WYOMING BIG SAGEBRUSH: EFFORTS TOWARDS DEVELOPMENT OF TARGET PLANTS FOR RESTORATION

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AUTHORIZATION TO SUBMIT THESIS

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Abstract

Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis*) is a dominant shrub throughout much of the interior western United States. It is a key component of sagebrush steppe ecosystems, which have been degraded due to European settlement, improper land use, and changing fire regimes resulting from the invasion of exotic annual grasses. Re-establishment of this shrub has relied largely on direct seeding, but success has been erratic; outplanting nursery grown seedlings may be a more effective method of establishment. This study was initiated to identify the effect of three container types on Wyoming big sagebrush seedling morphology and physiological status in the nursery, and to link those characteristics to performance following outplanting. A second objective was to determine the influence of field fertilization on outplanted Wyoming big sagebrush seedling growth and survival. One-year-old seedlings were grown in three types of Styroblock™ (Beaver Plastics, Acheson, Alberta, Canada) containers: 45/340; 60/250; and 112/105 (the first number indicates the number of cavities in a block and the second indicates the volume of each cavity), and then outplanted on three southern Idaho sites in March 2008 and their morphology and physiological status were monitored until October 2008. Cold hardiness was measured at the end of the nursery growing season and immediately prior to outplanting; seedlings were most cold hardy at the end of the growing season (November and December) and still cold hardy, but to a lesser degree, prior to outplanting in March. Initially larger seedlings, grown in larger volume containers, maintained significantly greater height (105: 10.4 ± 0.11 cm, 250: 15.9 ± 0.18 cm, 340: 18.7 ± 0.21 cm) and root-collar diameter (105: 2.04 ± 0.02 mm, 250: 2.68 ± 0.02 mm , 340: 3.05 ± 0.03 mm) and tended to show superior growth over seedlings grown in smaller containers. Only at the driest site did container size have an influence on seedling survival, with 250 (44% ± 0.04) and 340 (43% ± 0.04) seedlings showing higher survivorship than 105 seedlings (28% ± 0.04). Fertilization negatively impacted seedling survival, potentially due to high soil salt concentration caused by the fertilizer; however where seedlings survived, there tended to be a benefit in above-ground growth. These results indicate that container type should be used as a management tool in conjunction with anticipated outplanting conditions, and that field fertilization may not provide a benefit to outplanted seedlings.

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CHAPTER 1

Influence of container volume on Wyoming big sagebrush seedling morphology and cold hardiness

Abstract

Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis*) is a key component of sagebrush steppe ecosystems and is a dominant shrub throughout much of the interior western United States. These ecosystems have been degraded by fire, invasive species, and destructive land use. Attempts to direct seed Wyoming big sagebrush have failed, are costly, and could be resolved by planting seedlings. Frost sensitivity of seedlings is also a concern as these ecosystems are susceptible to periodic freezing in the spring and summer and it is important to managers that seedlings withstand such conditions. Developing a target plant for restoration of such degraded sites may aid in cost effective restoration. This study was conducted to 1) identify the effect of container volume on Wyoming big sagebrush seedling morphology, and 2) determine the level of cold hardiness attained during Wyoming big sagebrush seedling production. Seedlings were grown for one year (March 2007 to March 2008) in each of three different container volumes (cavity volume of 105, 250, and 340 ml) to investigate seedling quality with regard to cold hardiness, and the morphological characteristics of height, root-collar diameter, dry mass, root volume, shoot volume, and root:shoot. Cold hardiness was unaffected by container volume. Plant height, root-collar diameter, shoot volume, and dry mass increased with container volume. Seedling root volume in the two largest container volumes (340 and 250) was greater than that of seedlings grown in 105 containers. The results indicate the strong effect that container volume has on plant morphology. This information provides us with a greater ability to develop target plants for use in restoring degraded or harsh sites.

Introduction

Throughout much of the interior western United States, Wyoming big sagebrush (*Artemisia tridentata* ssp. *wyomingensis*) is a keystone species, serving an important ecological role in sagebrush steppe and Great Basin sagebrush vegetation types (Barbour and Billings 2000; Lambrecht et al. 2007; Meyer and Monson 1992). These two vegetation types occupy more than sixty-two million ha in the United States, with the sagebrush steppe occupying more than two thirds of that area (Barbour and Billings 2000). Sagebrush provides critical habitat for wildlife including sage-grouse and pronghorn (Kindschy et al. 1982; Rosentreter 2005). Sagebrush also contributes to ecosystem structure and function by modification of local environmental conditions for seed germination and seedling survival, soil nitrogen retention, environmental facilitation of microorganisms, and an increase of organic matter (Lysne 2005; Schlesinger and Pilmanis 1998; West 2000). These ecosystems have been degraded by fire, invasive species, and destructive land use including grazing pressure livestock inflicted in the past century (McIver and Starr 2001). Because of overgrazing and the low resilience of these ecosystems, invasive species, such as cheatgrass (*Bromus tectorum*), are able to establish, increasing wildfire size and frequency and promoting an unnatural fire cycle that prevents re-establishment of native vegetation (Mack and Thompson 1982; McIver and Starr 2001; Young et al. 1987).

Restoration of sagebrush ecosystems has only recently been emphasized due to loss of increasingly valued native communities, but plant establishment has focused predominately on direct seeding (Chambers 2000; Hou and Romo 1998b; Pierson et al. 2007). Seedling establishment is vital to restoration success; once established, Wyoming big sagebrush has shown relatively high rates of survival (Lysne 2005). Schuman and Belden (2002) found that after eight years, 59% of established seedlings survived. Kiger et al. (1987) found survival rates of 33% after 11 years. Success from direct seeding has been noted in seed-increase gardens (Welch 1997), however, there have been numerous failures from direct seeding (Lysne 2005; Monsen 2000). Field germination and establishment can be low due to a combination of factors including seed quality, animal foraging, water stress, and inadequate light or temperature conditions (Chambers 2000; Lysne 2005). Planting nursery-grown sagebrush seedlings could provide a more effective method of restoring sagebrush ecosystems, especially when cost and availability of locally adapted seeds are considered (Beyers 2004; Lysne 2005). The initial cost of planting with seedlings is higher than that of direct seeding, largely due to costs associated with nursery production and transportation of seedlings to the planting site (Stevens 1981). Container seedlings may, however, have greater establishment success in cold, arid site conditions and over time their use may result in less cost per established plant than direct seeding, as germination on direct-seeded field sites can be unpredictable and uneven (Romo and Young 2002; Young and Evans 1989).

Diurnal and seasonal temperature fluctuation in these ecosystems can be extreme (Lambrecht et al. 2007) and low temperatures may reduce establishment (Hou and Romo 1998a). Seedling physiological tolerance to such stresses may also influence survival. Length of exposure, often referred to as chilling hours, to colder conditions can contribute to dormancy induction (Naor et al. 2003). Cold hardiness refers to the capacity of a plant tissue to survive exposure to freezing temperatures (Burr 1990), and can be induced by photoperiod reduction or colder temperatures (Kozlowski and Pallardy 2002). Freezing temperatures are influential in determining big sagebrush distribution (Lambrecht et al. 2007) and young seedlings can be damaged or killed by or when exposed to such temperatures (Hou and Romo 1998a). Planting seedlings that are sufficiently cold hardy may aid in Wyoming big sagebrush establishment.

Field survival is a key component of planting success, but it is often difficult to predict. Typically, morphological attributes are used as a means of predicting planting survival with tree species. Nursery container dimensions and seedling density influence plant morphology during propagation (Endean and Carlson 1975; Simpson 1991; Timmis and Tanaka 1976) and are important when determining target seedling criteria for a site (Rose et al. 1990). Although larger containers yield larger plants (Endean and Carlson 1975; Kinghorn 1974; Paterson 1996), understanding the influence of container volume on seedling quality or survival (Grossnickle and Folk 1994) and has yet to be thoroughly studied for Wyoming big sagebrush. For other species, however, a larger seedling may be desired to overcome competing vegetation and/or to meet cost effectiveness in regards to establishment and growth (Grossnickle and Folk 1994; Jacobs et al. 2005; South and Mitchell 1999).

The initial cost of growing, handling, and planting container seedlings may, in the long term, yield higher establishment (in terms of numbers and distribution of plants per hectare) and/or improved seedling growth, and be more cost effective over time than direct seeding (Clements and Young 2000). Planted sagebrush seedlings produce seeds within three to five years (Lysne 2005; Meyer and Monsen 1992), indicating that once seedlings have successfully established, future regeneration may be achieved through natural establishment of this seed source. Therefore, study objectives were to 1) identify the effect of container volume on Wyoming big sagebrush seedling morphology, and 2) determine the level of cold hardiness attained in seedling production as a measure of seedling quality, to develop target plants for restoration of degraded sites.

Materials and Methods

Plant Materials

Seedlings were grown in Moscow, ID at the USDA Forest Service Rocky Mountain Research Station (46° 43.905' N, 117° 59.831' W). Seeds (Humboldt and Elko counties, NV sources) were sown May 17, 2007 into three types of Styroblock™ (Beaver Plastics, Acheson, Alberta, Canada) containers with uniform dimensions: 45/340; 60/250; and 112/105 (the first number indicates the number of cavities in a block and the second indicates the volume, in ml, of each cavity) (Table 1.1). Thinning and transplanting were conducted on June 6, 2007 to ensure that all cells were filled with a single germinant. Fertilizer was applied at initial seeding with irrigation at 100ppm N and switched to 25ppm N on June 4, 2007 prior to thinning and transplanting, for the rest of the growing season (Table 1.2). Seedlings received irrigation when the container medium dried to 65% of saturated block weight. Seedlings were moved to the University of Idaho Center for Forest Nursery and Seedling Research, in Moscow, ID (46° 43.388' N, 117° 57.431' W) on October 26, 2007 for hardening and overwintering in an open-wall greenhouse. Seedlings were extracted from the containers on March 11-12, 2008 to measure seedling morphology. This study was established as a completely randomized design with each individual seedling as the measurement unit. Seedling containers were periodically rearranged to ensure no undue influence of micro-environmental growing conditions occurred.

Plant morphology assessment

Height and root-collar diameter were measured on 480 randomly selected seedlings of each container volume. Concurrently, 40 randomly selected seedlings of each container volume were root-washed and root and shoot volume was measured using water displacement (Burdett 1979). Another random sample of ten seedlings from each container volume was destructively harvested to determine seedling dry mass following oven drying at 70°C for >72h (Grieve Industrial Oven NB-350, The Grieve Corporation, Round Lake, IL).

Cold hardiness assessment

Chilling hours were recorded to assess length of time exposed to critical temperatures for dormancy induction beginning September 1, 2007 using iButton Thermachron® temperature sensors (Maxim/Dallas SemiConductors, Dallas, TX) and ending March 19, 2007. Seedling cold hardiness was determined on four dates in 2007 (5 November, 19 November, 5 December, and 20 December) and one date in 2008 (19 March) using freeze-induced electrolyte leakage (FIEL; Flint et al. 1967). Leaf tissue samples, from the top third of the plant, were taken from five randomly selected seedlings per container volume at each test date. Tissue was cut into 1-cm lengths and one segment of tissue was placed into a vial containing 2.5-mL of deionized water and a grain of sand to help promote nucleation and decrease surface tension. At each test date, five test temperatures (2 [control], -10, -20, -30, and -40°C) were employed using a ScienTemp Lo-Cold Freezer (Scientemp Corp., Adran, MI). Replication was accomplished by testing each plant at each of the five temperatures. Starting at 2°C, the temperature was then decreased at -5°C each hour. Each successive test temperature was held for 30 minutes before removing the appropriate vials and then decreasing to the next test temperature. Electrolyte leakage (EL) was measured after thawing using a SevenEasy conductivity meter (Mettler Toledo, Columbus, OH) to quantify electrolyte leakage caused by freezing. Vials were then autoclaved 20 minutes at 121°C (Market Forge Sterilmatic, Vernon Hills, IL) to attain total cell death and total EL was measured. A lethal temperature value is often used to describe the level of cold hardiness, such as the LT_{50}, representing the minimum temperature at which 50 % of a specified tissue is killed (Burr

1990). To express cold hardiness in a manageable form, an LT_{50} was calculated by interpolating the temperature at which 50% of cell electrolyte leakage occurs (Burr 1990). Each LT_{50} was calculated by fitting simple quadratic regressions based on electrolyte leakage values at each of the five test temperatures.

