Influence of Container Size on Wyoming Big Sagebrush Seedling Morphology and Cold Hardiness

Kayla R. Herriman, Anthony S. Davis, R. Kasten Dumroese

Kayla R. Herriman is Graduate Research Assistant, Center for Forest Nursery and Seedling Research, College of Natural Resources, University of Idaho, Moscow ID 83844-1133; E-mail: kayla.traver@vandals.uidaho.edu. **Anthony S. Davis** is Assistant Professor of Native Plant Regeneration and Silviculture and Director of the Center for Forest Nursery and Seedling Research, College of Natural Resources, University of Idaho, Moscow ID 83844-1133; E-mail: asdavis@uidaho.edu. **R. Kasten Dumroese** is National Nursery Specialist, USDA Forest Service, Rocky Mountain Research Station, Moscow ID 83843; E-mail: kdumroese@fs.fed.us.

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Abstract: Wyoming big sagebrush (*Artemisia tridentata*) is a key component of sagebrush steppe ecosystems and is a dominant shrub throughout the western United States. Our objective was to identify the effect of container size on plant morphology of Wyoming big sagebrush. We used three different stocktypes (45/340 ml [20 in^3], 60/250 ml [15 in^3], 112/105 ml [6.4 in^3]) of 1-year old seedlings to examine seedling quality in regards to cold hardiness, height, root-collar diameter, dry mass, root volume, shoot volume, and root:shoot. Cold hardiness was measured four times in the fall and once in the spring. All other measurements were taken in the spring. Cold hardiness was not affected by container size. Plant height, root-collar diameter, and dry mass increased with container size. Shoot volume increased with container size, and root volume of seedlings from the two largest container sizes was greater than that of seedlings grown in 112/105 ml (6.4 in^3). Our results indicate the strong effect that container size has on plant morphology. This information provides us with a greater ability to develop target plants for use in restoring a particular site.

Keywords: seedling, nursery, stocktype, outplanting

Introduction

Throughout much of the western United States, Wyoming big sagebrush (*Artemisia tridentata*) is a signature species, serving an important ecological role in sagebrush steppe ecosystems (Meyer and Monson 1992; McIver and Starr 2001; Lambrecht and others 2007). Sagebrush is critical habitat for wildlife, including sage grouse (*Centrocercus urophasianus*) and pronghorn (*Antilocapra americana*) (Yoakum 1982; Rosentreter 2005). These ecosystems have been degraded by fire, noxious weeds, and land use patterns. Many of these ecosystems were exhausted by livestock grazing pressure between 1870 and 1900. Due to the many years of grazing and the low resilience of these ecosystems, exotic annual grasses, such as cheatgrass (*Bromus tectorum*), and noxious weeds were able to establish (Mack and Thompson 1982; Young and others 1987; Monsen and McArthur 1995; McIver and Starr 2001).

Restoration of sagebrush ecosystems has only recently increased in practice and has predominately focused on direct seeding (Hou and Romo 1998; Chambers 2000; Pierson and others 2007). Seedling establishment is paramount to restoration success. Once established, seedlings have shown relatively high rates of survival. Schuman and Belden (2002) found that after 8 years, 59% of seedlings survived. Kiger and others (1987) found long-term survival rates of 33% after 11 years. Direct seeding has shown success in long-term survival, as well as in seed-increase gardens (Welch 1997). In regards to outplanting, nursery-grown sagebrush seedlings could be a more effective method of restoring sagebrush ecosystems, especially with the influence of cost and seed availability (Beyers 2004).

The initial cost of nursery-grown seedlings is higher than that of direct seeding, mainly due to the cost of nursery production and costs associated with shipping plants. Container seedlings may, however, have greater establishment success in harsh site conditions, particularly where repeated direct-seeding operations are required to obtain desired results. Thus, the initial cost of growing, handling, and planting container seedlings may yield more desirable results (better plant establishment and growth) and be more cost effective over time than repeated, or perhaps even single, direct-seeding events (Clements and Young 2000). Sagebrush produces seeds within 3 to 5 years following establishment (Lysne 2005), indicating that surviving plants rapidly become a viable seed source, and able to further colonize the site. Our study objective was to identify the effect of container size on plant morphology of Wyoming big sagebrush so that appropriate target plant specifications can be developed for restoration of degraded sites.

