ADVANCING NURSERY PRODUCTION OF BIG SAGEBRUSH SEEDLINGS:

COLD STORAGE AND VARIATION IN SUBSPECIES GROWTH

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AUTHORIZATION TO SUBMIT THESIS

This thesis by Emily Carey Overton submitted for the degree of Master of Science with a Major in Natural Resources and titled "ADVANCING NURSERY PRODUCTION OF BIG SAGEBRUSH SEEDLINGS: COLD STORAGE AND VARIATION IN SUBSPECIES GROWTH," has been reviewed in final form. Permission, as indicated by the signatures and dates given below, is now granted to submit final copies to the College of Graduate Studies for approval.

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Abstract

Big sagebrush is an essential component of shrub-steppe ecosystems that is rapidly disappearing from the landscape. Recognizing this, it has become a target of restoration; however, current efforts using direct seeding have shown variable success and planting seedlings may provide a better option. Nonetheless, limited information is available on the nursery production of big sagebrush seedlings. This thesis aims to 1) further refine the cold storage component of sagebrush seedling nursery culture, and 2) investigate seed source variation in Wyoming big sagebrush (*Artemisia tridentata* ssp. *wyomingensis*) seedlings. Chapter 1 was a two-part interdisciplinary study that 1) surveyed current cold storage practices in nurseries throughout the Western United States, and 2) compared freezer and cooler storage methods on seedling survival and growth after transplanting. Surveys revealed few nurseries use refrigerated storage, but those that did preferred cooler storage. In the comparison study, freezer storage reduced survival by 18%; however, growth results indicate using coolers and freezers were both effective methods of seedling storage. The survey and comparison studies both point to mold being problematic in cooler storage; therefore, freezer storage may be preferred when longer-term storage is needed. Chapter 2 investigated growth, total biomass, and net photosynthesis among Wyoming big sagebrush seedlings grown from 5 disparate seed sources in a common greenhouse environment. The objective was to determine if plasticity could be detected at the subspecies level. Evidence of phenotypic expression and genetic effects were detected in measurements of total growth and biomass allocation; yet, no consistent trend was observed among populations in measurements of net photosynthesis. These results suggest that there is a possibility of local adaptations in populations of Wyoming big sagebrush and this should be considered in restoration planning.

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Chapter 1

Insights into Big Sagebrush Seedling Cold Storage Practices

Abstract

Big sagebrush is an essential component of shrub-steppe ecosystems in the Great Basin, 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 the western US found that only 13% of those that grow big sagebrush (*Artemisia tridentata* Nutt. [Asteraceae]) 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 by 18%, 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 temp maintains seedling vigor. Future work is needed on big sagebrush to determine the appropriate hardening regime and lifting dates for freezer storage.

Introduction

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 USA (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 (Sage Grouse (*Centrocercus urophasianus* Bonaparte [Phasianidae]), Sage Thrasher (*Oreoscoptes montanus* Townsend [Mimidae]), Sage Sparrow (*Amphispiza belli* Cassin [Emberizidae]), 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 this ecosystem's decline and a loss of big sagebrush habitat, Wisdom and others (2003) identified 253 species of plants as species of conservation concern. Due to the ecological importance of big sagebrush and the rate of disappearance, it is a species of interest.

Restoration of big sagebrush has often included the use of direct seeding, but planting seedlings could provide a better alternative. Direct seeding for restoration of big sagebrush on mine reclamation sites has shown erratic success with long-term survival rates of 59% after 8 y (Schuman and Belden 2002) and 28-32% after 11 y (Kriger and others 1987). Lysne and Pellant (2004) showed that post-fire aerial application of seed was ineffective for big sagebrush establishment. In comparison, first year survival of transplanted big sagebrush seedlings can be as high as 80% (Welch 2005), and when planted 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 expanded into the production of non-tree native plants and adapted tree seedling production practices to produce quality native plant material. These practices could provide a framework for the continued improvement of big sagebrush seedling quality. 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, they include: 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 a loss of seedling dormancy and helps maintain seedling quality. Refrigerated storage can be done in either coolers (2 to 4 °C (36 - 39 °F)) or freezers (0 to -2 °C (28 - 32 °F)) and the decision to use one method over another is dependent upon the species and the duration of storage (Landis and others 2010).

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 two-fold. Our first objective was to determine the number of native plant nurseries that grow big sagebrush and to investigate seedling cold storage practices. A survey was used to gather this information. We hypothesized that storage practices would be varied, with most nurseries implementing cold storage; additionally, when storage practices included freezer or cooler storage, we hypothesized that mold issues would be more prevalent in cooler storage than freezer storage. Our second objective was to investigate the effects of cooler and freezer storage on big sagebrush seedling quality and transplanting performance. We hypothesized that seedling growth would not be impacted by storage method, but that mold would likely be present in cooler-stored seedlings.

Materials and Methods

Nursery Questionnaire

We developed a telephone questionnaire to assess current big sagebrush storage practices among nurseries in the western US. The National Nursery and Seed Directory (www.rngr.net) 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 (Appendix). Subsequent data were summarized with descriptive statistics.

Cold Storage Comparison

Plant Material

Big sagebrush seeds, provided by the Bureau of Land Management and collected in southern Idaho, were sown into trays containing 36 Jiffy plugs on 3 June 2010, at the University of Idaho Pitkin Forest Nursery in Moscow, Idaho (latitude 46.732°; longitude -116.999°). Trays were randomly placed on 1 bench, and seeds were misted daily until germination and seedling establishment. Fertilization via subirrigation began 14 d after sowing and occurred once every 2 wk. For 10 wk, Peters® Professional Conifer Grower™ was applied at 150 ppm; the proportion of nutrients was constant: 100N (57.7 NO_3^-; 35.4 NH_4^+; 6.9 urea): 15.3P: 78.9K: 3.5Mg: 2Fe: 0.3Mn: 0.3 Zn: 0.3 Cu: 0.03 Mo: 0.13B. Seedlings were then given Peters® Professional Conifer Finisher™ for 5 wk at a rate of 25 ppm; the proportion of nutrients was constant: 100N (31.2NH_4 & 68.8Urea): 272.9P: 726.3K: 7.5Mg: 10Fe: 47.5S: 1.5Mn: 1.5Zn: 1.5Cu: 0.13Mo: 0.63B. Supplemental water was applied via overhead irrigation, using a gravimetric method when block weights reached 70% of field capacity.

Cold Storage

On 4 March 2011, 200 seedlings were randomly assigned to either a cooler (2 to 4 °C) or freezer (0 to -2 °C) treatment (n = 100 per storage treatment). In each cold storage treatment, seedlings were randomly separated into 5 replicates of 20 seedlings, and each replicate was placed in an un-waxed cardboard box fitted with a plastic bag liner. Within each box, seedlings were grouped into 4 bundles of 5 seedlings and placed in rolled-down plastic bags that covered the plug but not the shoot. Each bundle was stored upright inside the box and the bag liner was not sealed. Boxes were randomly scattered throughout either a walk-in cooler or freezer. Seedlings were checked for mold every 5 d and when it was discovered it was manually removed.