Data Analysis

Analysis of variance (ANOVA; SAS Institute Inc., version 9.1, Cary, NC) for a completely randomized design was used to identify differences among plants grown in the different container volumes. Means were separated using Tukey's honest significant difference (HSD) test ($\alpha = 0.05$). Quadratic regressions were prepared using SigmaPlot (version 11, Systat Software Inc., San Jose, CA) and Microsoft Excel (version 12 Microsoft Corporation, Redmond, WA).

Results

Plant morphology

Container volume significantly affected increased height, root-collar diameter, shoot volume, shoot dry mass, and root dry mass (340> 250>105; each $P<0.0001$; Figures 1.1 through 1.4). The two largest containers (340 and 250) yielded plants with root volumes significantly greater ($P<0.0001$) than those in 105 containers (Figure 1.4). Root:shoot based on biomass was unaffected by container volume, but root:shoot based on plant biomass volume revealed a significant difference ($P<0.0054$) between 340 and 105 containers (Figure 1.5).

Cold hardiness

At the first sample date (November 5, 2007), 65 chilling hours had accumulated at 5°C and 237 hours at 10°C. When seedlings were lifted (March 11-12, 2007), chilling hours at 5°C and 10°C had accumulated to 677 and to 1,217, respectively. Container volume had no effect on cold hardiness measured by the FIEL method when measured quantitatively. Despite the relatively low number of chilling hours at the first sample date, all three container volumes had initial LT_{50} values below -30°C (Table 1.3); this level of cold hardiness remained through the winter. During lifting in March, LT_{50} values indicated that seedling cold hardiness was decreasing (Table 1.3).

Discussion

As expected seedling height and root-collar diameter were significantly influenced by container volume because of differences in growing space and resource allocation (Endean and Carlson 1975; Pinto 2005; Timmis and Tanaka 1976). Larger seedlings grown in larger containers may be better able to survive stresses associated with planting and to overcome competition (Grossnickle and Folk 1994; Jacobs et al. 2005). Larger heights and root-collar diameters have been correlated with larger root volume in angiosperm seedlings (Jacobs et al. 2005). Seedlings with greater root volume could result in greater tolerance to drought (Blake and Sutton 1987; Haase and Rose 1993), a major contributor to planting stress, particularly in semiarid areas where the soil dries quickly, such as unvegetated sites and south-facing slopes (Bochet et al. 2007; García-Fayos et al. 2000). Competition can also inhibit seedling establishment (Schuman and Belden 2002). Thus taller seedlings, such as those grown in larger containers, may be able to outperform smaller seedlings because those with deeper roots and taller shoots can compete better with annual grasses (Meyer 2003). Larger root-collar diameter has also been correlated with higher field survival in slash pine (*Pinus elliottii*), loblolly pine (*Pinus taeda*), and Douglas-fir (*Pseudotsuga menziesii*) seedlings (Schneider et al. 1998; South and Mexal 1984; South and Mitchell 1999). Larger seedlings may not, however, always be best suited for the planting site. Stewart and Bernier (1995) found that larger seedlings, exposed to a warmer, drier environment, had a greater reduction in photosynthetic rate, and less ability to maintain it, than smaller seedlings. Therefore a seedling, regardless of container volume, better suited to withstand stresses associated with planting, such as water stress, may be best. As supported by the target seedling concept and other studies (Haase and Rose 1993; Jacobs et al. 2005; Rose et al. 1990), assessing a combination of seedling attributes could help to produce a more desirable seedling for a specified planting site.

The conventional forestry paradigm is that shoot:root imbalance is a main cause of transplanting shock and evaluation of root:shoot could estimate drought avoidance potential of container stock (Bernier et al. 1995). Seedling balance is a concern because seedlings must have an adequate root system for water uptake to meet transpirational demands of the shoot (Bernier et al. 1995). Seedling survival has been positively correlated to higher root:shoot when planted in dry soil conditions (Boyer and South 1987; Hasse and Rose 1993; Larsen et al. 1986). Seedlings grown in 340 containers could be more likely to suffer from water stress after planting due to the lower root:shoot (Bernier et al. 1995; Burdett 1990). Root volume was not significantly different among the two largest containers; perhaps, under this growing regime Wyoming big sagebrush cannot adequately fill the 340 cavity in one growing season, even though it had significantly larger shoot volume than the 250 cavity. A longer growing season or more fertilizer may remedy this (Landis et al. 1990; Timmer and Armstrong 1987). Lower proportions of roots can fail to meet transpirational demands of the shoot (Kriedemann et al. 1983), even though plants can regulate transpiration by means of high stomatal resistance under water stress (Meisner 1991). Root:shoot could be more critical at sites with high evaporative demand or those under drought conditions (Bernier et al. 1995). Although root:shoot has been considered more critical for bareroot stock than container stock (Mattsson 1996), it still may be a useful morphological trait because it portrays plant proportions.

Lambrecht et al. (2007) found that a single simulated episodic freezing treatment, two varieties of *A. tridentata,* damaged seedling photosynthetic tissues and effectively stopped growth. Therefore, cold hardiness is a critical factor in assessing seedling quality, particularly because seedlings may experience colder temperatures and greater diurnal fluctuations, especially in open areas that might be transplanted, than the mature plants around them (Boorse et al. 1998; Pratt et al. 2005; Sakai and Larcher 1987). It has been commonly noted that natural and seeded sagebrush seedlings are very susceptible to late frosts (Lambrecht et al. 2007). Because these seedlings were very cold hardy with very few chilling hours, Wyoming big sagebrush cold hardiness acquisition is likely driven through other environmental cues, such as photoperiod and moisture availability (Burr 1990). As expected, based on annual variation in plant hardening and dehardening (Fuchigami and Nee 1987; Kobayashi and Fuchigami 1983; Ritchie and Tanaka 1990), cold hardiness was lower when measured in spring at the time of lifting, likely due to the influence of rising temperatures and longer day lengths (Grossnickle et al. 1994; Kozlowski and Pallardy 2002). This agrees with observations of silver sagebrush (Hou and Romo 1998a). Using the degree growth stage model (Fuchigami and Nee 1987), which has been applied to tree species, could be a useful tool for producers and users of sagebrush seedlings to help them understand the relationship between seedling stress resistance and times of stress, such as handling and planting. Further examination of the cold hardiness cycle of sagebrush will provide insight to growers attempting to maximize storage and coordinate planting with times of higher stress resistance as indicated by cold hardiness (Burr 1990).

For coal mine restoration, the limited availability and increasing cost of native plant seeds has raised the question as to whether planting seedlings is a more feasible alternative to direct seeding for meeting desired shrub densities (Schuman et al. 1998; Schuman et al. 2005). This same question could be asked for sites impacted by other factors, such as fire (Lysne 2005), particularly because the demand for native shrub seeds over the past decade in the western United States has been high due to the millions of hectares of native rangelands requiring rehabilitation after wildfire (Schuman et al. 2005). Direct seeding has been a common practice in the past; however, more thorough, long-term studies to compare the costs and benefits of direct seeding versus planting have not yet been completed (Kleinman and Richmond 2000; Schuman et al. 2005). Thus, future studies should more closely compare the costs and success of direct seeding and planting of container seedlings. Research focusing on cost comparisons per established plant and success in meeting planting objectives including distribution of established seedlings for individual sites should be a topic for future research.

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Figure 1.1. Wyoming big sagebrush height (cm; + SE) as influenced by container volume (Table 1.1) after one growing season. Different letters indicate significance differences (α = 0.05); n=480 for each container volume.

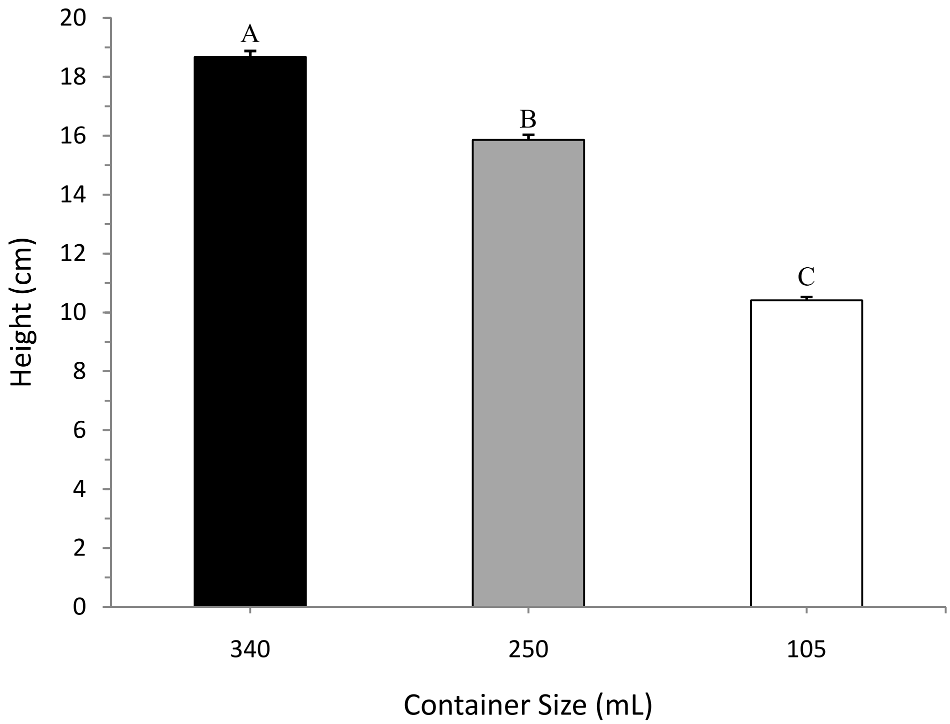

Figure 1.2. Wyoming big sagebrush root-collar diameter (mm; + SE) as influenced by container volume (Table 1.1) after one growing season. Different letters indicate significance differences (α = 0.05); n=480 for each container volume.