Materials and Methods

Plant Materials

Seedlings were started inside a greenhouse at the Rocky Mountain Research Station in Moscow, ID. Seeds (Humboldt and Elko Counties, Nevada sources) were sown 17 May 2007 into three sizes of Styroblock™ (Beaver Plastics, Acheson, Alberta, Canada) containers: (45/340 ml [20 in^3], 615A; 60/250 ml [15 in^3], 515A; 112/105 ml [6.4 in^3], 415B) (table 1). Thinning and transplanting was conducted on 6 June 2007 to ensure that all cells were filled with a single germinant. Fertilizer was initially applied with irrigation at 100 ppm nitrogen and switched to 25 ppm nitrogen on 4 June 2007 for the rest of the growing season. Seedlings were moved to the University of Idaho Center for Forest Nursery and Seedling Research (Moscow) on 26 October 2007 for hardening and overwintering. Seedlings were outplanted 14 and 15 March 2008 in southern Idaho to examine subsequent field performance.

Plant Morphology Assessment

Height and root-collar diameter were measured on all 480 seedlings of each stocktype following lifting from containers. Root and shoot volume were also measured at this time on a subsample of 40 seedlings of each stocktype using the water displacement method (Burdett 1979). A further subset of 10 seedlings from each stocktype was destructively harvested to determine seedling dry mass following oven-drying at 70 °C (158 °F) for >72 hours.

Cold Hardiness Assessment

Seedlings were tested on four dates in 2007 (5 November, 19 November, 5 December, and 20 December) and once in 2008 (19 March). At each date, cold hardiness was determined via freeze-induced electrolyte leakage (FIEL; Flint and others 1967). Tissue samples from 25 seedlings were randomly selected and five samples were used at each test date. Tissue was cut into 1-cm (0.4-in) lengths and divided into five replicates; one segment of plant was placed into a vial containing 2.5 ml (0.08 oz) 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 [36, 14, –4, –22, –40 °F) were used. In addition to FIEL, chilling hours were recorded beginning 1 September 2007 using iButton Thermachron® temperature sensors (Maxim/Dallas SemiConductors, Dallas, TX).

Data Analysis

We used SAS® software (SAS Institute Incorporated, Cary, NC) for analysis of variance (ANOVA) to identify differences among treatments. Treatment means were separated using Tukey's honest significant difference (HSD) test ($\alpha = 0.05$). SigmaPlot® (SYSTAT, San Jose, CA) and Microsoft Excel® (Microsoft Corporation, Seattle, WA) were used to calculate LT50s.

Results and Discussion

Plant Morphology

All sagebrush seedling parameters were significantly affected by container size (fig. 1, table 2), which we anticipated given the greater growing space among containers and subsequent resource allocation (Pinto 2005; Dominguez-Lerena and others 2006). Mean height, root-collar diameter, shoot volume, and dry mass for roots and shoots all significantly increased ($P < 0.0001$) as container size increased. Root volume showed no significant difference ($P = 0.0054$) between the two largest stocktypes, 45/340 ml (20 in^3) and 60/250 ml (15 in^3), although they were significantly different from the smallest stocktype, 112/105 ml (6.4 in^3). This could be attributed to the fact that, for one growing season under this growing regime, Wyoming big sagebrush could not adequately fill the cavity of a 45/340 ml (20 in^3) cell.

Cold Hardiness

Chilling hours accumulated by 5 November 2007 were 65 days at 5 °C (41 °F) and 237 days at 10 °C (50 °F). By the end of data recording, chilling hours at 5 and 10 °C (41 and 50 °F) had accumulated to 677 and 1,217 days, respectively. Stocktype had no effect on cold hardiness measured by the FIEL method and verified using the whole plant freeze test

Table 1. Specifications for containers used.