Transplanting

On 11 May 2011, all seedlings were removed from storage and thawed for 2 d prior to transplanting. Initial shoot volume (via water displacement; Burdett 1979), height, and root-collar diameter (RCD) were measured and seedlings were then transplanted into Tall One Treepots™ (2.83 l (0.75 gal), Stuewe & Sons, Corvallis, OR) containing 2:1 sand:vermiculite (v:v). The transplanted seedlings were grown in a completely randomized design, maintaining the storage replication structure (5 reps per treatment), under ambient greenhouse conditions, with average day/night temperatures of 27 °C (81 °F)/15.5 °C (60 °F), respectively. Seedlings were watered to field capacity every 3 to 4 d. The study ended 2 mo after transplanting when roots reached the bottoms of the pots. *In situ*, post-transplant measurements included: height, RCD, and survival. Seedlings were then carefully removed from the pots and roots were washed with tap water to remove the media. A final shoot volume measurement was taken before the shoots were separated from the roots, and dried at 60°C in an oven for 5 d. Growth was 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, Inc., Cary, NC, USA). 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 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 months. Five nurseries used cooler (0 to 3 °C) storage, while 1 used freezer storage (-1 to -2 °C).

The low proportion of nurseries using cold storage may be due 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, 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 (Landis and others 2010). Refrigerated storage is also expensive to install and operate, which may cause nurseries to use other methods.

Consistent with our hypothesis, mold was encountered at 4 of the 5 nurseries that use cooler storage, and all took steps to manage it. Only 1 nursery applied a fungicide prior to storage, but all nurseries employed multiple measures to increase airflow around seedlings. To improve airflow, 3 nurseries used un-waxed cardboard boxes and 4 used supplemental fans. Additionally, 3 nurseries stored seedlings upright and 3 ensured plastic bags were rolled down to leave shoots uncovered inside the boxes. The results of this study indicate that nursery storage practices are congruent with the recommendations of Kooistra (2004), who suggests increasing airflow to decrease humidity in storage units and only recommends the use of fungicides if other measures are 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). The deficit of information available to growers about this particular species and fear that freezer storage could impact subsequent growth and increase mortality may be the cause. Our survey was a first attempt to gather information on current methods of big sagebrush seedling storage, but our limited results suggest the need for a more detailed assessment. As illustrated, more information on all storage methods used and more general background information about sagebrush propagation and outplanting would further clarify future, pertinent storage research.

Cold Storage Comparison

Seedling survival was significantly affected by storage treatment ($P \leq 0.05$, χ^2 = 14.32). Despite the incidence of mold being higher in the cooler treatment, survival was 78% in the freezer and 96% in the cooler treatment (Table 1.1). In the cooler treatment, mold was discovered and removed from all 5 replicates after 3 wk in storage; however, in the freezer treatment, mold was found and removed from only 1 replicate after 6 wk in storage. Mold primarily occurred 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* Pers.Fr. [Sclerotiniaceae] 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).

Although not tested in this experiment, higher mortality in the freezer treatment could be due to a loss of cold hardiness. Cold hardiness is the ability of a plant to tolerate cold; it is variable by species, dependent upon 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, but Herriman and Davis (2012) found that cold hardiness in Wyoming big sagebrush seedlings had decreased by this time, which may be attributed to changes in photoperiod and moisture availability. At Lucky Peak Nursery (Boise, ID), both container and bare root 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 impacted by storage method. No significant differences were found between cold 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 1.1). Although this experiment yielded no differences in growth due to storage temperature, it should be noted 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 non-structural 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 seedlings. After 2 mo, freezer stored seedlings had significantly more stored carbohydrates than 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

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 more information on this topic. Our study reveals that few (13%) nurseries 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 potential mold growth, may result in a preference for freezer storage; however, further study is needed to quantify the appropriate hardening regime and lifting dates to improve seedling survival in freezer storage.

Literature Cited

Braun CE, Baker MF, Eng RL, Gashwiler JS, Schroeder MH. 1976. Conservation committee report on effects of alteration of sagebrush communities on the associated avifauna. Wilson Bulletin 88:165-171.

Burdett AN. 1979. A non-destructive method for measuring the volume of intact plants. Canadian Journal of Forest Research 9:120-122.

Burr KE, Tinus RW, Wallner SJ, King RM. 1989. Relationships among cold hardiness, root growth potential, and bud dormancy in three conifers. Tree Physiology 5:291-306.

Davies KW, Boyd CS, Beck JL, Bates JD, Svejcar TJ, Gregg MA. 2011. Saving the sagebrush sea: an ecosystem conservation plan for big sagebrush communities. Biological Conservation 144: 2573-2584.

Davis AS, Ross-Davis AL, Dumroese RK. 2011. Nursery culture impacts cold hardiness in longleaf pine (*Pinus palustris*) seedlings. Restoration Ecology 19:717-719.

Ellison AM, Bank MS, Clinton BD, and 17 others. 2005. Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. Frontiers in Ecology and the Environment 3:479-486.

Fleege CK. 2010. Protocols for sagebrush seed processing and seedling production at the Lucky Peak Nursery. In: Riley LE, Pinto JR, Dumroese RK, technical coordinators. National proceedings: forest and conservation nursery associations—2009. Fort Collins (CO): USDA Forest Service, Rocky Mountain Research Station. Proceedings RMRS-P-62. p 33-35.

Généré B, Garriou D, Omarzad O, Grivet JP, Hagège D. 2004. Effect of a strong cold storage induced desiccation on metabolic solutes, stock quality and regrowth, in seedlings of two oak species. Trees 18:559-565.

Herriman KR, Davis AS. 2012. Cold hardiness in Wyoming big sagebrush seedlings: implications for nursery production and outplanting. Ecological Restoration 30: 101-103.

[ITIS] Integrated Taxonomic Information System. 2011. URL: http://www.itis.gov (accessed 19 December 2011).

Jacobs DF, Wilson BC, Ross-Davis AL, Davis AS. 2008. Cold hardiness and transplant response of *Juglans nigra* seedlings subjected to alternative storage regimes. Annals of Forest Science 65:606.

Kooistra CM. 2004. Seedling storage and handling in Western Canada. In: Riley LE, Dumroese, RK, Landis TD, technical coordinators. National proceedings: forest and conservation nursery associations—2003. Fort Collins (CO): USDA, Forest Service, Rocky Mountain Research Station. Proceedings RMRS-P-33. p 15-21.

Kriger JA, Berg WA, Herron JT, Phillips CM, Atkinson RG. 1987. Shrub establishment in the mountain shrub zone. In: 4th Biennial Symposium Proceedings, surface mining and reclamation of the Great Plains; and 4th American Society for Surface Mining and Reclamation; 1987 March16-20; Billings, MT. Bozeman (MT): Montana State University, Reclamation Research Unit.