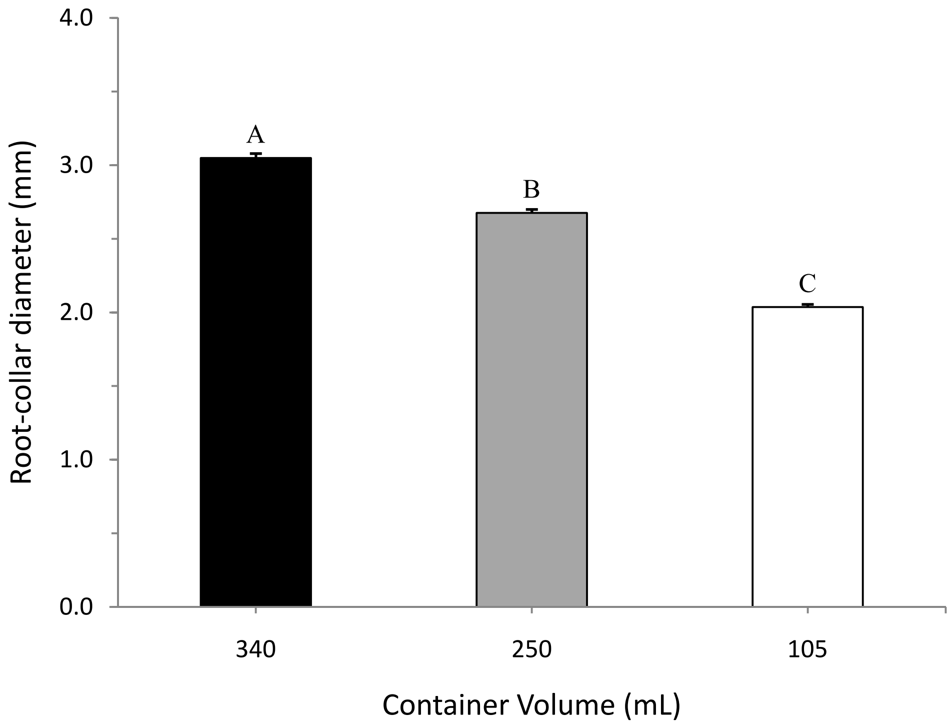

Figure 1.3. Wyoming big sagebrush dry mass (g; + SE) as influenced by container volume (Table 1.1) after one growing season. Different letters of the same case indicate significance differences ($\alpha = 0.05$); n=10 for each plant part and container volume.

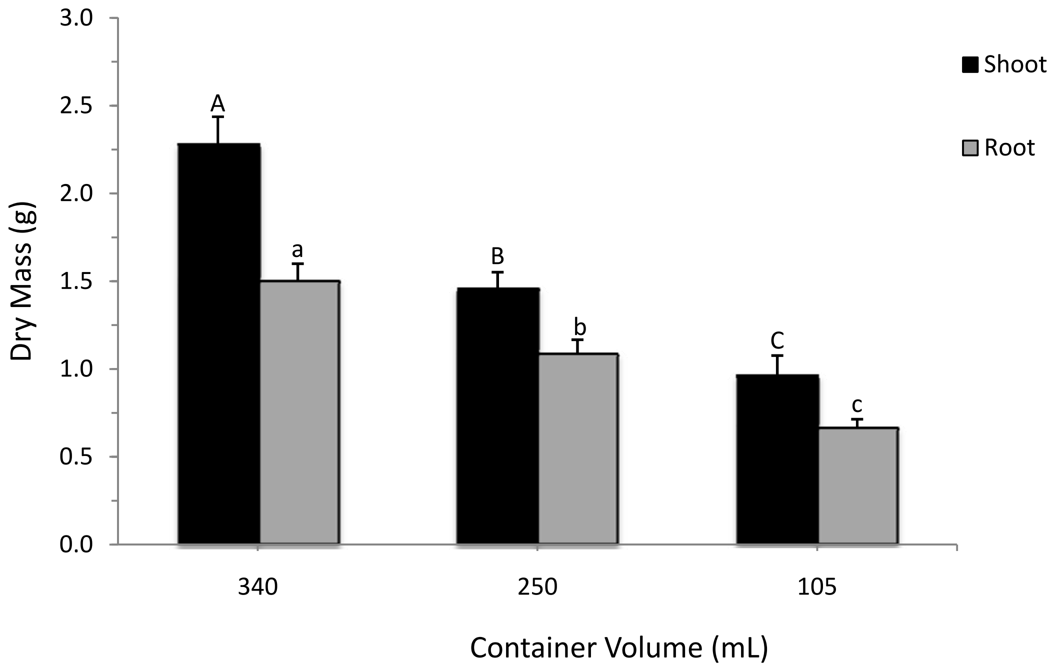

Figure 1.4. Wyoming big sagebrush volume (cm$^{3;}$ + SE) as influenced by container volume (Table 1.1) after one growing season. Different letters of the same case indicate significance differences (α = 0.05); n=40 for each plant part and container volume.

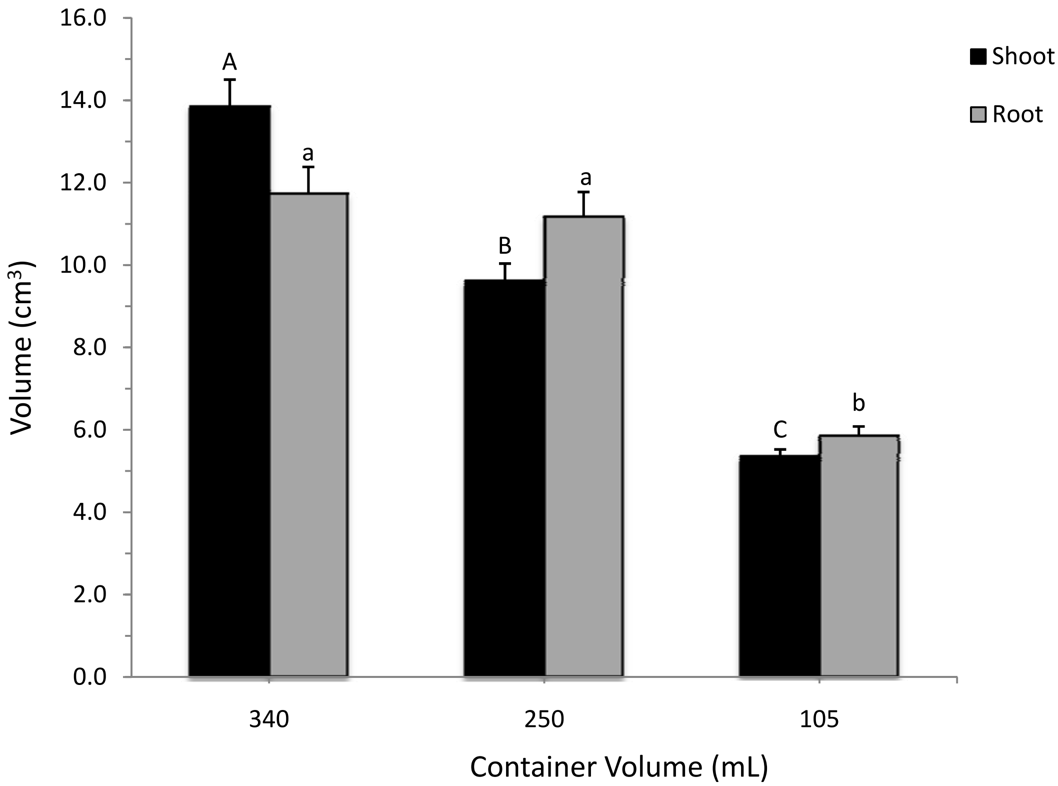

Figure 1.5. Wyoming big sagebrush root-to-shoot ratio (+ SE) as influenced by container volume (Table 1.1) after one growing season. Different letters indicate significance differences ($\alpha = 0.05$); Dry mass: n=10, volume: n=40 for each plant part and container volume.

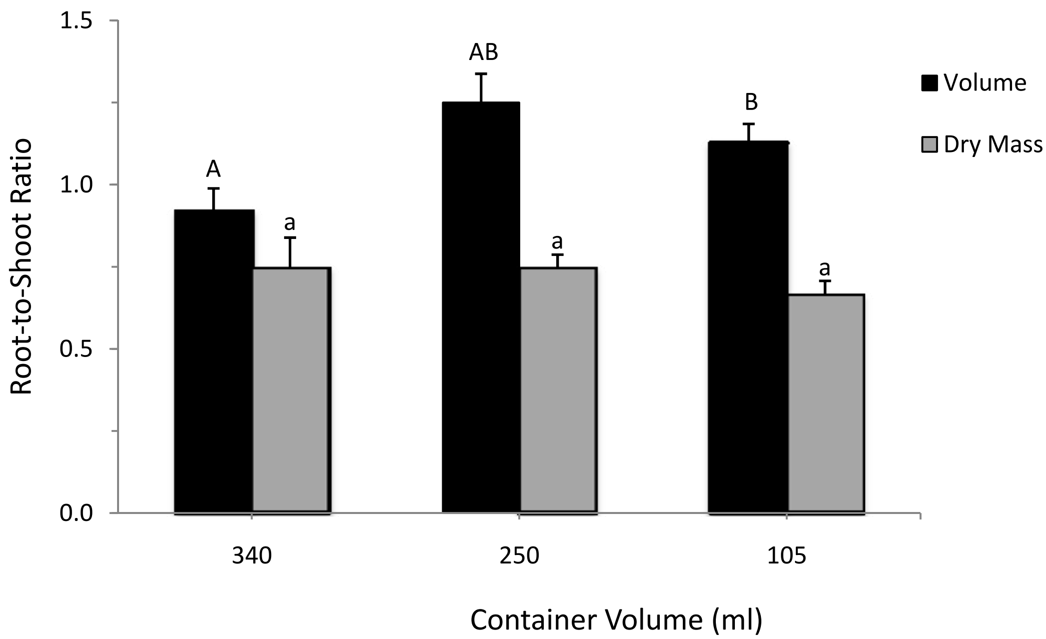

Table 1.1. Specifications for Beaver Plastics (Beaver Plastics, Acheson, Alberta, Canada) Styroblock™ containers used for Wyoming big sagebrush seedling production in the greenhouse

Container type		Top diameter		Depth		Volume		Cavity density	
		(mm)	(in)	(mm)	(in)	(cm^3)	(in^3)	(m^2)	(ft^2)
112/105	415B	36	1.4	148	5.8	105	6.6	530	49
60/250	515A	51	2.0	151	6.0	250	15.3	284	27
45/340	615A	59	2.3	151	6.0	340	10.5	213	20

Table 1.2. Specifications for fertilizer used during nursery production of Wyoming big sagebrush seedlings in the greenhouse.

Name	Elemental Chemistry	Manufacturer	Application at 25ppm Nitrogen	Application at 100ppm Nitrogen
Potassium nitrate	KNO_3	Champion®, Scotts-Sierra Horticultural Products Company, Marysville, OH	0.12 g	0.15 g
Monopotassium phosphate	KH_2PO_4	MoraLeaf®, Wilbur Ellis, Fresno, CA	0.18 g	0.23 g
Magnesium sulfate	$MgSO_4$	EpsoGrow®, Potash Import & Chemical Corporation, New York, NY	0.20 g	0.20 g
Ammonium sulfate	$(NH_4)_2SO_4$	American Plant Food Corp., Galena Park, TX	0.0 g	0.14 g
S.T.E.M.: Soluble Trace Element Mix	S, B, Cu, Fe, Mn, Mo, Zn	Peters Professional, Scotts-Sierra Horticultural Products Company, Marysville, OH	0.02 g	0.02 g
Sprint 330	Fe	Becker Underwood Incorporated, Ames, IA	0.02 g	0.02 g
Calcium nitrate	$Ca(NO_3)_2$	Viking Ship®, Hydro Agri North America, Tampa, FL	0.10 g	0.33 g
Phosphoric acid	H_3PO_4	Univar USA, Inc., Redmond, WA	0.16-mL	0.16-mL

Table 1.3. Cold hardiness (LT_{50}) according to container volume (Table 1.1) across five measurement dates. <-40°C indicates that LT_{50} was below -40°C and beyond the scope of measurement. LT_{50} represents the minimum temperature at which 50% of the plant tissue is killed.

