

Research Reports

Seed Germination of Roundleaf Buffaloberry (*Shepherdia rotundifolia*) and Silver Buffaloberry (*Shepherdia argentea*) in Three Substrates¹

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Abstract

Many western native plant species occur in areas characterized by well-drained soils low in organic matter. Some drought-tolerant native plant species exhibit poor seed germination. It was hypothesized that traditional growing substrates high in organic matter may impede their germination; therefore, stratified seeds of roundleaf buffaloberry (*Shepherdia rotundifolia*) and silver buffaloberry (*Shepherdia argentea*) were sown in three substrates differing in organic matter and drainage properties. Seed flats were irrigated twice daily to container capacity, and held on a greenhouse bench for 40 days. Seeds of roundleaf buffaloberry exhibited greatest total germination in a calcined montmorillonite calcined clay substrate (66%); seeds exhibited low germination in a commercial peat-based germination mix (13%) and in a self-prepared, locally popular substrate (22%) that contained sphagnum peat:perlite:calcined clay:sand (2:2:1:1 by vol). Seed germination of silver buffaloberry varied from 42 to 54% and was not different among the three substrates. When substrates are kept consistently moist, a calcined-clay substrate can improve germination of roundleaf buffaloberry, but not silver buffaloberry.

Index words: substrate properties, native plant production, sexual propagation, calcined clay, drought-adapted shrubs.

Species used in this study: roundleaf buffaloberry (*Shepherdia rotundifolia* Parry); silver buffaloberry (*Shepherdia argentea* (Pursh) Nutt.).

Significance to the Nursery Industry

With rapid development occurring in the arid West, many municipalities have faced unprecedented challenges in meeting demands for providing adequate water resources. To meet these demands, some have turned to regulating landscaping

methods to minimize water use. In addition, many residents desire landscapes that more closely match their natural surroundings, and they are including more native plant species in their landscape designs. For these reasons, demand for drought-adapted native plant species for ornamental use is on the rise. Growers struggle to meet demand for some native plant species due to limited knowledge of methods to optimize seed germination. Silver and roundleaf buffaloberry are attractive shrubs native to the Intermountain West. Roundleaf buffaloberry, in particular, is highly coveted by growers because of its drought tolerance and exceptional

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ornamental value. Its naturally rounded compact form, silver-green pubescent evergreen foliage, and tendency to cascade when planted in a terraced site make it highly desirable as a specimen plant. The deciduous silver buffaloberry has narrow silver-green leaves, attractive red berries in fall, and is adapted to wetter sites. This study was designed to test the most commonly used substrates for seed germination of these western plant species. Results suggest, under consistently moist conditions, calcined clay has the optimal combination of water-holding and drainage properties for germinating seeds of roundleaf buffaloberry. Seeds of silver buffaloberry can be germinated successfully in a peat-based substrate. Because of the high seed dormancy noted in this study, future research should investigate improved methods for release of seed dormancy in these species.

Introduction

Many municipalities in the arid western United States restrict how water is used in landscapes because of population growth and limited water supplies. There also is a growing desire among residents to more closely match their landscaping style to the natural beauty of the region. Recent surveys of both the Utah and Colorado green industries revealed demand for drought-tolerant native plant species is on the rise (7, 15). The Colorado survey also revealed commercial growers lack needed information about cost-effective methods to propagate and produce many desirable native plants (15). This lack of research limits widespread use of native plants. Some plants of interest to the green industry but not readily available are roundleaf buffaloberry (*Shepherdia rotundifolia*) and silver buffaloberry (*Shepherdia argentea*) (15).