Beaver Plastics Styroblock™ type		Top diameter		Depth		Volume		Seedling density per	
		mm	*in*	*mm*	*in*	*cm^3*	*in^3*	*m^2*	*ft^2*
112/105 ml (6.4 in^3)	415B	36	1.4	148	5.8	108	6.6	530	49
60/250 ml (15 in^3)	515A	51	2.0	151	6.0	250	15.3	284	26
45/340 ml (20 in^3)	615A	59	2.3	151	6.0	336	10.5	213	20

Figure 1. Wyoming big sagebrush grown in three different sizes of Styroblock™ containers.

Table 2. Influence of stocktype on Wyoming big sagebrush morphology, presented as mean, Tukey grouping, and standard error (SE). Different letters indicate significance within a column at α = 0.05.

Stocktype	Height (cm)	Root-collar diameter (mm)	Volume (cm³) Shoot	Volume (cm³) Root	Dry mass (g) Shoot	Dry mass (g) Root
45/340 ml (20 in³)	18.67 a (0.21)	3.05 a (0.03)	13.85 a (0.65)	11.73 a (0.65)	2.28 a (0.15)	1.50 a (0.10)
60/250 ml (15 in³)	15.86 b (0.18)	2.68 b (0.02)	9.62 b (0.42)	11.17 a (0.60)	1.46 b (0.09)	1.09 b (0.08)
112/105 ml (6.4 in³)	10.41 c (0.11)	2.04 c (0.02)	5.35 c (0.17)	5.85 b (0.23)	0.96 c (0.11)	0.66 c (0.05)

(data not shown). Despite the relatively low number of chilling hours, which typically induce cold hardiness (Christersson 1978; Kozlowski and Pallardy 2002), at the time FIEL measurement began, all three stocktypes had LT_{50} values below –30 °C (–22 °F) (table 3). This level of cold hardiness held for all fall measurements. When lifted on 19 March 2008, LT_{50} values indicated that seedling cold hardiness had decreased to between –10 and –20 °C (14 and –4 °F), which is logical, as dehardening usually occurs due to the influence of rising temperatures and change in day length (Kozlowski and Pallardy 2002). A minimal threshold of cold hardiness at outplanting may be necessary, as Lambrecht and others (2007) found that a single episodic freezing treatment on big sagebrush seedlings resulted in an arresting of growth and negatively affected photosynthetic tissues.

Conclusion and Future Directions ___

Wyoming big sagebrush seedling morphology was clearly influenced by container size, with plant size increasing as container size increased. Cold hardiness was unaffected by container size, but values at the end of the growing season (November/December) were higher (plants were hardier) than prior to lifting (March). Further examination of the cold hardiness cycle of sagebrush will provide insight to growers attempting to maximize storage and coordinate outplanting with times of higher stress resistance, for which cold hardiness is often a surrogate measure (Burr 1990).

For coal mine restoration, the limited availability and increasing cost of native plants seeds has raised the question as to whether outplanting seedlings is a feasible alternative to direct seeding for meeting desired shrub densities (Schuman and others 2005). This same question could be asked for sites impacted by other factors, such as fire. 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 in need of rehabilitation following wildfire (Schuman and others 2005). Direct seeding is perceived to have a greater seed:seedling efficiency. However, more thorough, long-term studies to examine the costs and benefits of direct seeding versus outplanting have not yet been completed (Kleinman and Richmond 2000; Schuman and others 2005).

Seedlings grown during this study were outplanted on sites in southern Idaho and will be tracked to evaluate the influence of container size on field performance of container-grown Wyoming big sagebrush. Future studies should compare the costs of direct seeding and planting of container seedlings with regard to meeting restoration objectives.

Table 3. Cold hardiness (LT_{50}) according to stocktype across five measurement dates; < –40 °C (–40 °F) indicates that LT_{50} was below –40 °C and beyond the scope of measurement.

	LT_{50} (°C) by Measurement date				
Stocktype	**5-Nov**	**19-Nov**	**5-Dec**	**20-Dec**	**19-Mar**
45/340 ml (20 in^3)	–37	< –40	< –40	< –40	–11
60/250 ml (15 in^3)	< –40	< –40	–40	< –40	–13
112/105 ml (6.4 in^3)	–35	< –40	< –40	< –40	–16

°F = (°C*9/5)+32

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