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Nursery Production of Selected Actinorhizal Species

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NURSERY PRODUCTION OF SELECTED

ACTINORHIZAL SPECIES

by

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ABSTRACT

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An important aspect of sustainable landscaping includes utilization of plants requiring few to no inputs once installed. Limited research exists for many of these species. For this research, we chose four with potential for use: Mexican cliffrose (*Purshia mexicana*), silver buffaloberry (*Shepherdia argentea*), roundleaf buffaloberry (*Shepherdia rotundifolia*), and seaside alder (*Alnus maritima*). All are actinorhizal, meaning they form a symbiotic relationship with soil-borne *Frankia* bacteria that fix atmospheric N_2 for plant use. Many actinorhizal species are also native to arid environments where soils have low organic matter (OM) content. We suspect that these species are detrimentally impacted by traditional growing substrates high in OM due to OM effects on water-holding porosity characteristics. We sowed previously stratified seeds of roundleaf buffaloberry and silver buffaloberry in three substrates with varying amounts of OM and found that roundleaf buffaloberry germination rate was maximized in a calcined clay (66.2%) containing no OM and had low germination rates (12.7 - 21.8%) in the substrates containing OM. Silver buffaloberry germination rates varied from 42.3 to 53.7%, and germination rates were similar across substrates. This shows that substrate water-holding properties can impact germination of some species native to arid environments. Poor seed quality made the research concerning Mexican cliffrose inconclusive.

We also investigated effects of controlled release fertilizer (CRF) on nodule formation in seaside alder and specifically sought to determine if previously inoculated plants treated with CRF would form nodules, and what level of CRF maximizes nodule formation. These plants were topdressed with CRF at rates from 0-32g, while a group of uninoculated plants received the manufacturer prescribed rate of 6 g. We found that 2 g CRF maximized root nodule formation (26 nodules; 110 mg dry weight). Plant growth parameters and leaf N content of 2-g treated plants were similar ($P < 0.10$) to those of uninoculated plants receiving the prescribed rate of CRF.

(66 pages)

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CHAPTER 1

INTRODUCTION, PREVIOUS WORK, AND LITERATURE REVIEW

Until recently, I worked at a local garden center and was responsible for helping clientele with appropriate selections of plant material for their homes. After several years of doing this, I found that the plant palette of most customers was very limited because they were unwilling to purchase plants unless they had seen them in another yard. Conversely, it was very refreshing when other customers wanted unique plants that were not seen elsewhere. I would often ask these customers what had sparked their interest in the particular plant(s) they were looking for; many would reply that they just wanted something different, but others would say that they were also looking for plants that required less maintenance than the commonly available plants used in yards today.

At the garden center, I showed customers unique and adaptable species from many areas of the world that required fewer inputs as compared to more traditional plants. However, there are many additional plants native to the U.S. and to Utah specifically that would grow as well as or better than the commonly available landscape plants.

Unfortunately, many potentially promising native plants are not available to the public because most wholesale growers do not have sufficient information to grow them efficiently, even though there is an increasing demand for such natives. Researchers in Colorado found that there is increasing interest in native plants, but there is a lack of knowledge of how to produce these plants cost-effectively (Potts et al., 2002). The

Southern Nevada Water Authority concluded that using water-wise landscaping plants and techniques could save consumers one-third the cost of irrigation and maintenance as compared to traditional landscaping plants and techniques (Sovocool et al., 2006). Researchers at New Mexico State University also found that consumers are interested in plants that require fewer inputs, and that they strongly identify with the use of native plants in the landscape (Hurd and Smith, 2005).