Roundleaf buffaloberry, a broadleaf evergreen shrub, is endemic to the Four-Corners region of the southwestern United States. It grows to 2 m (6 ft) high and 1–4 m (3–12 ft) wide, and its round downward-cupped leaves have a grayish-green cast and are pubescent on their undersides (1, 2). Silver buffaloberry, a deciduous shrub, grows naturally in riparian areas, but also can be found in drier locations, especially in the Great Plains (1). It can grow to 6 m (18 ft) high and 3 m (10 ft) wide, and has narrow grey-green leaves (1, 6). Characteristics common to these two species include their natural occurrence in alkaline soils and relative drought-tolerance once established (2, 12, 17). Both species have the capacity to utilize atmospheric nitrogen (N_2) by way of a beneficial relationship formed with *Frankia* Brunchorst soil bacteria (12). Plants having the capacity to form this symbiotic relationship are valuable in soil restoration and soil improvement, and they add plant-available nitrogen (N) to the soil, potentially benefiting neighboring plants (8, 12, 13). These qualities make the species valuable to communities in the West, where they could be used in low-water ornamental landscapes and survive with minimal inputs of N.

Some information is available on breaking dormancy and germinating seeds of these shrub species for habitat restoration. Zeidler and Justin (19) developed a protocol for seed germination of roundleaf buffaloberry, which involves sowing seeds outdoors in a thin layer of sand on mulched beds. The seeds are cold-stratified over winter and germinate in place in the spring. Similar techniques have been suggested for silver buffaloberry (1, 6); however, sowing seeds outdoors is impractical for commercial ornamental production in which it is crucial economically for maximum seed germination to occur over a relatively short time.

Little attention has been given to characteristics of growing substrates that can maximize seed germination of species native to arid regions. It has been suggested that substrates used to produce plants be formulated to a plant's growing requirements (9); however, most commercial substrates are designed to have organic matter content and water-holding characteristics optimal for production of conventional landscape plants adapted to wetter environments. These characteristics may be detrimental to seed germination of species that are endemic to arid climates with rocky or sandy soils low in organic matter. Some Intermountain West growers formulate and use their own growing substrates in an effort to improve seed germination and growth of native plant species, but these substrates have not been tested against conventional substrates for their efficacy. Therefore, the objective of this research was to determine germination success of the underused western native species, roundleaf buffaloberry and silver buffaloberry, in substrates with different water-holding properties under a controlled light, temperature, and irrigation regimen.

Materials and Methods

Seed germination. Seeds of silver buffaloberry were purchased from a commercial vendor (Granite Seed, Lehi, UT). Seeds of roundleaf buffaloberry were harvested from plants located 20 km (10 mi) west of Escalante, UT, in August 2005. Seeds were stored in a cloth sack in a room that is maintained at 22C (72F) and less than 30% relative humidity. On April 23, 2006, seeds of the two species were placed into cold-stratification (3C/37F) for 16 weeks in sealed 100 × 100 mm (4 × 0.4 in) plastic petri dishes between two Whatman™ #3 90-mm (3.5-in) filter papers (Whatman Inc., Piscataway, NJ), moistened with 2 ml (0.2 tsp) distilled water. On August 13, 2006, 48 stratified seeds of each of the two species were sown into 20.3 × 40.6 × 5 cm (8 × 16 × 2 in) seed flats, each filled with one of three substrates. Seeds of silver buffaloberry [3-mm (0.12-in) diameter] and roundleaf buffaloberry [6-mm (0.25-in) diameter] were sown to a depth of 0.64 cm (0.25 in) and 1.3 cm (0.5 in) deep, respectively. Seed flats were held on a greenhouse bench where 16-h photoperiods were provided by using 400-W, high-pressure sodium lamps. Each flat contained 16 seeds of each species, and there were three flats of each substrate, for a total of nine flats. Each set of 16 seeds in each flat was an experimental unit ($n = 3$). The experiment was arranged in a completely randomized design.

Seedling emergence was recorded daily for 40 days. Flats were hand-watered twice daily with tap water to saturation and allowed to drain to container capacity in order to keep substrates consistently moist. Mean day/night greenhouse temperatures during the experiment were 25.5/20C (78/68F), with an overall mean temperature of 22C (72F). Mean relative humidity was 48%, and mean daytime photosynthetically active radiation during the experiment was 646 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Environmental conditions were recorded by a Watchdog™ 2475 weather station (Spectrum Technologies, Inc., Plainfield, IL) mounted 1.4 m (4.5 ft) above the ground and 0.5 m (1.6 ft) from seed flats. After 40 days, a tetrazolium (TZ) test was performed on nongerminated seeds recovered from the substrates to ensure homogeneity of distribution of viable, nondormant seeds across flats. Tetrazolium testing involved nicking each seed with a razor blade to permit imbibition, and soaking the seed for 24 hr at 30C (86F) in 0.1% TZ. At

Table 1. Rate and total seed germination of roundleaf buffaloberry and silver buffaloberry in three substrates. Stratified seeds were sown August 13, 2006, and germination was recorded daily for 40 days ($n = 3$).