Container type		LT_{50} (°C) by Measurement date				
		5-Nov	19-Nov	5-Dec	20-Dec	19-Mar
112/105		-35	<-40	<-40	<-40	-16
60/250		<-40	<-40	-40	<-40	-13
45/340		-37	<-40	<-40	<-40	-11

CHAPTER 2

Outplanting performance of Wyoming big sagebrush seedlings as affected by container volume and field fertilization

Abstract

Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis*) is a dominant shrub throughout much of the interior the western United States and a key component of sagebrush steppe ecosystems. Due to changing and improper land use and altered fire regimes resulting from the invasion of exotic annual grasses (e.g. cheatgrass [*Bromus tectorum* L.]), Wyoming big sagebrush has been extirpated from more than 50% of the area it once occupied. Re-establishment of this shrub on wildland sites has focused on direct seeding, but establishment is often erratic. Outplanting nursery grown seedlings could provide a more effective method of plant establishment. This study was initiated to identify the effect of three container volumes on Wyoming big sagebrush seedling performance following outplanting. As outplanting allows one to know the exact location of plants in the field, additional practices such as fertilization are possible. Thus a second objective was to determine the influence of field fertilization on outplanted Wyoming big sagebrush seedlings. One-year-old seedlings were outplanted on two southern Idaho sites in March 2008 and their morphology and physiological status were monitored until October 2008. At the Mountain Home site there were two site conditions including untilled and tilled, which was induced by a mechanical seeder and simulates the site conditions following conventional seedling. Initially larger seedlings, grown in larger containers, maintained significantly greater height and root-collar diameter throughout the growing season at both Mountain Home sites. As container volume increased from 105 to 250 to 340, survival of seedlings increased from 28% ± 0.04 to 44% ± 0.04 to 43% ± 0.04 under tilled site conditions and there was no influence for untilled conditions. Fertilization negatively impacted seedling survival on a tilled site (Fertilized: 33% ± 0.03, Unfertilized: 44% ± 0.03) and a untilled site (Fertilized: 55% ± 0.03, Unfertilized: 64% ± 0.03), potentially due to high soil salt concentrations. Fertilizer increased root-collar diameter growth at the moist site (Fertilized: 4.28 ± 0.10, Unfertilized: 3.70 ± 0.09), but

had no major impact on nutrient status at Mountain Home. Container volume and field fertilization had no affect on gas exchange at any site; however seedlings that had media washed from root plugs prior to outplanting had lower transpiration (0.12 ± 0.10 mol H_2O $m^{-2}s^{-1}$) and stomatal conductance (0.01 ± 0.01 mmol H_2O $m^{-2}s^{-1}$) than those that were not root-washed (1.5 ± 0.27 mol H_2O $m^{-2}s^{-1}$and 0.08 ± 0.02 mmol H_2O $m^{-2}s^{-1}$ respectively). These results indicate that container type should be used as a management tool in conjunction with anticipated outplanting conditions, and that field fertilization may not provide a benefit to outplanted seedlings.

Introduction

Woody sagebrushes of subgenus Tridentatae of the *Artemisia* L. are landscape dominating species of the sagebrush steppe and Great Basin sagebrush vegetation types (Barbour and Billings 2000). In the western United States, big sagebrush (*A. tridentata* Nutt.) is the most common species of this subfamily (McArthur et al. 1979). Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis*) is the most xeric subspecies of big sagebrush (Lysne 2005) and is a candidate for restoration projects in southern Idaho. In recent decades, restoration of native communities has increased due to degradation of such ecosystems by noxious weeds, land use patterns, fire, and public concern for conserving native vegetation. Wyoming big sagebrush is particularly vulnerable as it does not resprout following fire and its seeds are short-lived (Cione et al. 2002; McIver and Starr 2001; Monsen and McArthur 1985). Sagebrush provides critical habitat and forage for many wildlife species (Rosentreter 2005; Welch 2005; West 2000). Sagebrush also contributes to ecosystem structure and function including soil nitrogen retention, environmental facilitation of microorganisms, and an increase of organic matter (Lysne 2005; Schlesinger and Pilmanis 1998; West 2000).

Wyoming big sagebrush is commonly re-established by aerial or drill seeding (Hou and Romo 1998; Lambrecht et al. 2007; Lysne 2005). Direct seeding can be unsuccessful because seed germination is often unpredictable and uneven due to seed morphological and physiological attributes, soil surface characteristics, and the fluctuating semiarid environmental conditions that determine the ability of seeds to emergence and establish (Chambers 2000; Young and Evans 1989). Use of seeding equipment such as drills, cultipackers, and imprinters (Haferkamp et al. 1987; Monsen and Meyer 1990; Monsen 1988), often referred to as mechanical seeding, has shown greater establishment success than aerial or broadcast seeding (Monsen 1988), with success depending on the seeding device used (Monsen and Meyer 1990). Given this uncertainty, plant establishment could be enhanced by outplanting nursery-grown seedlings (Lysne 2005; Welch et al. 1992), because it overcomes the combination of conditions critical to seed germination. Multiple processes, including production of viable seeds and dispersal through time and space, are involved in successful establishment. Site characteristics are also important: aspect, presence of wildlife, soil type, seedbed conditions, litter and other plants present, as well as presence of fungi or bacteria (Grubb 1977). The regeneration niche (Grubb 1977) where a new generation of plant is successfully able to fill space previously occupied by the species if all conditions are favorable supports the idea that many factors contribute to seedling establishment, therefore seedlings may be advantageous in such situations. Outplanted Wyoming big sagebrush seedlings have shown first year survival rates of at least 80 % (Welch et al. 1992). Container production of sagebrush seedlings has not been extensively practiced, but may become more accepted if higher and more consistent rates of plant establishment can be expected (Beyers 2004; Welch et al. 1992).

Field survival of planted seedlings can be difficult to predict, because it can be influenced by seedling size (Everett 1980; Johnson and Okula 2006; South and Mitchell 1999) and field conditions, including soil moisture and competition (Jobidon et al. 1998; South and Mitchell 1999). Larger seedlings may cope better with competing vegetation as well as stresses associated with outplanting, such as rough handling and limited soil moisture (Gilbert et al. 2001; Jacobs et al. 2005b; South and Mitchell 1999). Smaller seedlings, however, may be better able to mitigate the effects of water stress (Jobidon et al. 1998) due to decreased transpirational demand. A larger seedling may perform well if the root and shoot are effectively balanced (Bernier 1995).

Fertilization at time of outplanting is difficult to accomplish with direct seeding given the lack of specific knowledge of plant location as well as encompassing the large area in which seed is broadcast. Field fertilization may increase growth rates, a beneficial situation when rapid establishment is needed, and may also influence survival. If fertilization increases the growth rate of the target vegetation, the competitive ability of the plant may be enhanced. Because fertilization increases the salt concentration in the rhizosphere (Jacobs and Timmer 2005), there is the potential for negative effects on seedling development as woody plants are often sensitive to salt injury (Landis et al. 1989). Conversely, root proliferation has been noted in areas of high nutrient supply (Friend et al. 1990; Granato and Raper 1989).

Controlled-release fertilizer (CRF) is an attractive field amendment as it supplies a time-released source of nutrients in one application (Jacobs et al. 2005a). For this reason, CRF has been used experimentally, and in some cases operationally, in reforestation practices and may be beneficial to restoration projects involving other woody plants. CRF application increased seedling root growth in western hemlock (*Tsuga heterophyla* Raf.), on sites with well-balanced moisture (Carlson 1981) and positive diameter growth on dry sites for Jeffrey pine (*Pinus jeffreyii* Balf.) and singleleaf pinyon (*Pinus monophylla* Torr. & Frém; Walker 2002; Walker and Huntt 1992). Results are varied, however, and dependent on application rate, site conditions, species, and the interaction between those factors. Walker (2002) found that CRF had no impact on survival of Jeffrey pine seedlings on dry sites in the Sierra Nevada. However, Jacobs et al. (2003) found that higher CRF application rates negatively impacted Douglas-fir (*Pseudotsuga menziesii* Mirb.) seedling shoot and root growth, which can negatively influence plant establishment and survival.

Size of seedlings at outplanting and field fertilization can influence plant growth following outplanting (Dominguez-Lerena et al. 2006; South and Mitchell 1999; Svendsen and Tanino 2006), and thus potentially improve plant establishment. Determining a target seedling for outplanting may help in achieving plant establishment goals for restoration of degraded sites. Use of seedlings grown for specific site conditions allows for the effective evaluation of establishment costs. This in turn could help land managers to determine the cost-effectiveness of outplanting nursery grown seedlings vs. direct seeding Wyoming big sagebrush to re-establish the species. Better understanding factors contributing to establishment and survival of Wyoming big sagebrush seedlings could aid in evaluating the relative advantages or disadvantages of planting or seeding on these sites determining what may be best compared to direct seeding for optimal restoration of these sites. The study objectives were to 1) identify the influence of container volume on Wyoming big sagebrush outplanting establishment and survival without compromising growth, and 2) determine the influence of fertilizer on outplanted Wyoming big sagebrush seedlings in terms of growth and survival. The overall objective is to develop recommendations for seedling production and outplanting treatments to maximize sagebrush establishment on degraded sites, as a means of developing target plants for restoration.

Materials and Methods

Site description

This study was established in southern Idaho at two sites (Mountain Home and Orchard, ID) representing three distinct site conditions. All sites are representative of shrub-steppe communities dominated by Wyoming big sagebrush, with basin big sagebrush (*A. tridentata* Nutt. ssp. *tridentata*), antelope bitterbrush (*Purshia tridentata* Pursh DC.) and bunchgrass associations (Kinter et al. 2007), in need of restoration. They can be classified as an *Artemisia tridentata* ssp. *wyomingensis*/*Agropyron spicatum* (ARTRW/AGSP) habitat type (Hironaka et al. 1983). For this area mean air temperature is 45 to 55 °C, mean annual precipitation is 208 mm, and mean annual snowfall is 279 mm (NOAA). The Orchard site (43° 19.661' N, 115° 59.786' W) has soil that is part of the Lankbush Chardoton soil series complex. These soils are very deep and well-drained. They are classified as fine-loamy, mixed, mesic Xeric Haplagrids (Soil Survey Staff 1996). The Mountain Home site (42° 58.503' N, 115° 38.012' W) contained two field conditions, tilled and untilled. Tilled conditions were created by a mechanical seeder which altered the ground similar to conventional seeding. At this site, the soil is in the Scism series and is classified as a coarse-silty, mixed, superactive mesic Xereptic Haplodurid (Soil Survey Staff 1996). Each field condition will be referred to as Mountain Home Tilled, Mountain Home Untilled, or Orchard.

Plant materials

Wyoming big sagebrush seedlings were grown in Moscow, ID using seeds from Humboldt and Elko counties, NV. Seeds were sown May 17, 2007 into three types of Styroblock™ (Beaver Plastics, Acheson, Alberta, Canada) containers with uniform dimensions: 45/340; 60/250; and 112/105 (Table 2.1), hereafter referred to as 340, 250, and 105. Detailed production notes are provided in Chapter 1, this thesis. Seedlings were harvested from the containers on 11 and 12 March 2008 and transported to the field sites for outplanting.

