Insights into big sagebrush seedling storage practices

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ABSTRACT

Big sagebrush (*Artemisia tridentata* Nutt. [Asteraceae]) is an essential component of shrub-steppe ecosystems in the Great Basin of the US, where degradation due to altered fire regimes, invasive species, and land use changes have led to increased interest in the production of high-quality big sagebrush seedlings for conservation and restoration projects. Seedling storage is an important consideration in the production of high-quality planting stock, yet little is known about optimal storage conditions for this species. Our survey of nurseries in western US found that only 13% of those that grow big sagebrush in containers use cooler or freezer storage to maintain seedling quality. Of those nurseries, 80% rely on cooler storage. We tested cooler (2 to 4 °C) and freezer (0 to –2 °C) storage of big sagebrush in late spring and found that 2 mo of freezer storage reduced survival after transplanting from 96 to 78%, but seedling growth was unaffected. This suggests that seedlings may be more susceptible to freezer conditions because of reduced cold hardiness in spring, but that either storage temperature maintains seedling vigor. Future work is needed on big sagebrush to determine the appropriate hardening regime and lifting dates for freezer storage.

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KEY WORDS
overwinter storage, cooler storage, freezer storage, seedling quality, survey, Asteraceae

NOMENCLATURE
Plants: USDA NRCS (2011)
Birds and Fungi: ITIS (2011)

CONVERSIONS
(°C x 1.8) + 32 = °F
1 cm = 0.4 in
1 m = 3.3 ft
1 l = 0.26 gal

Big sagebrush (*Artemisia tridentata* Nutt. [Asteraceae]) is a semi-deciduous shrub and foundation species in shrub-steppe ecosystems in the Great Basin of the western US (Prevéy and others 2010). Foundation species facilitate stable conditions for associated biota and are vital to ecosystem processes and function (Ellison and others 2005). Roughly 100 species of birds use sagebrush for habitat and forage, with 4 species, Greater Sage-Grouse (*Centrocercus urophasianus* Bonaparte [Phasianidae]), Sage Thrasher (*Oreoscoptes montanus* Townsend [Mimidae]), Sage Sparrow (*Amphispiza belli* Cassin [Emberizidae]) (Figure 1), and Brewer's Sparrow (*Spizella breweri* Cassin [Emberizidae]), identified as obligates (Braun and others 1976). Big sagebrush ecosystems have been designated as critically imperiled due to altered fire regimes, invasion by exotic annual grasses, overgrazing, and land use changes (Noss and Peters 1995; Davies and others 2011). As a result of the decline of this ecosystem and a loss of big sagebrush habitat, Wisdom and others (2003) identified 253 species of plants as being of conservation concern. Because of the ecological importance of big sagebrush and its rate of disappearance, the species is of interest for restoration.

Figure 1. Sage Sparrows (*Amphispiza belli* Cassin [Emberizidae]) have an obligate requirement for sagebrush habitat. Photo by R Kasten Dumroese

Restoration of big sagebrush has often included the use of direct seeding, but planting seedlings could provide a better alternative. Direct seeding on mine reclamation sites has shown erratic success with long-term survival rates of 59% after 8 y (Schuman and Belden 2002) and 28 to 32% after 11 y (Kriger and others 1987). Lysne and Pellant (2004) showed that post-fire aerial application of seeds was ineffective for big sagebrush establishment. Unfortunately, few studies of the long-term survival of outplanted container-grown big sagebrush seedlings are reported; however, first-year survival of outplanted container or bareroot big sagebrush seedlings can be as high as 80% (Welch 2005). When outplanted in critical areas they can also serve as a source for subsequent seed dispersal and continued regeneration (Welch and others 1986; Shaw and others 2005).

To meet the needs of restoration efforts, many traditional forest tree seedling nurseries have modified their successful silvicultural models for producing nursery stock to support the production of high-quality, non-tree native plants (Dumroese and others 2005). These practices could provide a framework for the continued improvement in quality of big sagebrush seedlings. Seedling quality is defined as the ability of plants to establish and survive when outplanted and is dependent on nursery cultural practices (Landis and others 2010). One important component of seedling production that impacts seedling quality is storage. Overwinter storage is an operational practice that allows properly hardened seedlings to be held, in a dormant state, until conditions are favorable for outplanting (Généré and others 2004). Several types of overwinter storage are available for seedlings: open storage, sheltered storage, and refrigerated storage. Seedlings kept in open storage are left outside throughout the winter and may be covered in reflective plastic, Styrofoam sheets and panels, or left uncovered to allow snow to insulate and protect seedlings from temperature extremes. Seedlings in sheltered storage are placed in structures such as shadehouses, unheated greenhouses, polyhouses, and cold frames that provide protection from wind and allow for crude temperature control. Unlike open and sheltered storage, which are subject to variable weather conditions, refrigerated storage allows for precise control over the seedling environment. This prevents the potential loss of seedling dormancy, which, if it occurred, could result in a loss of subsequent seedling quality. Refrigerated storage can be in coolers kept at 2 to 4 °C or in freezers kept at 0 to –2 °C, and the decision to use one method over the other is dependent on the species, the duration of storage, and the potential for storage molds (Landis and others 2010). During storage, mold such as *Botrytis cinerea* Pers.: Fr. (Sclerotiniaceae) can proliferate on plant tissues and potentially lead to loss of seedling vigor or mortality. Storing seedlings in a freezer has been shown to slow and even prevent this problem (Mittal and others 1987; Ritchie 2004).