To aid in the availability of these plants, I chose to focus on four underutilized plants that could be adapted for use in the Utah landscape. These are seaside alder [*Alnus maritima* (Marsh) Muhl. ex Nutt.], Mexican cliffrose [*Purshia mexicana* (D. Don) Henrickson], silver buffaloberry [*Shepherdia argentea* (D. Don) Pursh], and roundleaf buffaloberry (*S. rotundifolia* Parry). None are readily available in the nursery trade, and all are actinorhizal, that is, have the ability to utilize atmospheric N_2 via a beneficial relationship formed with *Frankia* bacteria that live in root nodules (Ekblad and Huss-Danell, 1995; Paschke, 1997). The bacteria form this relationship with plants classified in eight families of angiosperms, but do not require a host plant to survive in soil (Batzli et al., 2004; Clawson et al., 2004). In fact, *Frankia* is especially common in newly formed soils and in soils of marginal quality (Clawson et al,. 1997). Therefore, plants that utilize this symbiotic relationship are also able to colonize these same areas, making them valuable in soil restoration and soil improvement. Actinorhizal plants actually add usable N to the environment that other non-actinorhizal species can then utilize (Ekblad and Huss-Danell, 1995; Fairchild and Brotherson, 1980; Hurd et al., 2001; Paschke, 1997). These same qualities also make actinorhizal plants potentially valuable to the public, wherein they can be transplanted and survive, once established, with little to no support from landowners, potentially reducing pollution to the environment and costs to homeowners.

Seaside Alder

Seaside alder was recognized as a species as early as 1785, but it is very rare in nature and is native to only three small, disjunct areas of the United States: Georgia, Oklahoma, and the Delmarva Peninsula. It is considered threatened or endangered in those areas (Schrader et al., 2006; Stibolt et al., 1977). One reason cited by researchers as to why the species is so rare is due to shade intolerance of seedlings which are less able to compete with a sympatric *Alnus* species (Schrader et al., 2006). Due to seaside alders' rarity and natural beauty, efforts have been made to further its utilization by the nursery industry. It can be effectively propagated from both seed and softwood cuttings (Schrader and Graves, 2000). It also shows potential for use in wet areas of the landscape, and could be utilized as a large screen or hedge due to its glossy green leaves, dense foliage and eventual size of twenty feet high and twenty feet wide (USDA, 2007). Schrader and Graves (2004a) investigated its potential for use in cold climates and found it to be hardy to USDA zone 3a.

Even though seaside alder is endemic to wet soils, it can thrive in drier situations. Hennessey and others (1985, p. 139) found that *A. maritima* growth under moderate drought stress is no different when compared to an adequately watered control. However, the same experiment yielded different results for another alder species, *A. glutinosa* (black alder), wherein leaf area and height were "markedly curtailed" under moderate drought conditions. This is important because black alder is commonly used in the landscape trade and apparently is not drought tolerant when compared to *A. maritima*. Schrader and others (2005) found that seaside alder can grow in field soil with vast differences in water saturation in which moisture content of the test plot soil moisture varies from 10% by volume to 100% saturated.

A cultivar of *A. maritima* called 'September Sun' has been introduced into the nursery industry. It is faster growing, more densely foliated and symmetrical than other individual plants observed in the trialing process (Schrader and Graves, 2004b). This cultivar is available from some growers, but it has not yet found wide acceptance in the nursery industry.

In other efforts to further the use of seaside alder in the nursery trade, Laws and Graves (2005), sought to characterize how the relationship between it and *Frankia* can be optimized during the nursery production of the species. Wall and others (2000) found that nodulation can be controlled within different species of alder when they are treated with various forms of N-fertilizer in solution. Similarly, Laws and Graves (2005) treated plants with assorted concentrations of liquid NH_4NO_3,-a fertilizer very common in the nursery industry. They found that levels between 4-8 mM (112-224 ppm N) prevented nodulation, but that lower levels between 0.5 and 2 mM (14-56 ppm N) fostered nodulation and also produced healthy plants. The N levels that prevented nodulation are actually within the realm of recommendations made by many manufacturers of liquid fertilizer products. Additionally, in this situation, growers could actually reduce the amount of N-fertilizer used, thereby reducing costs to them and reducing potential N pollution to the environment.

Alders are considered a model plant in the area of actinorhizal research. They are relatively easy to grow under greenhouse conditions and readily nodulate when exposed to *Frankia* bacteria. Ekblad and Huss-Danell (1995), achieved a 96% nodulation rate in one of their experiments. Conversely, a demand has been shown for other actinorhizal species that can be much more difficult to produce (Potts et al., 2002).