Experimental design

At each site, the study was a 3 container volume × 2 field fertilization × 4 replicate factorial randomized complete block design. The three container volumes (105, 250, and 340) and two fertilizer rates (0 or 7.5 g plant^{-1}) were randomly assigned rows within four 5 × 19 m blocks. Fertilizer (10-12-12 controlled-release plant food; Schultz™ Spectrum Brands, Atlanta, GA; 3-mo release rate at 21°C; Table 2.2) was placed in the bottom of the appropriate planting holes. Seedlings were planted at 1-m spacing with 20 seedlings per container volume-field fertilization-replicate combination, resulting in 480 seedlings per site.

Environmental monitoring

At Mountain Home, temperature, wind speed, wind direction, rainfall, and humidity were measured using a WatchDog 900 ET Weather Station (Spectrum Technologies, Inc., Plainfield, IL). Soil temperatures were recorded every hour using i-Button data loggers (Maxim/Dallas SemiConductors, Dallas, TX) with sensors initially buried at 5 and 24 cm depths in tilled and untilled conditions. Soil moisture was recorded using an EM-50 data recorder (Decagon Devices, Pullman, WA) with EC-5 soil moisture sensors buried at 5 and 24 cm depths (Figure 2.1). Soil electrical conductivity was assessed 21 February 2009 using a Field Scout Soil EC Probe & Meter (Spectrum Technologies, Inc., Plainfield, IL) at four randomly selected points surrounding each plant.

Measurement of plant morphology and physiological status

Seedlings were outplanted at all sites on 14 and 15 March 2008 and initial height and root-collar diameter were recorded. Seedlings were re-measured at the end of the growing season, 216 days after outplanting (13 October 2008), in addition approximately three seedlings from each container-fertilizer-replicate combination were harvested, root-washed, and shoot and root volume measured using the water displacement method (Burdett 1979). Dry mass was determined following oven-drying at 70 °C for > 72 h (Grieve Industrial Oven NB-350, Grieve Corporation, Round Lake, IL).

At Orchard, root and shoot volume were measured on the first five seedlings of each container-fertilizer-replicate combination prior to outplanting. These five seedlings were randomly selected, their roots were carefully washed, and, following measurements, the seedlings were kept cool and moist until outplanting as the first five plants in each container-fertilizer-replicate combination.

Seedling gas exchange (net photosynthetic assimilation, stomatal conductance, and transpiration) was measured with a LI-6400 portable photosynthesis system (Li-Cor Biosciences, Lincoln, NE) using one mature leaf from the top third of a seedling in each container-fertilizer-replicate combination at each site. Gas exchange was measured at Orchard on 12 April 2008, Mountain Home Tilled 13 April and 28 May 2008, and Mountain Home Untilled 28 May and 21 June 2008. Seedlings were measured between 1000-1400 h on relatively clear days using an internal light source that provided a source of photosynthetically active radiation at 1200 µmol m^{-2} s^{-1}. A controlled CO_2 source was sustained at 375 µmol mol^{-1} to provide a reference CO_2 level. A stable leaf temperature, close to ambient, was maintained. Leaves used for gas exchange measurement were collected and measured for leaf area using a LI-3100 (Li-Cor Biosciences, Lincoln, NE); leaf areas were used to adjust gas exchange values.

Samples for foliar nutrient analysis were collected 30 and 216 days following outplanting. Samples were oven-dried at 70°C for >72h (Grieve Industrial Oven NB-350, Grieve Corporation, Round Lake, IL), ground with a 1 mm screen (Cyclone Sample Mill, UDY Corporation, Fort Collins, CO), and analyzed by A&L Great Lakes Laboratory (Fort Wayne, IN) for: N, P, K, Ca, Mg, S, Zn, Mn, Fe, Cu, B, Al, and Na. Total N was measured by the Dumas combustion method (Dumas 1962) in a LECO FP-428 combustion analyzer (LECO corporation, St. Joseph, MI). All other minerals were analyzed using a Thermo Jarrell Ash model 61E ICP (Thermo Electron Corporation, Waltham, MA).

Data analysis

Experimental design was a 3 container volume × 2 field fertilization × 4 replicate factorial randomized complete block design. Analysis of variance (ANOVA, SAS Institute Inc., version 9.1, Cary, NC) was used to identify effects of container volume and field fertilizer (significant at $\alpha = 0.05$) on Wyoming big sagebrush seedling height and root-collar diameter, gas exchange, seedling dry mass and volume, root:shoot, and foliar nutrient status at 30 and 216 days following outplanting. For the Orchard site, a split plot analysis was used to test effects of root washing prior to outplanting on subsequent gas exchange ($\alpha = 0.05$). Treatment means were separated using Tukey's honest significant difference (HSD) test ($\alpha = 0.05$).

Results

Environmental conditions

At Mountain Home the mean daily temperature was 18.3 °C, with a maximal temperature of 41.6°C on 29 June 2008 at 1600 h. Average wind speed recorded was 10.3 km h^{-1}; the predominant wind direction was west southwest. The strongest wind, 59.5 km h^{-1} occurred 20 May 2008 at 1800 h, with gusts up to 91.7 km h^{-1}. Rainfall during the 216 days totaled 38.5 mm with maximum precipitation of 6.0 mm occurring on 28 May 2008 at 0100 h (Figure 2.2).

The high winds caused significant soil erosion. The soil moisture probes initially placed at the 5 cm depth were within 2 cm of the soil surface after 216 days, rendering observed values inaccurate because the probe reflected the dialect of the air. The moisture probe at 24 cm (Figure 2.1) identifies a drying condition over the growing season at the base of the seedling root plugs.

Soil electrical conductivity, measured 21 February 2009, did not differ ($p = 0.2365$) whether measured next to fertilized (0.71 ± 0.08 mS cm^{-1}) or unfertilized (0.54 ± 0.1 mS cm^{-1}) seedlings at the untilled condition. Similar results were seen for the untilled condition with soil electrical conductivity surrounding fertilized (0.31 ± 0.03 mS cm^{-1}) and unfertilized (0.32 ± 0.6 mS cm^{-1}) seedlings not significantly different ($p = 0.8281$).

Mountain Home Tilled

Survival

Container volume significantly influenced seedling survival ($p = 0.0026$); seedlings grown in the largest containers (340 and 250) had higher survival (44% ± 0.04 and 43% ± 0.04, respectively) than those grown in the 105 containers (28% ± 0.04). Field fertilization also significantly influenced survival ($p = 0.0084$); fertilized seedlings had lower (33% ± 0.03) survival than those that were not (44% ± 0.03).

Plant morphology

At the end of the growing season, 216 days after outplanting, container volume significantly influenced height ($p < 0.0001$) and root-collar diameter ($p < 0.0001$), with those parameters increasing as container volume increased (Table 2.3). Seedlings grown in the 105 containers had greater height growth (0.39 ± 0.57, $p = 0.0110$) but less root collar-diameter growth (1.00 ± 0.13, $p = 0.0005$) than those grown in 340 containers (-2.34 ± 0.57 and 1.71 ± 0.13 respectively), with neither being different from plants grown in the 250 containers (Table 2.3).

Shoot volume increased with container volume ($p < 0.0001$, Table 2.5), but shoot dry mass was improved only in the 340 containers ($p < 0.0001$, Table 2.7). Root volume of seedlings grown in the two largest containers was significantly greater than that of those grown in 105 containers ($p < 0.0001$, Table 2.5); root dry mass increased significantly as container volume increased ($p < 0.0001$, Table 2.7). No significant differences were observed among container volume for root:shoot based on volume ($p = 0.4300$, Table 2.5) or dry mass ($p = 0.0779$, Table 2.7).

Field fertilizer (Table 4) had no significant affect on height ($p = 0.8267$) or root-collar diameter ($p = 0.2516$) Fertilizer (Table 2.4) had no effect on height ($p = 0.2617$) or root-collar diameter ($p = 0.1334$). Fertilizer had no affect on shoot or root volume ($p =$ 0.3747, $p =$ 0. 1859, respectively; Table 2.6) or shoot or dry mass ($p = 0.3583$, $p =$ 0.2735, respectively; Table 2.8), or root:shoot by volume ($p = 0.4432$, Table 2.6) or dry mass ($p = 0.6133$, Table 2.8).

Data was analyzed for interactions; however both main effects were never significant for the same parameters measured at any one time.

Gas exchange and nutrient status

Neither container volume nor field fertilization influenced seedling gas exchange when measured on 13 April 2008 or 28 May 2008 ($p > 0.05$, Table 2.10). At 30 days following outplanting, nitrogen concentration was significantly higher ($p = 0.0021$) in fertilized seedlings whereas most other nutrients were not influenced ($p > 0.05$, Table 2.9). Fertilizer failed to affect nutrient concentration 216 days after outplanting ($p > 0.05$, Table 2.9).

Mountain Home Untilled

Survival

Seedling survival was unaffected by container volume ($p = 0.7604$). Survival for seedlings grown in the 340, 250, and 105 containers was 60%, 61%, and 57%, respectively. Survival (55% ± 0.03) was significantly lower ($p = 0.0391$) for field fertilized seedlings than unfertilized seedlings (64% ± 0.03).

Plant morphology

At the end of season, height ($p < 0.0001$) and root-collar diameter ($p < 0.0001$) significantly increased as container volume increased (Table 2.3). Seedlings grown in 340 and 250 containers had significantly less height growth (-0.4 ± 0.30 and -0.1 ± 0.42 respectively, $p = 0.0002$) than the 105 container (1.7 ± 0.41, Table 2.3), while the root-collar diameter growth of seedlings from the 250 (1.28 ± 0.08) and 105 (1.28 ± 0.11) containers was significantly less than the 340 container (1.66 ± 0.12, $p = 0.0078$, Table 2.3). Both height and root-collar diameter showed significantly greater growth over 216 days when fertilized ($p = 0.0109$ and $p = 0.0020$ respectively, Table 2.4). Final shoot volume was unaffected by container volume ($p = 0.0778$, Table 2.5). Final shoot dry mass of seedlings grown in the 340 containers was significantly greater than those grown in 105 containers, with no difference from the 250 container seedlings ($p = 0.0243$, Table 2.7); the same pattern held for root volume ($p = 0.0067$, Table 2.5). Final root dry mass of seedlings from the 340 and 250 containers was significantly ($p < 0.0001$) greater than that of seedlings grown in the 105 containers (Table 2.7). Root:shoot by volume was greater ($p = 0.0386$) for seedlings grown in 340 containers (Table 2.5); no difference in root:shoot occurred when investigating dry mass ($p = 0.3440$, Table 2.7).

Final height ($p = 0.2434$) was unaffected by field fertilization (Table 2.4). Height growth ($p = 0.0109$), root-collar diameter growth ($p < 0.0001$), and final root-collar diameter ($p < 0.0001$) were significantly higher with field fertilization (Table 2.4). Final shoot volume was significantly greater for seedlings that received field fertilization (p = 0.0194, Table 2.6). Shoot dry mass increased significantly with fertilization (p = 0.0177, Table 8). Fertilization decreased (p = 0.0255) root:shoot by dry mass (Table 2.8) but not by volume (p = 0.0692, Table 2.6). Fertilizer application also had no affect on root volume (p = 0.2562, Table 2.6) or root dry mass (p = 0.8482, Table 2.8).

Data was analyzed for interactions; however both main effects were never significant for the same parameters measured at any one time.