Substrate	Roundleaf buffaloberry			Silver buffaloberry		
	T_{30-90}^x	T_{50}^y	Total germination ^z (%)	T_{30-90}	T_{50}	Total germination (%)
Germination mix	18	16	12.7b ^w	12	13	53.7a
Native mix	25	4	21.8b	28	13	50.1a
Calcined clay	23	18	66.2a	34	5	42.3a

^xNumber of days from 30–90% of the measured germination rate.

^yNumber of days to reach 50% of the measured germination rate.

^zGermination is calculated as a percentage of viable, nondormant seeds.

^wMean separation within the germination columns by Fisher's LSD at $P < 0.05$.

the end of 24 hr, seeds were rinsed and cut in a manner that allowed visualization of the stained embryo using a dissecting microscope (10).

Substrates. The substrates used were Turface MVPTM (Profile Products, Buffalo Grove, IL), a calcined montmorillonite clay product (hereafter referred to as 'calcined clay'), SunshineTM Mix #3 (Sun Gro Hort., Bellevue, WA) (hereafter referred to as 'germination mix') and a self-prepared, locally popular substrate (hereafter referred to as 'native mix') containing sphagnum peat:perlite:calcined clay:sand (2:2:1:1 by vol). Total porosity, aeration porosity, and water-holding porosity were calculated for each substrate using methods easily duplicated by the nursery industry (3, 18). Substrate characteristics were measured using plastic 500-ml (1-pt) water bottles with the bottom removed so the new bottle volume was 400 ml (0.7 pt) and the height was 12 cm (4.7 in). The bottle was inverted, and a hose-end mesh filter was placed above the spout of the inverted bottle to prevent substrate from escaping from the bottle when the lid was removed. The bottles were filled with dry substrate, and water was poured slowly over the substrate until it was saturated. The amount of water poured into the column was recorded and represented substrate pore volume. Total porosity was determined as a percentage using the following equation: [(substrate pore volume / container volume) $\times$ 100]. Aeration porosity was obtained by covering the cut top of the bottle with aluminum foil to prevent water evaporation, and carefully removing the lid from the bottom of the inverted water bottle to allow the water to drain from the saturated substrate into a container under the column until the substrate no longer dripped water and had reached container capacity. The volume of water that drained from the substrate represented aeration pore volume. Aeration porosity also was determined as a percentage and was calculated using the following equation: [(aeration pore volume / container volume) $\times$ 100]. Water-holding porosity was determined using the following equation: [(total porosity – aeration porosity)]. Substrate measurements were performed three times to insure accuracy of technique. The height of the container after removing the bottom was 7 cm (3 in) greater than the height of the flats into which seeds were sowed. This is important to note because the ratio of air space to water changes with container height due to changes in moisture tension (3).

Statistics. Statistical analysis was performed using the SAS/STAT[®] software version 9.1 (2002–2003), using the general linear models procedure of SAS. Mean separation

was performed using Fisher's least significant difference (LSD), and Levene's test was used for evaluation of homogeneity of variance. Correlation between parameters was determined using the PROC CORR procedure of SAS.

Results and Discussion

Total seed germination of roundleaf buffaloberry was at least three times higher in calcined clay than in either of the other two substrates (Table 1). Total seed germination of silver buffaloberry was not different among the three substrates ($P = 0.8363$). Total germination of silver buffaloberry seeds was not different from that of roundleaf buffaloberry seeds (Table 2). Tetrazolium tests of nongerminated recovered seeds revealed that seeds of each species were of similar viability (Table 2). Levene's test revealed homogeneity of distribution across substrates among dormant, nonviable, and empty seeds of the two species (silver buffaloberry $P = 0.2433$, $P = 0.0787$ and $P = 0.1307$, respectively; roundleaf buffaloberry $P = 0.1301$, $P = 0.7026$ and $P = 0.5222$, respectively).