Mexican Cliffrose

Mexican cliffrose is native to arid areas of the Intermountain West (IMW) of the United States and is heavily browsed by deer and other wildlife (Booth, 1980; Paschke, 1997). It can reach 20 feet tall, but usually stays much shorter. It is evergreen and naturally grows in full sun exposure, which is uncharacteristic of most nonnative broadleaved evergreens that are grown in the arid climate of the IMW. Mexican cliffrose has beautiful spring flowers similar to that of shrubby cinquefoil (*Potentilla fruticosa* L). It also withstands heavy pruning. I have observed plants at the USDA shrub lab in Ephraim, UT and at Utah State University campus in Logan, UT that have been sheared into almost perfect globes that were not visibly affected by this.

Most of the research that has been performed involving Mexican cliffrose has occurred in the field, with the idea that the species is primarily for wildlife use and habitat restoration and not for its potential in the landscape. With this in mind though, some field research can potentially be applied to commercial propagation of the species (Table 1-1), but much of it is not efficient for commercial growers to utilize.

Table 1-1. Published methods of breaking seed dormancy and propagation of *Shepherdia argentea, S. rotundifolia* and *Purshia mexicana*

Species	Method	Reference
Shepherdia argentea	• Stratification for 60-90 days[z].	Baskin and Baskin 2003
	• Cold-stratification at 5° C for 30-60 days.	Smith 1986
	• Moist chill at 3° C for 90 days, or soak in sulfuric acid for 20-30 min.	USDA 2007
	• Stratify seeds by sowing outdoors in the fall and allow germination in the spring[y].	Zeidler and Justin 2003b
Shepherdia rotundifolia	• 15-30 min sulfuric acid soak[x], 30-60 days cold stratification.	Borland 1994
	• Soak seeds in sulfuric acid[x] for 15-30 min or moist chilling for 30-60 days at 3° C.	USDA 2007
	• Stratify seeds by sowing outdoors in the fall and allowing them to germinate in the spring[y].	Zeidler and Justin 2003c
Purshia mexicana	• Cold moist stratification for 30 days[z].	Baskin and Baskin 2002
	• Cold-stratification for 28-30 days at 1-3° C or 1-3 hour soak in 1-3% H_2O_2.	USDA 2007
	• Stratification for 1-2 months[z].	Dreesen 2003
	• Soak seeds in 98% pure sulfuric acid and then soak for 18 hours in sterile distilled water.	Kohls and others 1994
	• Soak seeds overnight in constantly stirred distilled water, after soaking nick seeds and surface sterilize.	Kyle and Righetti 1996, 1988
	• Unhulled seeds were planted in a layer of sterile sand placed over soil.	Righetti and Munns 1979
	• Achieved over 90 % when seeds soaked in 0.2% KNO_3 solution and chilled for 10 weeks[z].	Stidham and others 1980
	• Seeds sown at a depth of 1 cm with a seed drill in the fall and then germinated in the spring[y].	Zeidler and Justin 2003a

[z] no specific information given on stratification temperature.
[y] primarily for bare-root production.
[x] no specific concentration given

Silver Buffaloberry

Silver buffaloberry is native to much of Canada and the western half of the United States. Like seaside alder, it naturally grows in riparian areas but also can be found in dryer locations, especially in the Great Plains (USDA, 2007). It can grow to 6 m (20 ft) high and 6 m wide, and has glossy, grey-green leaves that are shed in the fall. The species is browsed by several wildlife species, with the exception of deer (and cattle), and the berries are consumed by humans. Native Americans used them as an accompaniment to buffalo meat, and the berries can also be made into jelly (Smith, 1986).

A current use of silver buffaloberry includes habitat reclamation, but researchers note that it could be used as an ornamental plant, especially as a windbreak, as a hedge, and in traffic control due to thorns being present on the branches (Paschke, 1997; Smith, 1986; USDA, 2007). Smith (1986) notes that silver buffaloberry is cold-hardy in all zones. Paul and others (1971) sampled soil where silver buffaloberry is endemic and found the soil samples had a clay consistency with a pH of 7.5, which suggests that the species could be grown in the alkaline soils of Utah's urban landscape which have similarly harsh characteristics. Little research exists pertaining to propagation of this species by commercial growers. A sample of propagation information found within the literature can be found in Table 1-1.