Landis TD, Dumroese RK, Haase DL. 2010. Seedling processing, storage, and outplanting. Volume 7, The Container Tree Nursery Manual. . Washington (DC): USDA Forest Service. Agriculture Handbook 674. 200 p.

Lindqvist HS. 2001. Effect of different lifting dates and different lengths of cold storage on plant vitality of silver birch and common oak. Scientia Horticulturae 88:147-161.

Lysne CR, Pellant M. 2004. Establishment of aerially seeded big sagebrush following southern Idaho wildfires. Boise (ID): USDI Bureau of Land Management. Technical Bulletin 2004-01.

Mittal RK, Singh P, Wang BSP. 1987. Botrytis: a hazard to reforestation. European Journal of Forest Pathology 17:369-384.

National Nursery and Seed Directory. 2011. URL: http://www.rngr.net/ resources/directory (accessed March 2011).

Noss RF, Peters RL. 1995. Endangered ecosystems: a status report on America's vanishing habitat and wildlife. Washington (DC): Defenders of Wildlife. 132 p.

Prevéy JS, Germino MJ, Huntly NJ. 2010. Loss of foundation species increases population growth of exotic forbs in sagebrush steppe. Ecological Applications 20:1890-1892.

Ritchie GA. 1982. Carbohydrate reserves and root growth potential in Douglas-fir seedlings before and after cold storage. Canadian Journal of Forest Research 12:905-912.

Ritchie GA. 1987. Some effects of cold storage on seedling physiology. Tree Planters' Notes 38:11-15.

Ritchie GA. 2004. Container seedling storage and handling in the Pacific Northwest: answers to some frequently asked questions. In: Riley LE, Dumroese, RK, Landis TD, technical coordinators. National proceedings: forest and conservation nursery associations—2003. Fort Collins (CO): USDA Forest Service, Rocky Mountain Research Station. Proceedings RMRS-P-33. p 3-7.

Schuman GE, Belden SE. 2002. Long-term survival of direct seeded Wyoming big sagebrush seedlings on a reclaimed mine site. Arid Land Research and Management 16:309-317.

Shaw NL, DeBolt AM, Rosentreter R. 2005. Reseeding big sagebrush: techniques and issues. In: Shaw NL, Pellant M, Monsen SB, compilers. Symposium proceedings, sage-grouse habitat restoration; 4–7 June 2001; Boise, ID. Fort Collins (CO): USDA, Forest Service, Rocky Mountain Research Station. Proceedings RMRS-P-38. p 99-108.

[USDA NRCS] USDA Natural Resources Conservation Service. 2011. The PLANTS database. URL: http://plants.usda.gov (accessed 19 December 2011). Greensboro (NC): National Plant Data Team.

Welch BL, McArthur ED, Nelson DL, Pederson JC, Davis J N. 1986. 'Hobble Creek'—a superior selection of low-elevation mountain big sagebrush. Ogden (UT): USDA Forest Service, Intermountain Research Station. Research Paper INT-370. 10 p.

Welch BL. 2005. Big sagebrush: a sea fragmented into lakes, ponds, and puddles. Fort Collins (CO): USDA Forest Service, Rocky Mountain Research Station. General Technical Report RMRS-GTR-144. 210 p.

Williamson B, Tudzynski B, Tudzynski P, Van Kan JAL. 2007. *Botrytis cinera*: the cause of grey mould disease. Molecular Plant Pathology 8:561-580.

Wisdom MJ, Rowland MM, LH Suring LH, L Schueck L, Wolff Meinke C, Wales BC, Knick ST. 2003. Procedures for regional assessment of habitats for species of conservation concern in the sagebrush ecosystem. La Grande (OR): USDA Forest Service, Pacific Northwest Research Station. March 2003 Report Version 1. 151 p.

Table 1.1. Cold storage effects on sagebrush (*Artemisia tridentata*) seedling growth, biomass, and survival 2 mo after transplanting. Root-collar diameter (RCD) is the diameter of the main seedling stem approximately 1 cm above ground-line. The analysis of variance Duncan's multiple range test was used on growth and biomass characteristics; The Pearson's chi-square test was used for survival.

Storage Method	Height Growth (cm)	RCD Growth (mm)	Shoot Volume Growth (ml)	Shoot Dry Mass (g)	Root Dry Mass (g)	Survival (%)
Cooler	3.53 a[a]	0.37 a	3.44 a	2.01 a	2.35 a	96 a
Freezer	3.61 a	0.36 a	3.43 a	2.20 a	2.45 a	78 b

[a]Column means with the same letters are not significantly different ($P \leq 0.05$)

Chapter 2

Subspecies Variation in Wyoming Big Sagebrush (*Artemisia tridentata* ssp. *wyomingensis*) Growth and Morphology

Abstract

Currently very little information is available on the variation that exists among Wyoming big sagebrush (*Artemisia tridentata* spp. *wyomingensis*) populations. This can be problematic because current restoration practices focus on matching the appropriate subspecies to a site even though site conditions can be disparate, as can the adaptive variation among populations within a subspecies. The objective of this study was to investigate subspecies plasticity with the goal to provide guidance for the selection of appropriate sources of plant material to improve the restoration of this ecologically important species. This experiment investigated the plasticity in total growth, biomass allocation, and net photosynthesis in Wyoming big sagebrush seedlings grown from 5 geographically distinct seed sources. Seedlings were grown in a common greenhouse environment for one growing season and then transplanted into larger pots for an additional growing season. Measurements of height, root-collar diameter, and seedling gas exchange were taken on transplanted seedlings. Seedlings were harvested and final dry weight measurements were taken of leaves, stems, and roots. Significant differences due to seed source were observed in measurements of total growth and biomass allocation; however, no consistent trend was observed among populations in measurements of net photosynthesis. Seedlings grown from Washington and Montana seed sources were the most different in total growth and seedling size, despite having similar patterns of biomass allocation. A low proportion of total biomass was allocated to roots (40%) in seedlings grown from a New Mexico seed source, which is uncommon in arid species that typically partition more biomass to roots. Differences in seedling growth and biomass allocation may be the result of genetic effects. Specific leaf area was the only trait tested that appears to be a modificative adaptation. Some plasticity occurred at the subspecies level, which indicates there is a possibility of local adaptation and this needs to be considered when restoring big sagebrush populations.

Introduction

Big sagebrush (*Artemisia tridentata* Nutt. [Asteraceae]) is a broadly distributed foundation species in Western North America. It is a dominant shrub throughout much of its range and as a foundation species is vital to stabilizing ecosystem processes and function (McArthur 2000; Ellison and others 2005; Prevéy and others 2010). Big sagebrush provides habitat and forage for an extensive spectrum of wildlife including 100 species of bird, several obligate mammals such as pygmy rabbits (*Brachylagus idahoensis* Merriam [Leporidae]), and pronghorn (*Antilocapra americana* Ord [Antilocapridae]) (Braun and others 1976; Noss and Peters 1995). It also aids in soil stabilization and if removed, has been shown to increase the establishment of exotic invasive forbs in sagebrush-steppe (Prevéy and others 2010).