<u>Gas exchange and nutrient status</u>

Seedling gas exchange, when measured on 28 May 2008 or 21 June 2008 was not influenced by container volume ($p > 0.0005$, Table 2.10). Net photosynthetic assimilation was significantly higher in May with fertilization (16.11 ± 1.71 µmol CO_2 $m^{-2}s^{-1}$, p = 0.0102) than without (2.96 ± 2.06 µmol CO_2 $m^{-2}s^{-1}$, Table 2.11). When foliar nutrient status was assessed 30 days following outplanting, fertilization had no effect for all nutrients ($p > 0.05$, Table 2.9). When reassessed 216 days following outplanting, magnesium was positively (p = 0.0001) influenced by fertilization, but zinc (p = 0.0419) and aluminum (p = 0.0483) concentrations were higher in unfertilized seedlings. No other differences occurred ($p > 0.05$, Table 2.9).

Orchard

Extensive animal browsing at Orchard resulted in extremely low seedling survival across all combinations (<15%). Therefore, data was not investigated in detail due to lack of sufficient sample sizes for statistical models. At 30 days following outplanting and prior to the onset of extensive mortality, seedling gas exchange was assessed. Washing roots significantly lowered seedling transpiration ($p < 0.0001$) and stomatal conductance compared to those seedlings that were not root washed ($p < 0.0001$), but photosynthetic assimilation for root washed seedlings (2.97 ± 0.44 µmol CO_2 m^{-2} s^{-1}) and seedlings that were not root washed (4.11 ± 0.87 µmol CO_2 m^{-2} s^{-1}) were not significantly different (p = 0.2066). Otherwise, gas exchange was not influenced by container volume ($p > 0.005$, Table 2.12) or field fertilization ($p > 0.05$, Table 2.13).

Discussion

At Mountain Home, field fertilization reduced seedling survival under tilled and untilled conditions. Given the potential for high fertilizer salt concentrations to damage seedling root development (Jacobs et al. 2003), and the likelihood for dry soil conditions to exacerbate that potential (Kozlowski 1987), it is not surprising that seedling survival decreased in the presence of fertilizer. The relatively low soil electrical conductivity measured in the present study could simply be a result of the fertilizer having dissipated; Jacobs and Timmer (2005) identified that for damage to occur to conifer roots, electrical conductivity readings would likely need to be > 2.5 mS cm^{-1}.

Container volume influenced survival on the tilled site. Thus, Wyoming big sagebrush seedling survival can be affected by nursery culture practices influencing initial seedling size, which concurs with findings for other plants (Everett 1980; Johnson and Okula 2006). Survival on the tilled site was approximately 50% lower across all treatment combinations than at the untilled site. This may be due in part to the extremely dry soil conditions (Figure 2.1); tilling is known to decrease soil moisture retention and increase susceptibility to erosion (Barton et al. 2004). Although site could not be examined as a factor given the experimental design employed in this study, it is possible that seedlings grown in larger containers are better able to withstand harsher conditions (Grossnickle and Folk 1994; Jacobs et al. 2005b). Larger seedlings may not, however, always be best suited for all sites because they can have difficulty maintaining photosynthetic rates in warm, dry environments (Stewart and Bernier 1995) although that did not appear to be the case in the present study. Using larger containers has been positively correlated with survival with pines in the southeastern United States (South and Mitchell 1999; South 1993), Englemann spruce (*Picea engelmannii* Parry ex Engelm.) in the Rocky Mountains (Hines and Long 1985), and native shrubs in the southwestern United States (Bean et al. 2004). Larger root-collar diameter at outplanting, a variable readily controlled by container volume/density (Chapter 1), has been correlated with higher field survival in Douglas-fir and slash pine (*Pinus elliottii* Engelm.) seedlings (Schneider et al. 1998; South and Mitchell 199l).

Seedlings that were larger at outplanting stayed so throughout the growing season under both Mountain Home conditions. This confirms the hypothesis tested and concurs with existing literature for many plants (Aphalo and Rikala 2003; Endean and Carlson 1975; Timmis and Tanaka 1976). For black spruce (*Picea mariana* Mill.) seedlings, greater initial seedling size positively influenced height and diameter growth (Jobidon et al. 1998). That held true for seedling root-collar diameter growth under both Mountain Home tillage regimes, but not for height growth; height growth was greatest with the smallest container size. The generally low, and often negative, height growth can be attributed to wind conditions at the sites and browsing by Townsend's ground squirrels (*Spermophilus mollis*) which commonly browse on sagebrush seedlings (Paige and Ritter 1999); visual assessment confirmed that browse damage was occurring. Given the overall increase in root-collar diameter that followed the container gradient, it was not surprising that shoot and root volume and shoot and root dry mass were greater with larger containers (Dominguez-Lerena et al. 2006; van den Driessche 1992). Container volume had no affect on root:ratio by dry mass and only affected root:shoot by volume at Mountain Home Untilled. Root:shoot provides information about the balance between shoot and root and is considered a predictor of field performance as well (Bernier et al. 1995; Boyer and South 1987; Haase and Rose 1993). In this case, the balance, achieved for the most part across all treatments, likely indicates that plants adjusted leaf area as needed to deal with environmental conditions.

Height and root-collar diameter growth, and final measurement, were unaffected by fertilizer at Mountain Home Tilled. Root-collar diameter was positively influenced by fertilization at Mountain Home Untilled, with fertilized seedlings having greater increase in root-collar diameter and end-of-season root-collar diameter than unfertilized seedlings. These variable results correspond with other studies. Shadscale (*Atriplex confertifolia* Torr. & Frém.) and big sagebrush response to N fertilization in the field varied depending on the year (James and Jurinak 1978). As well, Carpenter and West (1987) found that N fertilization did not affect growth of mountain big sagebrush (*A. tridentata* Nutt. ssp. *vaseyana* Rdyb. Beetle). In desert conditions, N fertilization did not affect perennial plant growth (Goodman 1973; Skujins 1981), but did promote growth of exotic annuals (Goodman 1973) and increase forage production and quality for wildlife (Barrett 1979). Fertilization positively influenced shoot volume and dry mass, but had no affect on root volume or dry mass at Mountain Home Untilled. Miller et al. (1991) also found addition of N to increase aboveground biomass, total shoot density, and individual shoot weight in Wyoming big sagebrush. Fertilizer increased root-collar diameter growth at Mountain Home Untilled, but had no major impact on nutrient status; this could be attributed to the ineffectiveness of the sampling time, as nutritional benefits may not have been present at the time foliar sampling occurred. Timing of measurements may also affect results and should be further studied to understand the release of fertilizer used, especially in arid ecosystems.

Seedling gas exchange was not influenced by container volume or fertilizer; this concurs with the lack of impact on foliar nutrient status and the similarity in root:shoot. Should plant nutrient status have been enhanced, it is possible that net photosynthetic assimilation would have been higher, as Gerdol et al. (2002) found that fertilization marginally increased net photosynthetic assimilation of some subalpine dwarf-shrubs. Similarly, because plants reduce leaf area to cope with drought conditions, it is not unexpected that without a difference in root:shoot, no difference in net photosynthetic assimilation, transpiration, or stomatal conductance would be observed.

At Orchard, container volume and fertilizer also had no influence on seedling gas exchange; however, a difference between root-washed seedlings and those that were not was observed. Root-washed seedlings had lower rates for transpiration and stomatal conductance. This could be due to increased handling stress caused by the root washing process (Carey et al. 2001), or by loss of the mediating environmental effect provided by the growing media in the root plugs resulting in drying (Maclaren 1993). One benefit of container-grown seedlings is the maintenance of an entire root plug at outplanting. This loss of vigor could be important for bareroot grown sagebrush seedlings and may warrant testing stress resistance prior to outplanting. As seen in Chapter 1, there was a loss of cold hardiness, often used as a surrogate measure for cold hardiness (Fuchigami and Nee 1987), between the end of the nursery growing season and the time of outplanting. Stomatal control allows the regulation of water loss, which is critical in arid ecosystems, and controlling the rate of CO_2 uptake necessary for photosynthesis (Taiz and Zeiger 2006). Higher transpiration and stomatal conductance indicate better water uptake in non-root-washed seedlings (Taiz and Zeiger 2006). From a research perspective, it is important to note the potential impact of handling for measurements on seedling performance.

Conclusion and Future Directions

Container volume can influence Wyoming big sagebrush field performance. On a drier site, survival was higher when seedlings produced in larger containers were used. That pattern did not hold true on a moister site, where survival was higher overall. On the moister site, root-collar diameter growth was enhanced with field fertilization, despite only minute differences in foliar nutrient concentration. The close relationship between size and survival can help nursery managers and land managers to determine the target plant and outplanting treatment for the restoration of Wyoming big sagebrush. Field fertilization had minimal contribution to seedling growth, while lower survival was recorded with addition of fertilizer. Thus, field fertilization must be evaluated in cost and appropriateness for the desired restoration site. In practice, for restoration of sagebrush ecosystems on particularly dry sites, it may be advisable to produce seedlings with larger root-collar diameter given the resultant higher survival and growth rate. Furthermore, the maintenance of a balanced root:shoot ratio appears to be important for seedlings to withstand the water stress of these arid ecosystems. Seedlings suited for survival can aid in plant establishment resulting in accomplishment of restoration goals. Additional research in the area of developing specific seedling guidelines for particular site conditions will benefit restoration programs and may result in lower costs per established plant than direct seeding.

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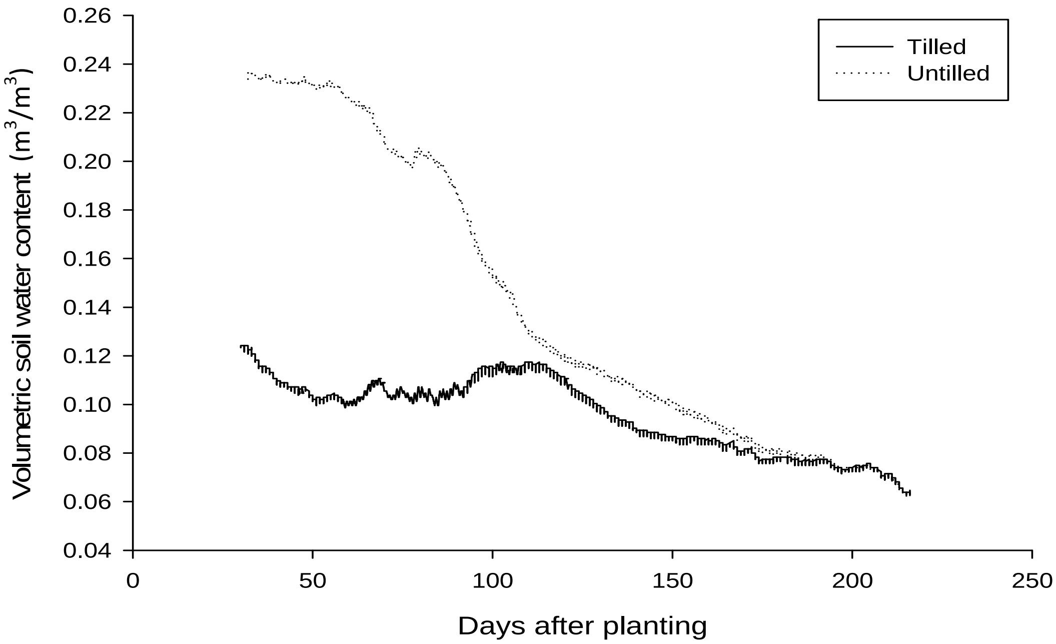

Figure 2.1. Soil moisture at 24 cm, measured as volumetric soil water content, at Mountain Home Tilled (solid line) and Mountain Home Untilled (dotted line) recorded for 216 days after outplanting.

