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Cold Hardiness in Wyoming Big Sagebrush Seedlings: Implications for Nursery Production and Outplanting

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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 (Lysne 2005, Lambrecht et al. 2007). Over the past century, these ecosystems have been degraded by fire, invasive species, and destructive land use including livestock-inflicted grazing pressure. 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 (Lysne 2005, Mack 2010).

Restoration of sagebrush ecosystems has only recently been emphasized due to the loss of increasingly valued native communities, but plant establishment has focused predominantly on direct seeding. Once established, Wyoming big sagebrush maintains a relatively high rate of persistence; however, seedling establishment can be erratic, and there have been numerous failures from direct seeding (Lysne 2005). Germination and establishment can be low due to seed quality, animal foraging, water stress, and inadequate light or temperature conditions.

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. Although the initial cost of planting with seedlings is higher than that of direct seeding, largely due to costs associated with nursery production and material, this strategy may prove to be more cost effective over time. Container seedlings have greater establishment success in cold, arid site conditions, while establishment on direct-seeded field sites can be unpredictable and uneven (Romo and Young 2002).

Producing nursery grown seedlings requires some separation from natural environmental conditions. The physiological condition of seedlings at the time they leave the nursery can dramatically impact plant establishment success after outplanting. Given the larger diurnal and seasonal temperature fluctuations to which Wyoming big sagebrush plants are exposed following outplanting (Lambrecht et al. 2007), understanding tolerance to such stresses during nursery culture is of great importance. Cold hardiness refers to the capacity of a plant tissue to withstand exposure to freezing temperatures and can be induced by factors such as photoperiod reduction, decreased soil moisture, or colder temperatures. Young seedlings can be damaged or killed when exposed to temperatures that exceed their cold hardiness level (Hou and Romo 1998); therefore, planting seedlings that are sufficiently cold hardy may aid in Wyoming big sagebrush establishment.

We initiated a study to characterize the level of cold hardiness of Wyoming big sagebrush across a period of seedling production with the aim of improving overall seedling quality for restoration of degraded sites. In May 2007, we sowed 5–10 seeds (Humboldt and Elko counties, NV sources) into Styroblock™ (Beaver Plastics, Acheson, Alberta, Canada) containers at the United States Department of Agriculture Forest Service Rocky Mountain Research Station greenhouse (Moscow, ID; 46° 43.905' N, 117° 59.831' W). Once seedlings had emerged (approximately 3 wks after sowing), we thinned and transplanted them to ensure that each container was filled with a single plant. At approximately 24 wks following sowing, we moved the seedlings to the University of Idaho Franklin H. Pitkin Forest Nursery (Moscow, ID; 46° 43.388' N, 117° 57.431' W) for hardening and overwintering in an open-wall greenhouse. Throughout the growing season we applied fertilizer and irrigation as needed based on operational standards. We arranged seedling containers in a completely randomized design with each individual seedling as the measurement unit and periodically rearranged containers to ensure there was no undue influence of micro-environmental growing conditions. From September 2007–March 2008, we recorded chilling hours to assess length of time exposed to critical temperatures for dormancy induction using iButton Thermachron® temperature sensors (Maxim/Dallas SemiConductors, Dallas, TX).

We determined seedling cold hardiness on 4 dates in 2007 (5 November, 19 November, 5 December, and 20 December) and once in 2008 (19 March) using freeze-induced electrolyte leakage. We collected leaf tissue samples from the top third of each of 15 randomly selected plants at each test date. Using 1 cm lengths of tissue, we placed one segment of tissue into a vial containing 2.5 mL of deionized water and a grain of sand to help promote nucleation and decrease surface tension. Using a programmable freezer (ScienTemp Corp., Adrian, MI) we employed 5 test temperatures (2 [control], -10, -20, -30, and -40°C). Starting at 2°C, we then decreased the temperature by -5°C each hour. Each successive test temperature was held for 30 min before removing the appropriate vials and then decreasing to the next test temperature. We measured electrolyte leakage (EL) after thawing using a SevenEasy conductivity meter (Mettler Toledo, Columbus, OH) to quantify electrolyte leakage caused by freezing. Vials were then autoclaved 20 min at 121°C (Market Forge Sterilmatic, Vernon Hills, IL) to attain total cell death, and we measured total EL. This process was replicated 5 times at each test date.

To describe the level of cold hardiness, we calculated lethal temperature (LT), in this case the LT_{50}, which represented the temperature at which 50% of the tissue was killed. We calculated each LT_{50} by fitting simple quadratic regressions based on EL values at each of the 5 test temperatures. We prepared quadratic regressions using SigmaPlot (version 11, Systat Software Inc., San Jose, CA) and Microsoft Excel (version 12 Microsoft Corporation, Redmond, WA).

At the first sample date, 65 chilling hours had accumulated at 5°C and 237 h at 10°C. When seedlings were lifted (March 11–12, 2007), chilling hours at 5°C and 10°C had accumulated to 677 h and to 1,217 h, respectively. Despite the relatively low number of chilling hours at the first sample date, LT_{50} values indicated that seedlings were extremely cold hardy for early November (-37°C) and maintained similar cold hardiness with LT_{50} values < -40°C for the subsequent 2 sample periods. At lifting in March, LT_{50} values indicated that seedling cold hardiness was decreasing (-13°C).

Lambrecht and colleagues (2007) found that a single simulated episodic freezing treatment damaged Wyoming big sagebrush 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). It has been commonly noted that natural and seeded sagebrush seedlings are very susceptible to late spring frosts (Lambrecht et al. 2007).

Because these seedlings were very cold hardy with exposure to relatively few chilling hours, Wyoming big sagebrush cold hardiness acquisition is likely driven by environmental cues such as photoperiod and moisture availability rather than temperature. As expected, based on annual variation in plant hardening and dehardening, 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. This agrees with observations of silver sagebrush (*Artemisia cana*) (Hou and Romo 1998).

Nursery growers and restoration practitioners may find it necessary to track seedling cold hardiness over time to better predict the ability of such seedlings to withstand cold temperatures following outplanting. Further research should elucidate the relative contribution of chilling hours and photoperiod to seedling cold hardiness. Our results indicate that there is a high degree of cold hardiness in the late-fall and a subsequent dramatic loss of cold hardiness. That this decrease occurs in conjunction with typical spring planting seasons tells us seedling growers and restoration practitioners may benefit from adjusting planting windows to capture higher degrees of cold hardiness, and using cold or freezer stored seedlings to preserve cold hardiness at a higher level.

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