Six distinct subspecies of big sagebrush vary in their ability to tolerate the large amount of environmental and ecological variation that exists throughout an extensive range. The distribution of big sagebrush includes 14 US states and stretches from southern British Columbia to northern Baja Mexico (Welch 2005). It is found from 100 to 3,400 m in elevation with annual precipitation varying threefold within its range (McArthur and others 1981; West 1983; Kolb and Sperry 1999). Studies have focused on comparisons between the most widely distributed subspecies: *tridentata*, *vaseyana*, and *wyomingensis* (Stanton and others 2002). These studies show that Wyoming big sagebrush (ssp. *wyomingensis*) occupies drier, shallow soils, while work by Kolb and Sperry (1999) suggest that it has adapted to these drought conditions through increased resistance to xylem cavitation. This subspecies is a tetraploid, short in stature (typically 1m to <0.5 m tall), and grows slowly, reaching a yearly maximum growth rate earlier in the season (Booth and others 1990; Barker and McKell 1986; Kolb and Sperry 1999; McArthur and Sanderson 1999; Meyer and Monsen 1992). It has a distribution that spans from North Dakota to Washington and extends south to Arizona and New Mexico (Mahalovich and McArthur 2004). Despite the large amount of research that has been conducted comparing subspecies, very little information is available on the plasticity within a subspecies.

Big sagebrush is disappearing from the landscape and due to its ecological importance has become a target species for restoration. Urbanization, conversion to agriculture, over-grazing, altered fire regimes, and the presence of exotic invasive species are causing the degradation of sagebrush ecosystems (Davies and others 2011). It is estimated that nearly half of sagebrush steppe communities have disappeared, causing them to being designated as critically imperiled (Noss and Peters 1995). Recognizing this, and knowing big sagebrush is an important contributor to these ecosystems, this species has been targeted for restoration. Unfortunately, current restoration projects produce inconsistent results. For example, restoration efforts, using direct seeding and seedling planting, have shown variable success with survival rates of 32-80% (Welch and others 1986; Schuman and Belden 2002).

Many factors contribute to the success or failure of restoration revegetation efforts. Seed zone selection, nursery culture, and outplanting techniques are just a few of these factors; yet, despite the identified need for restoration and increases in big sagebrush seedling production, nursery and outplanting practices have not been refined to provide practitioners with strong guidance regarding these factors. The Target Plant Concept (TPC) addresses all of these factors and provides conceptual guidance for planting seedlings successfully for any type of project (Landis and others 2010). One major component of the TPC advocates proper plant material selection. Studies by Herriman (2009) and Aghai (2012) provide insight into new types of big sage plant materials and alternative container production methods; however, successful implementation of those practices requires a better understanding of species and subspecies characteristics.

An increased understanding of the role that seed source plays at the subspecies level may help to increase the success of restoration projects. This study is the first to investigate subspecies level plasticity in Wyoming big sagebrush. Our study objectives were to determine if Wyoming big sagebrush (ssp. *wyomingensis*) seedlings grown from geographically distinct seed sources vary in growth. Due to the large distribution of this subspecies, we hypothesized variation in total growth, net photosynthetic assimilation, and biomass partitioning would exist between populations. To determine if potential differences in growth and morphology were a genetic response, seedlings were grown in a greenhouse common garden. The impact this could have on future nursery culture and outplanting practices is discussed.

Materials and Methods

Plant Material

Wyoming big sagebrush (ssp. *wyomingensis*) seeds collected from 1 family (mother plant) at 5 sites within the current range were obtained from the Provo Shrub Sciences Laboratory in Provo, Utah. Seed sources are referred to by state, and collections were made in: Montana, Idaho, New Mexico, Utah, and Washington (MT, ID, NM, UT, and WA, respectively). Climate data at each collection site was generated using PRISM data explorer (Parameter- elevation Regressions on Independent Slopes Model, PRISM Climate Group, Oregon State University, Corvallis, OR, http://www.prismclimate.org [accessed 26 August 2012]) and is presented as average of 60 yr of data (Table 2.1).

Five individuals from each site (n = 25) were grown at the University of Idaho (UI) Pitkin Forest Nursery (PFN) in Moscow, Idaho (latitude 46.732; longitude -116.999°). First year nursery culture involved growing seedlings in 164 ml Ray Leach Cone-tainers (SC10, Stuewe & Sons, Inc., Tangent, OR) using a peat-vermiculite commercial potting mix (40-50% peat, vermiculite, and bark, Sunshine Custom Blend #1, Sun Gro Horticulture, Bellevue, WA). Seeds were covered with a light layer of media and misted daily until germination and seedling establishment. Biweekly fertilization via subirrigation began 14 d after sowing. For 8 wk, Peters® Professional Conifer Finisher ™ (The Scotts Company, Marysville, Ohio; $4N{:}25P_2O_5{:}35K_2O$) was applied at 25 mg $N\cdot l^{-1}$. For the next 6 wk, Peters® Professional Conifer Grower ™ ($20N{:}7P_2O_5{:}19K_2O$) was applied at 150 mg $N\cdot l^{-1}$, and for 4 wk seedlings were again given Peters® Professional Conifer Finisher™ ($4N{:}25P_2O_5{:}35K_2O$) at a rate of 25 mg $N\cdot l^{-1}$. Supplemental water was applied via overhead irrigation every 7 d. After 20 wk of nursery culture, seedlings were placed in freezer storage (0 to -2 °C) on 4 March 2011.

Transplant Experiment

On 2 June 2011, all seedlings were removed from storage and allowed to thaw for 2 d before being transplanted into 9.65 l pots (TP818, Stuewe & Sons Inc., Tangent, OR). The pots were filled with sand and vermiculite (2:1, v:v), mixed with 0.14 g Scotts® Professional Osmocote® ($19N{:}6P_2O_5{:}12K_2O$), and to watered field capacity every 5 d. Seedlings were grown under ambient light conditions and average greenhouse temperatures throughout the study were 25 ºC max/14 ºC min. A completely randomized design was used to organize the populations of transplanted seedlings in the UI PFN greenhouse.

Growth and Biomass Allocation Measurements

Prior to transplanting, morphological measures were made to summarize nursery cultural practices on growth. Nondestructive, initial measurements of height (from root collar to apical meristem of the tallest leader), root-collar diameter (RCD), and shoot volume (using water displacement, Burdett 1979) were recorded. The seedlings were then removed from their Cone-tainers, roots were washed to remove media, and root volumes were measured (same water displacement technique mentioned above). On 26 October 2011, after 21 wk of growth, final morphological measurements of seedling height, RCD, and root and shoot volumes were measured (using the same techniques mentioned above). Growth parameters were calculated by subtracting the initial morphological measure (post nursery culture) from the final one (post transplanting culture). Morphological measures of leaf area and biomass were also acquired. Total leaf area was obtained for each plant by removing all the leaves and scanning them on a flatbed scanner; ImageJ software (Schneider and others 2012) was used to calculate total leaf area. Root, stem, and leaf dry weights were determined after placing plant material in a 60 °C oven and for 5 d. Measurements of specific leaf area (SLA; total plant leaf area / total leaf dry weight) and leaf area ratio (LAR; total plant leaf area/ total plant biomass) were calculated.