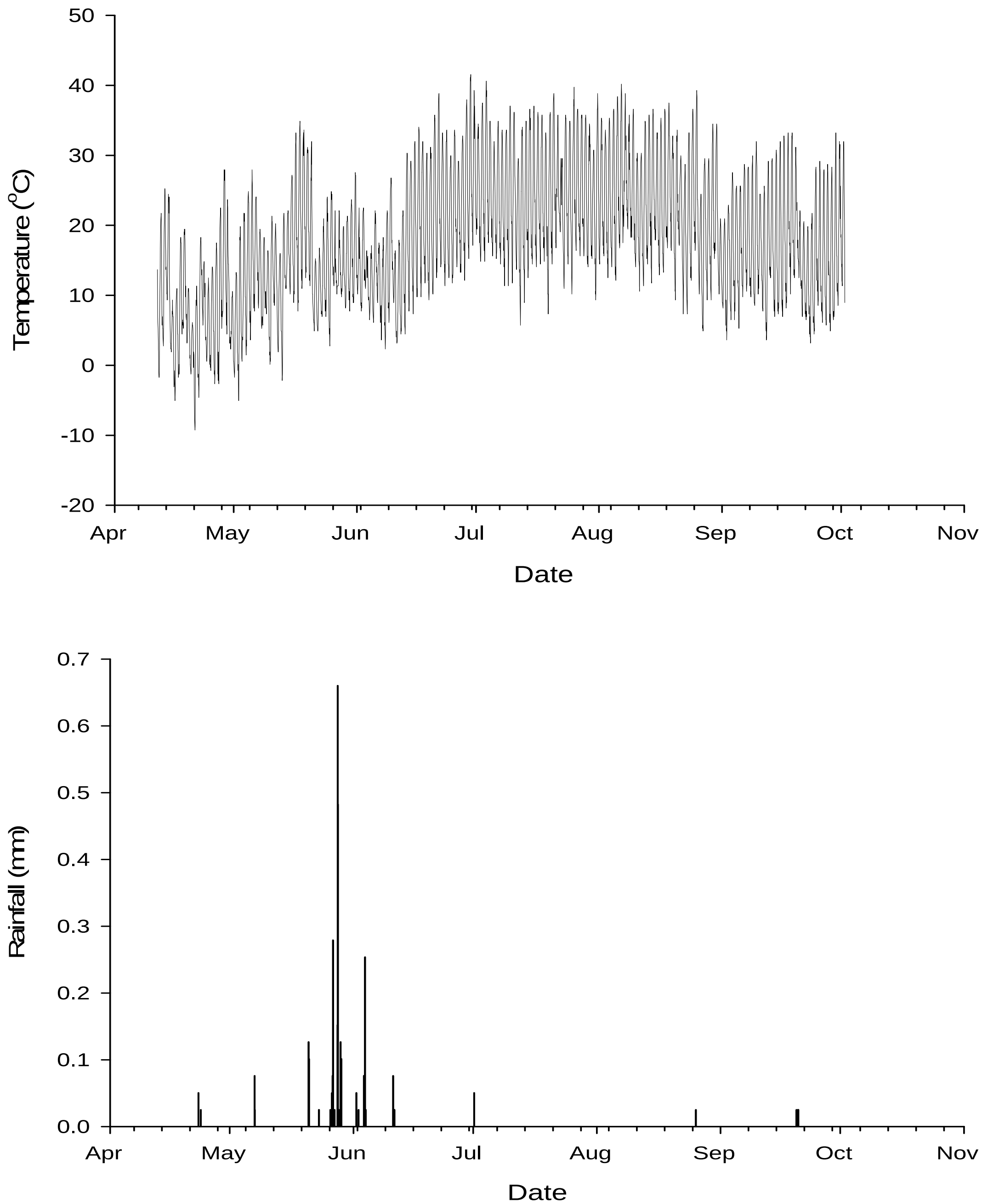

Figure 2.2. Air temperature (top) and precipitation (bottom), recorded hourly for 216 days after outplanting, at Mountain Home.

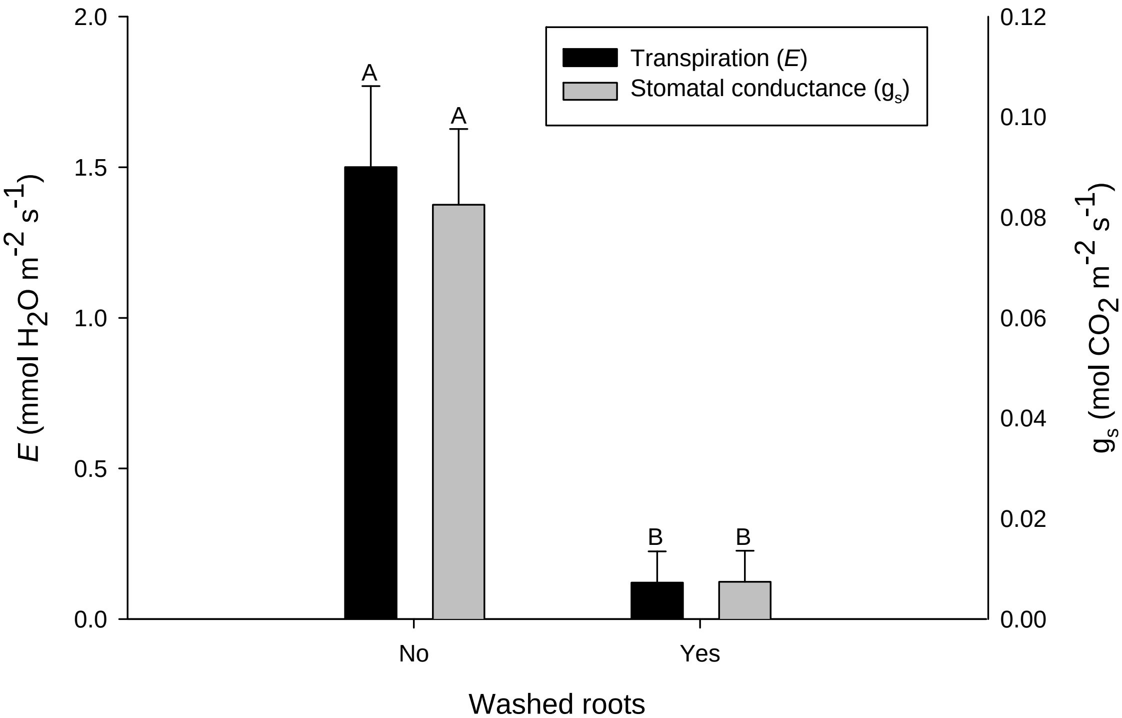

Figure 2.3. Influence of root washing on Wyoming big sagebrush seedling transpiration (E, dark bars) and stomatal conductance (g_s, light bars) as measured on 12 April 2008 at Orchard. Different letters indicate significance differences for each measurement separated using Tukey's HSD at $\alpha = 0.05$.

Table 2.1. Specifications for Beaver Plastics (Beaver Plastics, Acheson, Alberta, Canada) Styroblock™ containers used. Containers vary in density of seedlings, top diameter, and volume. Container type is noted by number of cavities in the container and the volume of such cavities.

Container type		Volume		Top diameter		Cavity density		Depth	
		(cm^3)	(in^3)	(mm)	(in)	(m^{-2})	(ft^{-2})	(mm)	(in)
112/105	415B	105	6.6	36	1.4	530	49	148	5.8
60/250	515A	250	15.3	51	2.0	284	27	151	6.0
45/340	615A	340	10.5	59	2.3	213	20	151	6.0

Table 2.2. Nutritional composition of Schultz™ (Schultz™ Spectrum Brands, Atlanta, GA) 10-12-12 Controlled-Release Plant Food used for field fertilization. Percentage by weight.

Nutrient	Percentage (%)
Nitrogen (N)	10
Phosphate (P_2O_5)	12
Potash (K_2O)	12
Sulfur (S)	2.8
Boron (B)	0.02
Copper (Cu)	0.05
Iron (Fe)	1
Manganese (Mn)	0.05
Molybdenum (Mo)	0.0005
Zinc (Zn)	0.05

Table 2.3. Wyoming big sagebrush (mean ± SE) height and root-collar diameter as influenced by container volume. Different letters indicate significance differences for each site separated using Tukey's HSD at α = 0.05.

Site	Container	Height (cm)											
		Initial				Final				Growth			
		Mean		SE		Mean		SE		Mean		SE	
Mountain	340	19.8	±	0.4	A	18.2	±	0.66	A	-2.3	±	0.57	B
Home	250	15.9	±	0.3	B	15.0	±	0.68	B	-1.4	±	0.55	AB
Tilled	105	10.0	±	0.2	C	10.4	±	0.60	C	0.4	±	0.57	A
Mountain	340	18.2	±	0.3	A	18.1	±	0.52	A	-0.4	±	0.30	B
Home	250	16.4	±	0.3	B	15.6	±	0.45	B	-0.1	±	0.42	B
Untilled	105	10.6	±	0.2	C	12.2	±	0.45	C	1.7	±	0.41	A
		Root-collar diameter (cm)											
		Initial				Final				Growth			
		Mean		SE		Mean		SE		Mean		SE	
Mountain	340	2.90	±	0.05	A	4.67	±	0.13	A	1.71	±	0.13	A
Home	250	2.52	±	0.04	B	3.90	±	0.12	B	1.31	±	0.12	AB
Tilled	105	1.92	±	0.03	C	2.93	±	0.13	C	1.00	±	0.13	B
Mountain	340	2.97	±	0.04	A	4.61	±	0.13	A	1.663	±	0.12	A
Home	250	2.68	±	0.04	B	3.93	±	0.08	B	1.278	±	0.08	B
Untilled	105	2.05	±	0.03	C	3.33	±	0.11	C	1.28	±	0.11	B

Table 2.4. Wyoming big sagebrush (mean ± SE) height and root-collar diameter as influenced by field fertilization. Different letters indicate significance differences for each site separated using Tukey's HSD at α = 0.05.

Site	Fertilized	Height (cm)											
		Initial				Final				Growth			
		Mean		SE		Mean		SE		Mean		SE	
Mountain Home Tilled	No	15.26	±	0.36		14.73	±	0.57		-1.67	±	0.42	
	Yes	15.18	±	0.36		15.57	±	0.70		-0.90	±	0.56	
Mountain Home Untilled	No	15.19	±	0.31		14.95	±	0.43		-0.17	±	0.27	B
	Yes	14.93	±	0.30		15.83	±	0.42		0.99	±	0.37	A
		Root-collar diameter (cm)											
		Initial				Final				Growth			
		Mean		SE		Mean		SE		Mean		SE	
Mountain Home Tilled	No	2.44	±	0.04		3.86	±	0.11		1.28	±	0.10	
	Yes	2.46	±	0.04		4.09	±	0.14		1.53	±	0.12	
Mountain Home Untilled	No	2.57	±	0.04		3.70	±	0.09	B	1.14	±	0.08	B
	Yes	2.57	±	0.04		4.28	±	0.10	A	1.72	±	0.10	A

Table 2.5. Wyoming big sagebrush (mean ± SE) shoot volume, root volume, and root:shoot as influenced by container volume 216 days following outplanting. Different letters indicate significance differences for each site separated using Tukey's HSD at α = 0.05.

