decreased seed weight in the original seed collection. In most cases, the original seed collection was derived from mature stands where plants were in close intra- and interspecific competition. As expected given competition, the original collection had more negative effects on seed weight than common gardens (Fig. 4). At Orchard, other factors may be at work affecting seed weight. At this warm and dry garden, growth rate was much slower than at Majors Flat, and therefore, plant competition appears to be negligible. However, Orchard weather could be a factor. This garden received almost half the precipitation of Majors Flat and experienced longer, drier summers (Appendix: Table A1). In 2012 and 2013, ten populations at Orchard did not produce any seed. Despite these extreme conditions, the environmental effect on seed weight was only slightly negative at this garden (Fig. 4).

Previous studies of intraspecific seed weights have largely shown a significant genetic effect. Our seed weight results of big sagebrush show significant genetic effects between subspecies-cytotype groups. In addition, within groups, populations and families had significant effects, although these factors have an interaction with environment (Table 2). Our results further support the large body of literature that seed characteristics are under genetic control (Biere 1991, Castro 1999, Halpern 2005, Völler et al. 1999).
2012) and have high heritability (Zas and Sampedro 2014). As described elsewhere (Zas and Sampedro 2014), partitioning maternal genetic versus maternal environment effects requires methodology beyond the scope of this study. However, the variation in seed weight exhibited in original maternal environment and seed from daughters in common gardens of contrasting environments showed seed weight is consistent across environments compared to other traits. For example, the Orchard garden had a 42% reduction in growth and 84% reduction in seed yield compared to Majors Flat (Richardson, unpublished data).

Beyond being a diagnostic trait between 2x-
tridentata and wyomingensis, it is not certain how seed weight could influence plant fitness. For instance, does seed size have a functional role in seedling establishment? No formal study in big sagebrush has been completed on seed size and its effect on seedling growth, but other studies support seed size having a positive effect on establishment, survival and growth (Bonfil 1998, Larson et al. 2014). However, it is possible negative trade-offs (e.g., seed predation) may adversely affect seed dispersal and establishment (Meyer and Carlson 2001, Gómez 2004). Observations from a broader range of taxa in the Tridentatae may support the importance of seed weight. For example, seed weights of low sagebrush, A. arbuscula and black sagebrush, A. nova, which occur in more xeric sites (McArthur and Stevens 2004), are considerably heavier than big sagebrush (Jorgensen and Stevens 2004, Meyer 2008), suggesting greater aridity may have a positive effect on seed size in this subgenus.

Restoration implications

The evaluation of commercial seed lots shows the mixed success in the seed collection and certification of big sagebrush subspecies. Subspecies vaseyana certified seed lots were largely correctly collected and labeled. Of the seven vaseyana seed lots, six have higher UV fluorescence ratings (≥3), within the expected range for this subspecies (Appendix: Table A2). Similarly, four out of five tridentata seed lots had seed weights within the expected range. However, seed certification of wyomingensis seed has largely failed over the two years that were evaluated in this study. We suspect the results from 2013 and 2014 commercial seed lots, where wyomingensis seed lots were comprised largely of seeds from 2x-tridentata, likely reflect commercial seed collections from previous years. While onsite certification can determine if a particular area is suitable for collecting a given subspecies, certification agencies do not have the personnel to monitor seed collectors. It is our intent that seed weighing of basin subspecies can be used not only to evaluate the composition of subspecies in commercial collections, but also inform the seed industry and land management. Seed weight results could be useful to the seed industry in determining the best sites for collection. Land managers could utilize seed weights to infer subspecies composition (i.e., tridentata versus wyomingensis), choosing a seed lot that best suits the restoration site, matching proportion of tridentata and wyomingensis habitat to composition found in the seed lot.

This research brings into question whether many of the previous restoration efforts in wyomingensis habitat were destined to fail due to planting the wrong subspecies, 2x-tridentata, regardless of other factors important to establishment. Recent monitoring and landscape analyses have shown that the Emergency Stabilization and Rehabilitation (ESR) program methods (BAER Guidebook version 4, 2006) failed to produce adequate establishment of sagebrush and sage-grouse cover, especially for sites in wyomingensis habitat (Arkle et al. 2014, Knutson et al. 2014). In light of our research, it is likely that planting the wrong subspecies of big sagebrush in the wrong habitat with exotic seeded competitors (e.g., Crested wheatgrass, Agropyron cristatum) would certainly provide poor odds for the establishment of big sagebrush cover. The BLM and U.S. Forest Service, which oversee much of the land occupied by sagebrush, have issued policies that direct the use of genetically appropriate plants for restoration (USDA 2008, USDI 2008). Along with the development of seed transfer zones, our proposed application of assessing subspecies tridentata and wyomingensis based on seed weight will aid in fulfilling these policies.
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SUPPLEMENTAL MATERIAL

ECOLOGICAL ARCHIVES

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