Gas Exchange Measurements

One mo after transplanting, gas exchange measurements were initiated to quantify carbon assimilation. For each seed source, light response curves and diurnal measurements were taken, using a Li-6400 portable photosynthesis analysis system (Li-COR, Inc., Lincoln, NE), to find light saturation points and determine the appropriate time of day for subsequent gas exchange measurements. Data indicated the best time of day to measure was 0800-1300 hr, and photosynthetically active radiation (PAR) should be set to 1200 µmol photon $m^{-2} s^{-1}$. During gas exchange measurements, conditions inside the leaf chamber of the Li-6400 were set to a relative humidity of 60-70%, a PAR level of 1200 µmol photon $m^{-2} s^{-1}$, and CO_2 concentration of 400 µmol mol^{-1}. Measurements were made in a 2 × 3 cm leaf chamber equipped with a LED light source (6400-02B LED) that uses infrared gas analyzers to detect changes in H_20 and CO_2 when compared to a reference sample.

Biweekly gas exchange measurements were taken on all 25 seedlings in July and August, followed by monthly measurements in September and October. Because sage leaves were not big enough to fill the Li-6400 chamber, leaf area had to be determined to correctly adjust gas exchange values. Following gas exchange measurements, the leaves placed inside the chamber were excised from the plant and placed in a flatbed scanner to determined leaf area (same technique mentioned above). Gas exchange measurements were then scaled to the whole-plant level using the total leaf area values acquired from the biomass allocation measurements.

Data Analysis

Seedlings were placed in a completely randomized design and 5 seedlings were grown for each of the 5 populations (n = 25). All data was analyzed using SAS software (SAS Inc., Cary, NC, 2010). Normality was assessed visually using diagnostic plots. A Levene's test was used to test the assumption of homogeneity of variance. The variable stem dry weight was found to have unequal variance, so a Satterthwaite approximation was used to find approximate F statistics, and means were compared using the Tukey's method. A one-way analysis of variance was used to determine the effect of seed source on height growth, RCD growth, leaf dry weight, root dry weight, whole plant dry weight, SLA, and LAR. When differences by seed source were found to be significant a Tukey's HSD test ($\alpha = 0.05$) was used to separate means.

Photosynthesis data was gathered from all 25 seedlings on 7 measurement periods. Each measurement period was analyzed separately using an analysis of variance, and when differences among seed sources were detected, a Tukey's HSD test ($\alpha = 0.05$) was used to separate means.

Results

Overall differences in growth were detected between seed sources (Table 2.2). The WA seed source had significantly greater height growth than the MT site with an average of 12.8 cm more growth. RCD growth was significantly larger in seedlings grown from the WA and NM seed sources than those grown from the MT source. The WA source also had greater RCD growth than seedlings from the ID source (Table 2.4).

Seedlings grown from each seed source showed significant differences in total biomass and biomass allocation (Table 2.2 and 2.3). Seedlings grown from the WA seed source had significantly more total plant biomass than those from the NM, ID, and MT sources (Table 2.5). Those from the UT collection site also had a greater whole plant dry weight than those from the MT site. Leaf dry weight was the same among seedlings from the WA, NM, and UT seed sources and was significantly greater than seedlings grown from the MT seed source. Stem dry weight was the greatest in seedlings grown from the WA collection site. Stems of seedlings from the NM collection site were significantly larger than the MT and ID sites. Root dry weight was significantly higher in seedlings from the WA seed source than those from the NM and MT sources.

Seedlings grown from the UT and ID seed sources allocated more biomass to roots than shoots than seedlings from the NM source (Figure 2.1). Allocation to roots as percent of total biomass was 67% and 62% for the ID and UT seed sources respectively; however, the NM site allocated only 40% (Figure 2.1). Allocation to leaf area as a proportion of total biomass (LAR) was greater in seedlings from the NM seed source than the WA source (Table 2.5).

Due to large significant differences in total biomass, leaf level gas exchange measurements of net photosynthesis were scaled to the whole-plant level with the assumption that final leaf area was representative of leaf area throughout the growing season. Whole-plant net photosynthesis was significantly different among seed sources 7 and 11 weeks after transplanting, but not on any other measurement period (Table 2.6). In general, photosynthesis decreased throughout the growing season as seedlings moved to a more quiescent status in October. Among the dates that yielded significant differences, the MT seed source was consistently low in net carbon allocation, while the NM seed source was always high (Figure 2.2).

Discussion

We detected significant differences in growth and total biomass in seedlings grown from different seed sources, which indicates there is some plasticity at the subspecies level. Seedlings in this study were grown in a common environment, and that allows for speculation on whether the differences detected might be an expression of phenotypic plasticity or genetic effects. The greatest number of differences in growth and total biomass occurred between seedlings grown from the MT and WA seed sources. These seed sources also have large differences in the total amount of annual precipitation with the MT seed collection site receiving 45% more annual precipitation than the WA site (Table 2.1). It is possible WA seedlings were able to capitalize on the absence of a water limitation and this may account for their significantly larger size.

Another potential genetic adaptation was observed in seedlings grown from the NM seed source. Seedlings from the NM seed source did not follow the same pattern of increased biomass partitioning to roots that is typically observed in shrubs from arid environments (Lloret and others 1999; Schwinning and Ehleringer 2001). They allocated only 40% of total biomass to roots (Figure 2.1) and this may be explained by a genetic adaptation to seasonal rainfall patterns at the collection site. The NM site receives over half of its precipitation from June to October; the same time frame this study was conducted. Conversely, MT, ID, UT, and WA seed sources receive a majority of their precipitation from November-July. In their meta-analysis of over 1300 studies of plants in arid regions, Schenk and Jackson (2002) found shrubs receiving precipitation during the summer months tended to have shallower root systems than those that received the majority of precipitation in the winter. The results of this study indicate that a similar pattern of differences in root architecture may occur in populations of Wyoming big sagebrush. A reciprocal common garden experiment would help validate whether differences detected in total biomass and biomass allocation are the result of genetic adaption (Kawecki and Ebert 2004). If certain traits prove to be the expression of genetic effects, this knowledge may help to further define seed transfer guidelines for big sagebrush subspecies.