Site	Container type		Shoot volume (cm^3)					Root volume (cm^3)					Root:shoot			
			Mean		SE			Mean		SE			Mean		SE	
Mountain	340		7.84	±	0.69	A		8.33	±	0.78	A		0.62	±	0.05	
Home	250		5.37	±	0.49	B		6.85	±	0.63	A		0.82	±	0.09	
Tilled	105		3.09	±	0.47	C		3.06	±	0.32	B		0.77	±	0.07	
Mountain	340		7.93	±	1.10			13.65	±	0.49	A		2.27	±	0.51	A
Home	250		7.03	±	0.50			8.55	±	0.67	AB		1.31	±	0.12	B
Untilled	105		5.28	±	0.82			3.92	±	0.26	B		1.24	±	0.19	B

Table 2.6. Wyoming big sagebrush (mean ± SE) shoot volume, root volume, and root:shoot as influenced by field fertilization 216 days following outplanting. Different letters indicate significance differences for each site separated using Tukey's HSD at α = 0.05.

Site	Fertilized	Shoot volume (cm^3)			Root volume (cm^3)			Root:shoot		
		Mean	SE		Mean	SE		Mean	SE	
Mountain Home Tilled	No	5.21 ±	0.51		5.76 ±	0.52		1.31 ±	0.11	
	Yes	5.81 ±	0.62		6.62 ±	0.73		1.45 ±	0.20	
Mountain Home Untilled	No	5.60 ±	0.51	B	10.07 ±	2.41	A	1.94 ±	0.32	B
	Yes	7.89 ±	0.82	A	7.34 ±	0.76	B	1.27 ±	0.20	A

Table 2.7. Wyoming big sagebrush (mean ± SE) shoot dry mass, root dry mass, and root:shoot as influenced by container volume 216 days following outplanting. Different letters indicate significance differences for each site separated using Tukey's HSD at α = 0.05.

Site	Container type		Shoot dry mass (g)					Root dry mass (g)					Root:shoot		
			Mean		SE			Mean		SE			Mean		SE
Mountain	340		2.91	±	0.31	A		1.57	±	0.11	A		0.62	±	0.05
Home	250		1.65	±	0.15	B		1.16	±	0.07	B		0.82	±	0.09
Tilled	105		1.03	±	0.15	B		0.62	±	0.06	C		0.77	±	0.07
Mountain	340		2.56	±	0.40	A		1.82	±	0.18	A		0.98	±	0.15
Home	250		2.02	±	0.18	AB		1.52	±	0.11	A		0.81	±	0.07
Untilled	105		1.44	±	0.23	B		0.79	±	0.07	B		0.81	±	0.09

Table 2.8. Wyoming big sagebrush (mean ± SE) shoot dry mass, root dry mass, and root:shoot as influenced by field fertilization 216 days following outplanting. Different letters indicate significance differences for each site separated using Tukey's HSD at α = 0.05.

Site	Fertilized		Shoot dry mass (g)					Root dry mass (g)					Root:shoot			
			Mean		SE			Mean		SE			Mean		SE	
Mountain Home Tilled	No		1.78	±	0.18			1.09	±	0.08			0.75	±	0.05	
	Yes		2.00	±	0.26			1.18	±	0.11			0.72	±	0.07	
Mountain Home Untilled	No		1.61	±	0.17	B		1.36	±	0.11			1.00	±	0.07	A
	Yes		2.40	±	0.29	A		1.39	±	0.14			0.74	±	0.10	B

Table 2.9. Influence of fertilizer on Wyoming big sagebrush seedling foliar nutrient concentration at day 30 and day 216 after planting at both Mountain Home sites. Presented as mean ± standard error (SE), (*) indicates a significant difference at α = 0.05 for that date of measurement.

	Tilled													
	30 day							216 day						
Nutrient	Fertilized			Unfertilized				Fertilized			Unfertilized			
	Mean		SE	Mean		SE		Mean		SE	Mean		SE	
Nitrogen (N) %	1.93	±	0.07	1.77	±	0.04		1.70	±	0.05	1.59	±	0.04	
Phosphorus (P) %	0.47	±	0.03	0.49	±	0.02		0.22	±	0.01	0.22	±	0.01	
Potassium (K) %	1.99	±	0.05	2.02	±	0.07		1.66	±	0.04	1.67	±	0.04	
Calcium (Ca) %	0.72	±	0.06	0.68	±	0.04		0.45	±	0.02	0.50	±	0.01	
Magnesium (Mg) %	0.32	±	0.02	0.31	±	0.01		0.24	±	0.01	0.19	±	0.01	*
Sulfur (S) %	0.21	±	0.01	0.21	±	0.01		0.13	±	0.00	0.13	±	0.00	
Sodium (Na) %	0.02	±	0.00	0.03	±	0.00		0.02	±	0.00	0.02	±	0.00	
Zinc (Zn) ppm	61.28	±	3.77	56.00	±	2.81		32.89	±	1.03	37.28	±	0.98	*
Manganese (Mn) ppm	125.18	±	7.99	94.75	±	5.74		101.97	±	3.71	104.64	±	2.46	
Iron (Fe) ppm	387.00	±	22.40	378.83	±	38.10		588.22	±	41.57	630.47	±	40.37	
Copper (Cu) ppm	79.27	±	4.06	78.33	±	5.48		102.19	±	13.79	108.31	±	6.79	
Boron (Bn) ppm	34.18	±	2.12	30.92	±	1.04		21.14	±	0.78	22.00	±	0.67	
Aluminum (Al) ppm	440.45	±	31.46	444.75	±	40.88		581.22	±	41.93	678.50	±	40.50	*

	Untilled													
	30 day							216 day						
Nutrient	Fertilized			Unfertilized				Fertilized			Unfertilized			
	Mean		SE	Mean		SE		Mean		SE	Mean		SE	
Nitrogen (N) %	1.65	±	0.14	1.40	±	0.09	*	1.50	±	0.05	1.44	±	0.05	
Phosphorus (P) %	0.42	±	0.05	0.39	±	0.03		0.22	±	0.01	0.22	±	0.01	
Potassium (K) %	1.99	±	0.18	1.94	±	0.06		1.66	±	0.04	1.72	±	0.04	
Calcium (Ca) %	0.73	±	0.07	0.66	±	0.04		0.51	±	0.01	0.54	±	0.01	
Magnesium (Mg) %	0.30	±	0.03	0.27	±	0.02		0.20	±	0.01	0.22	±	0.01	
Sulfur (S) %	0.19	±	0.03	0.18	±	0.01		0.12	±	0.00	0.12	±	0.00	
Sodium (Na) %	0.03	±	0.00	0.03	±	0.00		0.02	±	0.00	0.02	±	0.00	
Zinc (Zn) ppm	51.25	±	6.74	47.42	±	4.93		28.30	±	1.19	27.77	±	1.74	
Manganese (Mn) ppm	73.25	±	15.71	67.83	±	11.05		83.58	±	3.77	74.91	±	3.53	
Iron (Fe) ppm	395.00	±	50.18	424.00	±	39.11		664.00	±	28.20	678.86	±	30.15	
Copper (Cu) ppm	71.75	±	8.43	77.00	±	5.78		135.45	±	6.72	125.66	±	5.74	
Boron (Bn) ppm	31.33	±	3.42	29.17	±	1.96		19.45	±	1.14	19.69	±	0.77	
Aluminum (Al) ppm	422.58	±	62.00	430.92	±	57.37		652.24	±	29.90	664.29	±	38.67	

Table 2.10. Wyoming big sagebrush seedling gas exchange [net photosynthetic assimilation (*A*), transpiration (*E*), and stomatal conductance (g_s)], as influenced by container volume, at both Mountain Home sites. Measurements were conducted 13 April, 28 May and 21 June 2008. Presented as mean ± standard error (SE).

Site	Month	Container type	*A* (µmol CO_2 $m^{-2}s^{-1}$)			*E* (mmol H_2O $m^{-2}s^{-1}$)			g_s (mol H_2O $m^{-2}s^{-1}$)		
			Mean		SE	Mean		SE	Mean		SE
Tilled	April	340	5.09	±	2.17	1.39	±	0.45	0.08	±	0.03
		250	8.15	±	1.57	1.64	±	0.26	0.10	±	0.02
		105	5.63	±	1.82	1.10	±	0.47	0.07	±	0.03
	May	340	12.78	±	3.27	4.71	±	0.64	0.45	±	0.07
		250	18.45	±	2.56	4.74	±	1.49	0.47	±	0.14
		105	18.92	±	4.25	4.04	±	1.73	0.44	±	0.22
Untilled	May	340	8.34	±	4.45	4.94	±	0.61	0.42	±	0.06
		250	10.99	±	5.21	5.28	±	1.03	0.45	±	0.10
		105	9.28	±	3.72	6.60	±	1.67	0.67	±	0.23
	June	340	1.33	±	2.28	-0.74	±	0.37	-0.05	±	0.01
		250	6.94	±	1.58	-0.69	±	0.35	-0.01	±	0.01
		105	2.79	±	0.55	-0.82	±	0.33	-0.02	±	0.01

Table 2.11. Wyoming big sagebrush seedling gas exchange [net photosynthetic assimilation (*A*), transpiration (*E*), and stomatal conductance (g_s)] as influenced by fertilizer, at both Mountain Home sites. Measurements were conducted 13 April, 28 May and 21 June 2008. Presented as mean ± standard error (SE).

			A (µmol CO_2 $m^{-2}s^{-1}$)				*E* (mmol H_2O $m^{-2}s^{-1}$)			g_s (mol H_2O $m^{-2}s^{-1}$)		
Site	Month	Fertilized	Mean		SE		Mean		SE	Mean		SE
Tilled	April	No	4.70	±	1.53		0.95	±	0.28	0.05	±	0.05
		Yes	7.88	±	1.36		1.81	±	0.31	0.11	±	0.02
	May	No	17.84	±	2.59		5.13	±	1.30	0.54	±	0.15
		Yes	15.59	±	3.10		3.87	±	0.77	0.37	±	0.08
Untilled	May	No	2.96	±	2.06	B	5.29	±	1.21	0.50	±	0.17
		Yes	16.11	±	1.71	A	5.93	±	0.62	0.53	±	0.06
	June	No	3.48	±	1.65		-0.98	±	0.25	-0.02	±	0.01
		Yes	3.89	±	1.29		-0.52	±	0.28	-0.01	±	0.01

Table 2.12. Wyoming big sagebrush seedling gas exchange [net photosynthetic assimilation (*A*), transpiration (*E*), and stomatal conductance (g_s)] as influenced by container volume, at Orchard. Measurements were conducted 12 April 2008.

Container type	*A* ($\mu mol\ CO_2\ m^{-2}s^{-1}$)			*E* ($mmol\ H_2O\ m^{-2}s^{-1}$)			g_s ($mol\ H_2O\ m^{-2}s^{-1}$)		
	Mean		SE	Mean		SE	Mean		SE
340	2.42	±	0.61	0.39	±	0.25	0.02	±	0.01
250	3.65	±	0.88	1.01	±	0.37	0.06	±	0.02
105	4.55	±	0.98	1.04	±	0.27	0.06	±	0.01

Table 2.13. Wyoming big sagebrush seedling gas exchange [net photosynthetic assimilation (A), transpiration (E), and stomatal conductance (g_s)] as influenced by field fertilization at Orchard. Measurements were conducted 12 April 2008.

Fertilized	*A* (µmol CO_2 $m^{-2}s^{-1}$)			*E* (mmol H_2O $m^{-2}s^{-1}$)			g_s (mol H_2O $m^{-2}s^{-1}$)		
	Mean		SE	Mean		SE	Mean		SE
	Mean		SE	Mean		SE	Mean		SE
No	4.15	±	0.77	0.96	±	0.25	0.05	±	0.01
Yes	2.93	±	0.59	0.66	±	0.25	0.04	±	0.01