Many studies have investigated the effects of cooler and freezer storage on tree seedlings (Ritchie 1982; Lindqvist 2001; Généré and others 2004; Jacobs and others 2008), but little information exists on storage of big sagebrush (Fleege 2010). This knowledge deficit has been acknowledged and reinforced by growers in the Intermountain West who have expressed a need for additional information on overwinter storage for this particular species. Therefore, our study objectives were 1) to characterize storage practices in native plant nurseries that grow big sagebrush; and 2) to investigate cooler and freezer storage effects on mold occurrence, survival, and transplanting performance of big sagebrush seedlings.

MATERIALS AND METHODS

Nursery Questionnaire

We developed a telephone questionnaire to characterize and assess current big sagebrush storage practices among nurseries in the western US. The National Nursery and Seed Directory (2011) was used to generate a sample population that was limited to states within big sagebrush range. Within each respective western state, all nurseries were contacted and a person familiar with nursery operations was asked to complete the survey. Each nursery was called a maximum of 3 times, and if there was no response, a copy of the questionnaire was mailed to the nursery. If there was no response to the mail survey, it was considered incomplete. The first survey question asked if the nursery grew sagebrush in containers. If the answer was "yes," subsequent questions investigated the following: storage technique, packing technique, temperature and duration of storage, and mold issues. Subsequent data were summarized with descriptive statistics.

Storage Comparison

Plant Material

Big sagebrush seeds were sown into fully expanded Jiffy forestry pellet containers (maximum dimensions 13 cm × 5 cm, Jiffy Products of America, Norwalk, Ohio) on 3 June 2010 at the University of Idaho Pitkin Forest Nursery in Moscow, Idaho (lat 46.732°, long –116.999°). Trays containing 36 plugs were randomly placed on one bench, and seeds were misted daily until germination and seedling establishment. Fertilization by way of subirrigation began 14 d after sowing and occurred once every 2 wk. For 10 wk, Peters Professional Conifer Grower (20N:7P_2O_5:19K_2O; The Scotts Company, Marysville, Ohio) was applied at 150 ppm; the proportion of nutrients was constant: (100 N [57.7 NO_3^-; 35.4 NH_4^+; 6.9 urea]: 15.3 P: 78.9 K: 3.5 Mg: 2 Fe: 0.3 Mn: 0.3 Zn: 0.3 Cu: 0.03 Mo: 0.13 B). Seedlings were then given Peters Professional Conifer Finisher (4N:25P_2O_5:35K_2O; The Scotts Company) for 5 wk at a rate of 25 ppm; the proportion of nutrients was constant: 100 N (31.2 NH_4; 68.8 urea): 272.9 P: 726.3 K: 7.5 Mg: 10 Fe: 47.5 S: 1.5 Mn: 1.5 Zn: 1.5 Cu: 0.13 Mo: 0.63 B. Supplemental water was applied by way of overhead irrigation, using a gravimetric method when tray weights reached 70% of field capacity.

Storage

On 4 March 2011, 200 seedlings were randomly assigned to either a cooler (2 to 4 °C) or a freezer (0 to –2 °C) treatment (100 seedlings in each). Because of limitations in time and availability, freezer and cooler storage units were not statistically replicated. To minimize the likelihood of an error due to pseudoreplication, large, walk-in cooler (3.4 m × 8.2 m) and freezer (12.2 m × 12.2 m) units were selected for storage; this allowed for reasonable variability in storage conditions and more adequately captured variation that can occur in operational situations. For each storage treatment, seedlings were randomly separated into 5 replicates of 20 seedlings, and each replicate was placed in an unwaxed cardboard box fitted with a plastic bag liner. Within each box, seedlings were grouped into 4 bundles of 5 seedlings and placed in plastic bags that covered the plugs but not the shoot. Seedlings were stored upright inside the box, and the box liner was not sealed. Boxes were then randomly placed in the appropriate storage units, representing a broad cross section of spatial arrangement (high, low, front, and back of the storage units). Seedlings were checked for mold every 5 d; if mold was present, it was removed by hand.