Specific leaf area was similar among populations of Wyoming big sagebrush when placed in greenhouse growing conditions, which may indicate this trait is a phenotypic response to environment. SLA is an important plant functional trait that is strongly correlated with relative growth rate (Lambers and others 1998). It is a plastic trait that often varies in response to limiting resources (Poorter and others 2009). Scheepens and others (2010) investigated SLA differences in *Campanula thyrsoides* populations, grown at different altitudes, to see whether the trait was the result of phenotypic plasticity or genetic effects. They found SLA variation was primarily attributed to phenotypic plasticity and that genetic effects were comparatively small; however, genetic effects were still the result of local adaptation. Seedlings in this study were placed in conditions that attempted to satisfy resource needs, which could explain the similarity in SLA among populations.

Consistent differences in whole-plant net photosynthesis among populations of Wyoming big sagebrush were not observed in this experiment, despite large differences in growth and total biomass. It is possible the timing of measurement periods may not have accurately reflected the maximum growth period. Vegetative growth of big sagebrush species occurs in the beginning of the season due to the timing of moisture (Evans and Black 1993). Additionally, Booth and others (1990) showed Wyoming big sagebrush height growth rates were the greatest in mid-march. Gas exchange measurements in this experiment did not begin until July, so it is possible that the periods of maximum growth were not detected.

The apparent differences in seedling size and biomass allocation observed in this experiment have implications for nursery production of Wyoming big sagebrush. Seedlings in this study were grown in a common environment, but produced seedlings of significantly different sizes and morphology. Further understanding of the amount of plasticity at the subspecies level will help nursery growers create seed source specific culturing regimes. This is common practice in many tree species, but has not been considered in sagebrush subspecies. Refining cultural practices will help to create target seedlings that meet the needs of restoration plantings (Landis and others 2010).

This study provides some evidence that local adaptations may occur in populations of Wyoming big sagebrush and this needs to be considered in restoration planning. Further understanding subspecies level plasticity will help to determine which environmental factors drive population differentiation and will help to improve the success of restoration plantings in both the short term and the long term. In the short term, using locally adapted seed sources will help to increase establishment as seedlings will better be able to cope with environmental stresses. In the long term, this will ensure that genetic diversity is maintained and that local adaptations are not extirpated through heterosis or outbreeding depressions (Hufford and Mazer 2003; McKay and others 2005).

Literature Cited

Aghai MM. 2012. Container configuration influences western larch and big sagebrush seedling development [MSc Thesis]. Moscow (ID): University of Idaho. 68p.

Barker JR, McKell CM. 1986. Differences in big sagebrush (Artemisia tridentata) plant stature along soil-water gradients: genetic components. Journal of Range Management 39:147-151.

Booth GD, Welch BL, Jacobson TLC. 1990. Seedling growth rate of 3 subspecies of big sagebrush. Journal of Range Management 43:431-436.

Braun CE, Baker MF, Eng RL, Gashwiler JS, Schroeder MH. 1976. Conservation committee report on effects of alteration of sagebrush communities on the associated avifauna. Wilson Bulletin 88:165-171.

Burdett AN. 1979. A non-destructive method for measuring the volume of intact plants. Canadian Journal of Forest Research 9:120-122.

Davies KW, Boyd CS, Beck JL, Bates JD, Svejcar TJ, Gregg MA. 2011. Saving the sagebrush sea: an ecosystem conservation plan for big sagebrush communities. Biological Conservation 144: 2573-2584.

Ellison AM, Bank MS, Clinton BD, and 17 others. 2005. Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. Frontiers in Ecology and the Environment 3:479- 486.

Evans RD, Black RA. 1993. Growth, photosynthesis, and resource investment for vegetative and reproductive modules of *Artemisia tridentata*. Ecology 74:1516-1528.

Herriman KR. 2009. Wyoming big sagebrush: efforts towards development of target plants for restoration [MSc thesis]. Moscow (ID): University of Idaho. 70 p.

Hufford KM, Mazer SJ. 2003. Plant ecotypes: genetic differentiation in the age of ecological restoration. TRENDS in Ecology and Evolution 18:147-155.

[ITIS] Integrated Taxonomic Information System. 2011. URL: http://www.itis.gov (accessed 19 December 2011).

Kawecki TJ, Ebert D. 2004. Conceptual issues in local adaptation. Ecology Letters 7:1225-1241.

Kolb KJ, Sperry JS. 1999. Differences in drought adaptation between subspecies of Sagebrush (*Artemisia tridentata)*. Ecology 80:2373-2384.

Lambers H, Chapin S, Pons TL. 1998. Plant physiological ecology. New York (NY). Springer. 540 p.

Landis TD, Dumroese RK, Haase DL. 2010. Seedling processing, storage, and outplanting. Volume 7, The Container Tree Nursery Manual. . Washington (DC): USDA Forest Service. Agriculture Handbook 674. 200 p.

Lloret F, Casanovas C, Peñuelas J. 1999. Seedling survival of Mediterranean shrubland species in relation to root:shoot ratio, seed size., and water and nitrogen use. Functional Ecology 13:210-216.

Mahalovich MF, McArthur ED. 2004. Sagebrush (*Artemisia* spp.) Seed and Plant transfer Guidelines. Native Plants Journal 5: 141-147.

McArthur ED. 2000. Sagebrush systematics and distribution. In: Entwistle PG, DeBolt AM, Kaltenecker JH, Steenhof K, compliers. Proceedings: steppe ecosystems symposium; 2000. Boise, (ID): Bureau of Land Management. BLM/ID/PT-001001+1150. 6p.

McArthur ED, Pope CL, Freeman DC. 1981. Chromosomal studies of subgenus *Tridentatae* of *Artemisia*: evidence for autopolyploidy. American Journal of Botany 68:589-605.

McArthur ED, Sanderson SC. 1999. Cytogeography and chromosome evolution of subgenus *Tridentatae* of *Artemisia* (Asteraceae). American Journal of Botany 86: 1754-1775

McKay JK, Christian CE, Harrison S, Rice KJ. 2005. “How local is local?”- A review of practical and conceptual issues in the genetics of restoration. Restoration Ecology 13: 432-440.

Meyer SE, Monsen SB. 1992. Big sagebrush germination patterns: Subspecies and population differences. Journal of Range Management 45:87-93.

Noss RF, Peters RL. 1995. Endangered ecosystems: a status report on America’s vanishing habitat and wildlife. Washington (DC): Defenders of Wildlife. 132 p.

Poorter H, Niinemets U, Poorter L, Wright IJ, Villar R. 2009. Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. New Phytologist 182:565-588.

Prevéy JS, Germino MJ, Huntly NJ. 2010. Loss of foundation species increases population growth of exotic forbs in sagebrush steppe. Ecological Applications 20:1890-1892.

Scheepens JF, Frei ES, Stocklin J. 2010. Genotypic and environmental variation in specific leaf area in a widespread Alpine plant after transplantation to different altitudes. Oecologia 164:141-150.

Schenk HJ, Jackson RB. 2002. Rooting depths, lateral root spreads and below-ground/above-ground allometries of plants in water-limited ecosystems. Journal of Ecology 90:480-494.

Schneider CA, Rasband WS, Eliceiri KW. 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9: 671-675.