Seed germination of roundleaf buffaloberry may have been optimal in calcined clay because of the pore-size distribution of this substrate. Due to the relatively large particle size [between 1 and 2 mm (0.04 and 0.08 in)] of calcined clay, water is held in capillary pore spaces inside the particles, leaving noncapillary pore spaces between particles largely filled with air (9). This results in excellent drainage of water from the substrate. Such a well-drained substrate would help buffer roundleaf buffaloberry seeds from the effects of frequent irrigation. Water held within the capillary pore spaces, while not directly in contact with seeds, creates a 100% humidity environment for seed germination. These conditions, in which optimal seed germination of this species occurred, are similar to those to which seeds of roundleaf buffaloberry

Table 2. Results of tetrazolium testing of nongerminated seeds recovered from each seed flat ($n = 3$).

Species	Seed quality			
	Germination (%) ^x	Dormant (%)	Nonviable (%)	Empty (%)
Silver buffaloberry	44.0a ^y	21.5a	1.4a	32.6a
Roundleaf buffaloberry	25.7a	31.2a	13.2a	30.0a

^xGermination is expressed as a percentage of all seeds of each species (a total of 48 seeds per species).

^yMean separation within columns by Fisher's LSD at $P < 0.05$.

would be exposed in its native habitat. Fairchild and Brotherson (5) analyzed soil samples from several locations where roundleaf buffaloberry is native, and found average content of sand, silt, and clay was 83, 12, and 5%, respectively. The high sand content suggests the soil was well drained. They did not analyze soil for organic matter content. In earlier work, we obtained soil samples from beneath roundleaf buffaloberry growing in its native habitat in southern Utah. Analysis of our samples indicated it was a sandy loam with an organic matter content of 1.3%.

Organic matter is sometimes added to potting substrates to increase water-holding porosity (9). Drzal et al. (4) studied characteristics of various substrates and reported a peat mix of sphagnum peat:horticultral grade vermiculite (1:1 by vol) contained particles sizes ranging from 0.5 to 2 mm (0.02 to 0.08 in). They reported particles of this size distribution created a substrate with a high water-holding porosity, and the pores within this peat mix were capable of storing water that was readily available for uptake by plants or seeds. Similarly, our test of substrate properties revealed germination mix, a peat-based substrate, had the highest total porosity, the greatest proportion of which was due to water-holding porosity (Table 3). There was a significant negative correlation between germination of roundleaf buffaloberry seeds and substrate water-holding porosity ($r^2 = -0.72$; $P = 0.0301$) (data not presented), and germination of roundleaf buffaloberry seeds was low in the two substrates that contained high amounts of organic matter (Tables 1 and 3). Analysis of substrate properties showed native mix had a lower water-holding porosity than germination mix, and it had the highest aeration porosity of the three substrates. Germination of roundleaf buffaloberry seeds in native mix, however, was less than one third of that in calcined clay. It was also observed, in earlier tests of substrate properties, that component separation (mostly settling of sand) occurred when water was applied too quickly to native mix during measurement of substrate properties. This created a perched water table in the columns that would impede water drainage, and this substrate potentially could have acted similarly in seed flats. This could have adversely affected germination of roundleaf buffaloberry seeds in this substrate by holding available water in direct contact with seeds.

The organic matter content and, therefore, the pore-size distribution of native mix, germination mix (29 and 71%

organic matter, respectively; Table 3), and most other conventional potting mixes are out of the range of organic matter content to which many plants endemic to intermountain western soils are exposed. In general, soils in the arid Intermountain West are characterized by low organic matter content, in the range of 1–3% (G. Cardon, personal communication). It may be that seeds of roundleaf buffaloberry are sensitive to high water-holding porosity and require the excellent drainage provided by a substrate such as calcined clay.