Transplanting

On 11 May 2011, all seedlings were removed from storage, and freezer-stored seedlings were thawed for 2 d indoors at room temperature (21 °C). Initial shoot volume (assessed by water displacement; Burdett 1979), height, and root-collar diameter (RCD) were measured and seedlings were then transplanted into Tall One Treepots (10 cm × 36 cm; 2.83 l; Stuewe & Sons, Tangent, Oregon) containing 2:1 sand:vermiculite (v:v). Transplanted seedlings were grown in a completely randomized design maintaining the storage replication structure (20 seedlings per replicate; 5 replications per treatment) and under ambient greenhouse conditions, with average day/night temperatures of 27 °C/15.5 °C, respectively. Seedlings were watered to field capacity every 3 to 4 d. Most roots had reached the bottom of the pots 2 mo after transplanting, and final measurements of height, RCD, and survival were recorded. We then carefully removed seedlings from the pots and washed the roots with tap water to remove the media. A final shoot volume measurement was obtained before shoots and roots were separated and oven-dried (60 °C for 5 d). For our analysis, growth is defined as the difference between final and initial measurements.

Data Analysis

A 2 × 2 contingency table and Pearson's chi-square test were used to analyze survival data. Growth and dry weight data were analyzed using SAS Software (SAS Institute, Cary, North Carolina). An ANOVA was performed using PROC GLM with replicates nested within storage treatment. A Duncan's multiple range test was used to separate means.

RESULTS AND DISCUSSION

Nursery Questionnaire

Of the 229 nurseries we attempted to contact, 201 completed the survey for a response rate of 91%. Nine nurseries were no longer in business, and 19 did not complete the mailed questionnaire. Data from the completed surveys showed that 46

TABLE 1

The effect of storage method on height growth, root-collar diameter (RCD) growth, shoot volume growth, shoot and root dry mass, and survival 2 mo after transplanting.

Storage method	Height growth (cm)	RCD growth (mm)	Shoot volume growth (ml)	Shoot dry mass (g)	Root dry mass (g)	Survival (%)
Cooler	3.53 (0.23) a	0.37 (0.04) a	3.44 (0.22) a	2.01 (0.10) a	2.35 (0.15) a	96 a
Freezer	3.61 (0.32) a	0.36 (0.04) a	3.43 (0.26) a	2.20 (0.13) a	2.45 (0.19) a	78 b
P value	0.7432	0.8556	0.8840	0.3153	0.9384	<0.0001

Notes: Values are an average of growth (standard errors are in parentheses). Among growth parameters, means with the same letter are not significant ($P \leq 0.05$), by Duncan's multiple range test. Among survival data, means with the same letter are not significant ($P \leq 0.05$), by Pearson chi-square test.

nurseries grow sagebrush seedlings in containers but only 6 (13%) place seedlings in cold storage. Seedlings were lifted from September to March, and subsequent duration of storage ranged from less than a month to 9 mo. Five nurseries used cooler (0 to 3 °C) storage, while one used freezer storage (–1 to –2 °C).

The low proportion of nurseries using cold storage may be attributable to the timing of outplanting windows in specific regions. For example, if fall outplanting is possible, overwinter storage is not needed. Additionally, this survey focused on refrigerated storage, but some nurseries may overwinter their seedlings using another method, such as in a cold frame or shadehouse, or outdoors. The use of alternative methods may be more prevalent in regions where temperatures are consistently below freezing, which prevents a loss of seedling dormancy and unwanted growth (Landis and others 2010). Refrigerated storage is expensive to install and operate, which may cause nurseries to use other methods. Space and expertise considerations are prominent as well. As methods are further developed for producing high-quality native plants, the nuanced practice of overwinter storage is likely to be developed in a successful manner.

Our assessment found that mold was encountered at 4 of the 5 nurseries that use cooler storage, and all took steps to manage it. Only one nursery applied a fungicide prior to storage, but all nurseries employed multiple measures to increase airflow around seedlings. To improve airflow, 3 nurseries used unwaxed cardboard boxes and 4 used supplemental fans. Additionally, 3 nurseries stored seedlings upright and 3 ensured that plastic bags were rolled down to leave shoots uncovered inside the boxes. The results of this study indicate that nurseries are following the recommendations of Kooistra (2004), who suggested increasing airflow to decrease humidity in storage units, and only recommended the use of fungicides if other measures were not sufficient to control storage molds. Interestingly, cooler storage was used more frequently than freezer storage despite information that storage mold growth is slowed at temperatures below freezing (Ritchie 2004; Landis and others 2010). This contradictory behavior may occur because too little information is available to growers about this particular species or because of fears that freezer storage could impact subsequent growth and increase mortality. Our survey was a first attempt to gather information on current methods of big sagebrush seedling storage. Our limited results suggest the need for a more detailed assessment that includes all storage methods used and more general background information about sagebrush propagation and outplanting to further understand the needs and capacity around storage research.