Schuman GE, Belden SE. 2002. Long-term survival of direct seeded Wyoming big sagebrush seedlings on a reclaimed mine site. Arid Land Research and Management 16:309-317.

Schwinning S, Ehleringer JR. 2001. Water use trade-offs and optimal adaptations to pulse-driven arid ecosystems. Journal of Ecology 89:464-480.

Stanton DJ, McArthur ED, Freeman DC, Goldenberg EM. 2002. No genetic substructuring in *Artemisia* subgenus *Tidentatae* despite strong ecotypic subspecies selection. Biochemical Systematics and Ecology 30: 579-593.

[USDA NRCS] USDA Natural Resources Conservation Service. 2012. The PLANTS database. URL: http://plants.usda.gov (accessed September 2012). Greensboro (NC): National Plant Data Team.

Welch BL. 2005. Big sagebrush: a sea fragmented into lakes, ponds, and puddles. Fort Collins (CO): USDA Forest Service, Rocky Mountain Research Station. General Technical Report RMRS-GTR-144. 210 p.

Welch BL, McArthur ED, Nelson DL, Pederson JC, Davis J N. 1986. 'Hobble Creek'—a superior selection of low-elevation mountain big sagebrush. Ogden (UT): USDA Forest Service, Intermountain Research Station. Research Paper INT-370. 10 p.

West NE. 1983. Great Basin-Colorado Plateau sagebrush semi-desert. Pages 331–349 *in* N. E. West, editor. Ecosystems of the world: temperate deserts and semi-deserts Volume 5. Elsevier, New York, New York, USA.

Table 2.1. Seed collection location and climate data for 5 populations of Wyoming big sagebrush. Total precipitation and temperature values are an average of 60 years of annual data[a]

Seed Source	Latitude	Longitude	Elevation (m)	Total Annual Precipitation[a] (mm)	Annual Temperature Max/Min[a] (°C)
Miles City, MT	46.3223	-105.8261	757	329.30	15.5/0.4
Oregon Trail, ID	43.0931	-115.6528	963	258.55	18.2/2.7
Penistaja, NM	35.9802	-107.226	2122	322.21	17.4/-0.6
Scooby, UT	41.8566	-113.0499	1438	242.41	15.8/-0.3
Saddle Mountain, WA	46.7672	-119.473	297	180.71	17.6/4.0

[a]PRISM Normals 1951-2011, Parameter-elevation Regressions on Independent Slopes Model, PRISM Climate Group, http://www.prismclimate.org [accessed 15 Feb 2012]

Table 2.2. One-way analysis of variance mean square values testing the effect of seed source on Wyoming big sagebrush seedling growth, biomass, and leaf characteristics (specific leaf area, SLA; leaf area ratio, LAR). Root-collar diameter (RCD) is the diameter of the main seedling stem approximately 1 cm above ground-line.

Source	df	Height Growth	RCD Growth	Leaf Dry Weight	Root Dry Weight	Whole Plant Dry Weight	SLA cm^2/ g	LAR cm^2/g
Seed Source	4	129.46*	4.20***	1.22**	25.13**	65.65**	53.21	53.15**
Error	20	36.17	0.41	0.13	5.81	8.01	90.41	12.01

Significance levels, *P ≤0.05, ** P ≤0.01, *** P ≤0.0001

Table 2.3. Approximate F statistics, determined by a Satterthwaite approximation, testing the effect of seed source on Wyoming big sagebrush seedling stem dry weight.

Variable	Numerator df	Denominator df	F statistic
Stem Dry Weight	4	9.17	58.33***

Significance levels, *P ≤0.05, ** P ≤0.01, *** P ≤0.0001

Table 2.4. Seed source effects on mean height and root-collar diameter (RCD) growth of transplanted Wyoming big sagebrush seedlings grown in greenhouse conditions for 21 wk.

Seed Source	Height Growth (cm)	RCD Growth (mm)
MT	8.9 b[a]	1.78 bc
ID	11.5 ab	1.49 c
NM	16.9 ab	2.83 ab
UT	17.3 ab	2.70 abc
WA	21.7 a	3.79 a

[a]Column means with the same letters are not significantly different ($P \leq 0.05$), by Tukey's HSD.

Table 2.5. Seed source effects on mean biomass and leaf characteristics (specific leaf area, SLA; leaf area ratio, LAR) of transplanted Wyoming big sagebrush seedlings grown in greenhouse conditions for 21 wk.

Seed Source	Stem Dry Weight (g)	Leaf Dry Weight (g)	Root Dry Weight (g)	Whole Plant Dry Weight (g)	SLA (cm^2/g)	LAR (cm^2/g)
MT	1.07 c[a]	0.93 b	2.66 b	4.67 c	76.98	15.96 ab
ID	1.37 bc	1.34 ab	5.56 ab	8.28 bc	78.53	13.96 ab
NM	2.84 b	1.99 a	3.20 b	8.02 bc	78.61	19.99 a
UT	2.11 b	2.00 a	6.67 ab	10.77 ab	71.39	11.34 ab
WA	4.50 a	2.01 a	7.92 a	14.42 a	79.48	13.49 b

[a]Column means with the same letters are not significantly different ($p \leq 0.05$), by Tukey's HSD.

Table 2.6. Wyoming big sagebrush seed source effects on mean whole-plant net photosynthesis (µmol CO_2 plant $^{-1}$ s^{-1}) at 7 and 11 wk after transplanting.

Seed Source	Measurement Period (Weeks After Transplanting)	
	7	11
MT	0.1735 b[a]	0.1625 b
ID	0.2748 ab	0.1430 b
NM	0.3684 a	0.2585 a
UT	0.3263 ab	0.2402 ab
WA	0.3243 ab	0.1806 ab

[a]Column means with the same letters are not significantly different ($p \leq 0.05$), by Tukey's HSD.