Roundleaf Buffaloberry

Unlike other members of the *Shepherdia* genus, roundleaf buffaloberry is endemic to arid regions of southern Utah, northern Arizona, and northern New Mexico. It grows to 2 m (6 feet) high and 2-4 m (6-12 ft) wide, and its leaves are grayish-green. It is mostly evergreen in contrast to other members of the genus (Borland, 1994). This characteristic potentially makes *S. rotundifolia* a valuable landscape plant due to a lack of other broadleaved evergreen species that thrive in the harsh climates of the IMW. Roundleaf buffaloberry also thrives in areas with alkaline soil and can improve soils by accumulating the nutrients N and K in its root-zone (Fairchild and Brotherson, 1980).

Like Mexican cliffrose and silver buffaloberry, little research has been done pertaining to propagation of roundleaf buffaloberry, and most of what exists pertains to breaking seed dormancy and not germination in greenhouse settings. It is known that all buffaloberry species exhibit both a physiologically dormant embryo and physical dormancy due to a hard seed coat (USDA, 2007). Table 1-1 provides further information on what others have done to break dormancy of roundleaf buffaloberry.

Research Performed

With the demand for native species increasing and the lack of production information available to growers, we have chosen to focus on these four species to further their use within the nursery industry. We want our research to be directly applicable to commercial growers. To do so, we utilized products and production methods that those within the industry already use or could potentially use in their operations. For example, certain actinorhizal species may only form a symbiotic relationship with specific strains of *Frankia* bacteria, and soils in the natural environment do not always contain compatible forms for every species (Clawson et al., 1997). Because of this, if growers could produce previously nodulated plants using compatible *Frankia* in a nursery setting, they could potentially reduce costs to themselves and the public by being able to use reduced amounts of N-fertilizer. Reduced N-fertilizer use would also diminish potential environmental contamination.

To facilitate production of previously nodulated alders, we performed an experiment similar to one described by Laws and Graves (2005). However, we used a controlled release fertilizer (CRF) as a nutrient source due to its availability within the nursery industry and because it is uncertain how its use impacts nodulation in actinorhizal species. Many researchers have found that nutrient release from CRF increases as temperatures rise and also with heavy watering (Cabrera, 1997; Dou and Alva, 1998; Engelsjord et al., 1997; Huett and Gogel, 2000; Husby et al., 2003). We applied CRF to alders previously inoculated with *Frankia* bacteria at rates ranging from 0-32 g per plant. Plants were irrigated to container capacity twice daily for 45 days, and NO_3-N levels from the leachate were monitored every 14 days of the experiment. Plants treated with CRF at rates below the manufacturer's recommendation of 6 g per plant were both healthy and formed nodules, especially in the 2-g treatment.

Information about commercial production of roundleaf buffaloberry, silver buffaloberry, and Mexican cliffrose is also very limited. These plants are native to areas where soil organic matter levels are characteristically low. Because of this, we suspected that utilizing traditional growing substrates high in organic matter would be detrimental to germination in these species and others native to the arid West.

To determine if water-holding porosity was detrimental to germination of silver buffaloberry, roundleaf buffaloberry, and Mexican cliffrose, we placed seeds of each species in three different growing substrates: the first, a traditional germination mix high in organic matter (71% by volume); another, a locally popular self-blended mix common in the local native plants industry (29% organic matter by volume); and a calcined Montmorillonite clay product (0% organic matter). Roundleaf buffaloberry germination, was highest (above 60%) in the calcined clay, and much lower in the other two substrates. Silver buffaloberry germination varied from approximately 40 to 50% in all substrates; and Mexican cliffrose seed quality was of low quality, and germination was excessively low in all substrates.

Because roundleaf buffaloberry germinated at its highest levels in the calcined clay, we sought to further refine techniques involving breaking seed dormancy to maximize its germination when sown within calcined clay. To do so, we cold-stratified groups of roundleaf buffaloberry seed for 0, 4, 8, or 16 weeks. In addition to this, groups of seed were scarified for 10 min in sulfuric acid and then cold-stratified for 0, 4, or 8 weeks. Groups of seed were also scarified using boiling water and then stratified for 0, 4, or 8 weeks.