Storage Comparison

Seedling survival was significantly affected by storage treatment ($P \leq 0.05$, $\chi^2 = 14.32$). Survival was 78% in the freezer and 96% in the cooler treatment (Table 1), despite a higher incidence of mold in the cooler treatment. Mold was discovered and removed from all 5 replicates after 3 wk in storage in the cooler; however, in the freezer treatment, mold was found and removed from only 1 replicate after 6 wk in storage. Mold occurred primarily on dead leaf tissue on the tops of plugs and was removed before it could transfer to the stem. If left unchecked, molds such as *Botrytis cinerea* can eventually move to healthy tissue on the stem, cause cankers, and may lead to seedling mortality (Mittal and others 1987; Williamson and others 2007).

Cold-stored seedlings can also suffer mortality from desiccation and cold injury in refrigerated storage (Landis and other 2010). In both storage environments, seedling desiccation was prevented by maintaining high relative humidity in the storage unit, packing seedlings in plastic bags that covered the plugs, and using plastic bag liners inside the cardboard boxes. In-box temperatures were monitored throughout the experiment and remained within the recommended range of 1 to 2 °C for cooler storage and –2 to –4 °C for freezer storage (Landis and others 2010). Cold injury may still have occurred, however, in freezer-stored seedlings because of a loss of seedling cold hardiness; unfortunately, cold hardiness was not measured in this experiment. The ability of a plant to tolerate cold is variable by species, dependent on cultural regimes and changes throughout the growing season (Ritchie 1987; Burr and others 1989; Davis and others 2011). Seedlings in this study went into storage treatments in March, and Herriman and Davis (2012) found that cold hardiness in Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis* Beetle & Young [Asteraceae]) seedlings had decreased by this time, which may be attributed to changes in photoperiod and moisture availability. Seasonal accumulation and loss of cold hardiness has been well described by Fuchigami and others (1982); in the present study a loss of cold hardiness may explain the increased mortality of freezer-stored seedlings. At the USDA Forest Service Lucky Peak Nursery (Boise, Idaho), both container- and bareroot-grown seedlings are placed in freezer storage in the fall and stored for 5 mo, suggesting that fall may be a more appropriate time to lift if freezer storage is used (Fleege 2010). Further study is needed to evaluate physiological status before and after storage to determine the appropriate lifting windows for big sagebrush.

We found subsequent growth was not affected by storage method. No significant differences were found between storage treatments in any of the growth parameters measured ($P \leq 0.05$; height, RCD growth, shoot volume growth, root dry weight, and shoot dry weight; Table 1). Although this experiment yielded no differences in growth due to storage temperature, note that the duration of storage was only 2 mo. Storage duration can impact seedling quality and is often species dependent (Ritchie 1982; Jacobs and others 2008). During storage, plants use nonstructural stored carbohydrate reserves to maintain respiration. These stored carbohydrates are what plants draw from to produce new tissue after outplanting and serve as one measure of seedling quality. Rates of respiration, and therefore stored carbohydrate depletion, are dependent on temperature, with higher temperatures causing increased depletion (Ritchie 2004; Landis and others 2010). Ritchie (1982) showed that 2 mo in storage was a pivotal point for coastal Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii* [Pinaceae]) seedlings. After 2 mo, freezer-stored seedlings had significantly more stored carbohydrates than did cooler-stored seedlings. The impact of storage temperature and duration on carbohydrate reserves and subsequent transplanting performance of big sagebrush seedlings needs further investigation.

CONCLUSION AND FUTURE DIRECTIONS

Our study reveals that few nurseries (13%) store big sagebrush seedlings under cooler or freezer conditions. We found that growth after transplanting was unaffected by storage temperature, indicating seedlings can be stored for short durations using either cooler or freezer methods. Additional considerations such as the potential for mold may result in a preference for freezer storage, but further study is needed to quantify the appropriate hardening regime and lifting dates to improve seedling survival in freezer storage. Few studies have focused on refining cultural practices for growing big sagebrush in container nurseries, and our study is one of the first that attempts to provide growers with more information on this topic.

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