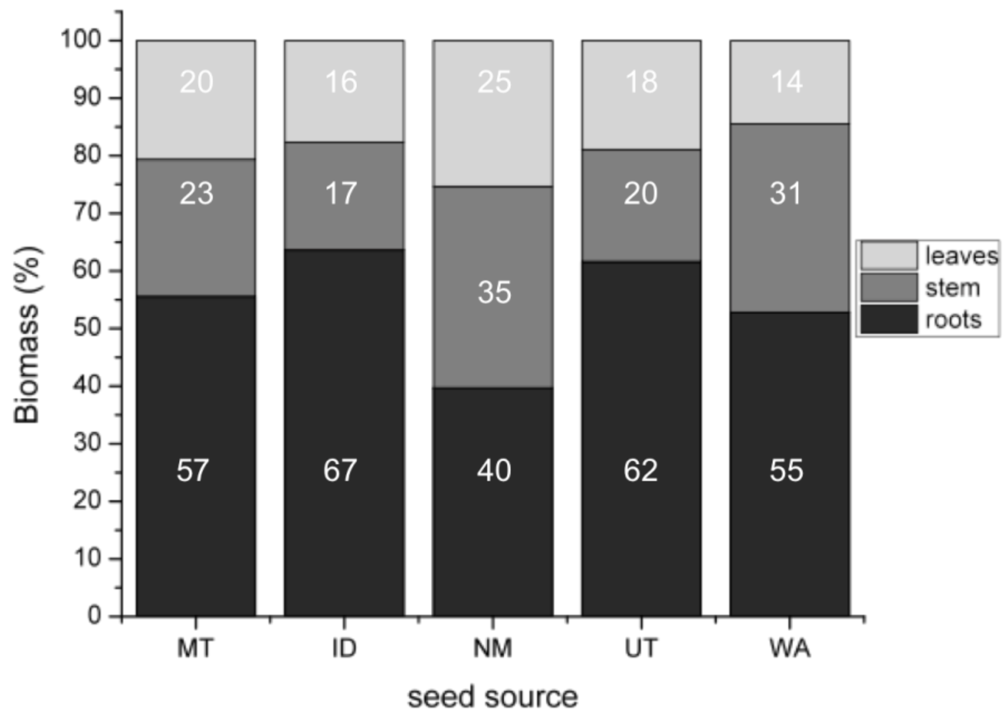

Figure 2.1. Biomass partitioning of Wyoming big sagebrush seedlings grown from different seed sources. Numbers represent the percent of total biomass allocated to each plant part.

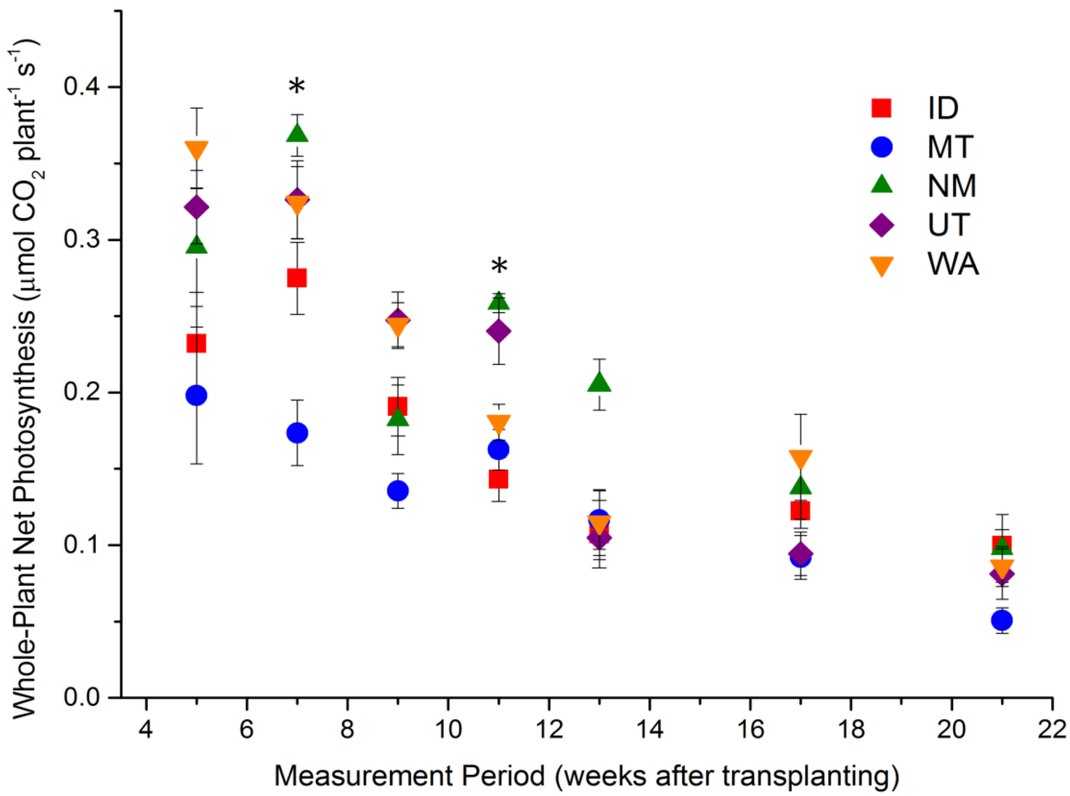

Figure 2.2. Seed source effects on mean whole-plant net photosynthesis of transplanted Wyoming big sagebrush seedlings grown in greenhouse conditions. An analysis of variance was performed on each measurement period separately. Significant differences were observed 7 and 11 weeks after transplanting (indicated by *).

Appendix

Nursery Telephone Questionnaire

Hello. My name is Emily Overton and I am a graduate student at the University of Idaho researching sagebrush. I'm calling regional nurseries to identify if they grow sagebrush and if so, to determine if and how the seedlings are cold-stored.

1. Do you grow sagebrush at this nursery? YES NO

If NO → STOP! Thank you for your time.

If YES → Next question

2. Do you grow it in containers? YES NO

If NO → STOP! Thank you for your time.

If YES → Next question

3. Do you cold store your sagebrush seedlings prior to shipping? YES NO

IF NO → STOP! Thank you for your time

If YES→ Next question

Are you able to answer questions about sagebrush seedling production and cold storage? (If NO → Can I speak with or make an appointment to speak with the employee knowledgeable about sagebrush seedling production and cold storage?)

Do you have time to participate in a five-minute survey? All of the following questions refer to sagebrush seedlings specifically. Before we begin I would like you to know that the results of this survey are anonymous and will be pooled for analysis and presentation.

4. During which months do you typically lift your sagebrush seedlings?

____

5. Do you store your sagebrush seedlings in a cooler or a freezer?

Cooler → At what temperature and for how long?

TEMP_________________DURATION_________________

Freezer → At what temperature and for how long?

TEMP_________________DURATION_________________

6. Do you remove dead foliage before packing? YES NO

7. Are the seedlings kept in complete darkness during storage? YES NO

8. Have you encountered mold problems during storage? YES NO

If NO → STOP! Skip to question 9.

If YES → Next question

9. Have you taken measures to manage mold problems? YES NO

If NO → STOP! Skip to the end of the survey.

If YES → Next question

10. Have you ever applied fungicide to the sagebrush seedlings? YES NO

If NO → STOP! Skip to question 12.

11. If YES → Have you applied fungicide:

a. Before packing for storage? YES NO

b. While seedlings were in storage? YES NO

c. After storage prior to shipping? YES NO

12. **Have you taken measures to improve airflow during storage? YES NO**

If NO → STOP! Skip to the end of the survey.

13. If YES →

a. Do you use un-waxed boxes? YES NO

b. Do you store seedlings upright? YES NO

c. Do you fold bags down to allow more air to flow? YES NO

d. Do you use a fan? YES NO

e. Do you do anything else I haven't mentioned to improve airflow? YES NO

____

Thank you for your time. I am planning to compile this information for my thesis but would also like to share it through presentations and other publications. Again, any information I report will be a combination of responses and will not be traceable to any particular nursery. Do I have your consent to release this information?