Data herein demonstrate that silver buffaloberry can be readily germinated in all three substrates (Table 1). This suggests that this species is relatively insensitive to substrate water-holding properties. Indeed, Canadian researchers were able to produce more than 16,000 seedlings of silver buffaloberry in a peat-based substrate [sphagnum peat:sand (2:1 by vol)] in one year for use in land reclamation (14). Successful commercial propagation also is possible in an outdoor setting for bare-root production of silver buffaloberry (6). Given its occurrence mostly in riparian habitats, silver buffaloberry is likely adapted to soils higher in organic matter with increased water-holding porosities, although growth and survival is possible in drier soils (1).

Other researchers have similarly found that production of native species can be enhanced by providing conditions that mimic native habitats. For instance, a study of several native and exotic plants growing in southern California salt marshes found seed germination of species native to that habitat was sensitive to temporal variation in salinity and moisture, whereas exotic species were not (11). In a study with plains cottonwood (*Populus deltoides* Marshall subsp. *monilifera*), Shafroth et al. (16) examined environmental characteristics influencing seed germination of the species, and reported greatest germination occurred under moist conditions in direct sunlight, conditions that closely replicated those of its native habitat.

Decisions about which substrate to use for plant production are usually based on availability and cost of substrate components (9). However, for some high-value species that are resistant to conventional production methods, the cost of custom-prepared substrates with higher cost components may be offset by the potential increased expense of plant losses during production. Increased rate of germination is considered an advantage in seed propagation. In the present investigation, the time to reach 50% germination (T_{50}) was only 4 days for seeds of roundleaf buffaloberry in native mix (Table 1); however, more complete germination occurred in calcined clay, resulting in fewer seed losses. Seed availability of roundleaf buffaloberry varies from year to year, and current demand is high for plants of this species (15). Despite the current higher cost of calcined clay compared to more economical substrates (9), it may be to a grower's advantage to choose a calcined-clay-based substrate for propagation of roundleaf buffaloberry by seed when frequent irrigation is used. The T_{50} for germination of silver buffaloberry seed was only 5 days in calcined clay, but the time from 30 to 90% germination was only 12 days in germination mix, and completeness of germination was relatively high in all three substrates (Table 1). In the case of silver buffaloberry, a peat-based substrate may be most economical for propagation of this species.

Relatively high rates of seed dormancy were recorded in this study (22 to 32%), despite cold-moist stratification for 16 weeks prior to germination. We have noticed in previous

Table 3. Relative differences in pore-space distribution and organic matter content among the three substrates used for seed germination of roundleaf buffaloberry and silver buffaloberry (n = 3).

Substrate	Substrate properties ^a			
	Total porosity (%)	Aeration porosity (%)	Water-holding porosity (%)	Organic matter ^b (%)
Germination mix	71.3a ^c	16.3b	55.0a	71.0
Native mix	61.6b	19.3a	42.3b	29.0
Calcined clay	55.0c	15.5b	39.5c	0.0

^aCalculated at a column height of 12 cm (4.7 in).

^bOrganic matter contents are estimates based on the proportion of sphagnum peat in the germination substrate.

^cMean separation within the first three columns by Fisher's LSD at $P < 0.05$.

work with these species that seeds often germinate during the latter part of cold-stratification and, once sown, sometimes continue to germinate well beyond 40 days. It may be that the species have adapted to harsh, unpredictable moisture conditions by extending the period in which seeds are capable of germinating. Alternatively, seeds could be exhibiting double-dormancy, and may require release of both physical and physiological dormancy. In any case, more effective means for releasing seed dormancy in these species should be investigated.

In summary, seed germination of the high-value western native species, roundleaf buffaloberry, can be optimized by propagation in a calcined clay substrate. On the other hand, silver buffaloberry seed can be propagated economically in a peat-based substrate. Our results also provide insight into substrate properties that may optimize seed germination of other drought-tolerant western native plant species.

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Errata

It has come to our attention that the issue number was incorrectly listed in the footers of the June issue. The correct footer for each manuscript should have read:

J. Environ. Hort. 27(2):pp–pp. June 2009.

We apologize for any inconvenience or confusion resulting from this error.