We found no statistical differences in germination among treatments, wherein germination varied from 0% in the 0 weeks stratification, and no scarification treatment to 16% in the treatment stratified for 8 weeks and heat-scarified. These germination rates were well below that experienced in the previous experiment. Tetrazolium (TZ) tests at experiment's end revealed upwards of 60% seed viability in some of the treatments. The seed used in this experiment was of a different lot than that used in our previous experiment. We are not certain as to why it germinated at such low rates. A certificate of seed quality was not included with our purchase from the vendor, and we were unable to check germination before the experiment due to a lack of available seed.

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CHAPTER 2

SEED GERMINATION OF *SHEPHERDIA ROTUNDIFOLIA* AND *SHEPHERDIA ARGENTEA* IN THREE SUBSTRATES[1]

Abstract

Many western native plant species occur in areas characterized by well-drained soils low in organic matter, and we suspect traditional growing substrates high in organic matter may impede their germination. Silver buffaloberry (*Shepherdia argentea* (Pursh) Nutt. [Elaeagnaceae]) and roundleaf buffaloberry (*Shepherdia rotundifolia* Parry [Elaeagnaceae]) seeds were sown in three substrates that differed in organic matter and drainage properties. Roundleaf buffaloberry exhibited greatest germination in a calcined montmorillonite clay substrate (66.2%); it had low germination rates in a commercial germination mix (12.7%) and a self-prepared locally popular mix (21.8%). Silver buffaloberry germination rates varied from 42.3 to 53.7%, but rates in different substrates were not different statistically. We conclude that a calcined clay can improve germination of roundleaf buffaloberry.

Significance to the 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, many entities have turned to regulating landscaping methods to minimize water loss. Many residents have also begun to identify with their surroundings and have started landscaping in ways that more closely match the local

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[1] Co-authored by Taun Beddis and Heidi A. Kratsch.

natural environment where inclusion of native drought-adapted plants is an important aspect.

However, many who desire to use native-adapted plants face a lack of availability due to growers' limited knowledge of methods to optimize germination of these species. We studied germination of two genetically related nitrogen-fixing, drought-adapted species, silver and roundleaf buffaloberry. Nitrogen-fixing species are adapted to low nutrient soils and add nitrogen to the environment. We planted seeds of both species in three substrates: a germination mix, a locally popular native plant mix, and a calcined montmorillonite clay product. Roundleaf buffaloberry germination was maximized in this calcined clay which contained no organic matter. The other two substrates, which had greater water holding capacities, negatively affected germination of this species.

Introduction

Many municipalities in the arid western United States have had to restrict how water is used in landscapes because of population growth and limited water supplies. There also is a growing desire by 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 found that demand for drought-tolerant native plant species is on the rise (9, 17). The Colorado survey also found that commercial growers lack needed information about cost-effective methods to propagate and produce many desirable native plants (17). This lack of research limits widespread use of native plants. Some plants of interest to the green industry that are not readily available are silver buffaloberry (*Shepherdia* *argentea* (Pursh) Nutt. [Elaeagnaceae]) and roundleaf buffaloberry (*Shepherdia rotundifolia* Parry [Elaeagnaceae]) (17).

Silver buffaloberry, a deciduous shrub that grows naturally in riparian areas, but it also can be found in drier locations, especially in the Great Plains (23). It can grow 6 m (20 ft) high and 6 m (20 ft) wide, and has glossy, grey-green leaves. Roundleaf buffaloberry is native to the Four-Corners area of the southwestern United States, grows to 2 m (6 ft) high and 2-4 m (6-12 ft) wide, and its round evergreen leaves have a grayish-green cast and are pubescent on their undersides (2, 23).

Characteristics common to these two species include their natural occurrence in alkaline soils and drought-tolerance once established (2, 14, 16, 20). Both species have the capacity to utilize atmospheric nitrogen by way of a beneficial relationship formed with *Frankia* soil bacteria (14). Plants that utilize this symbiotic relationship are valuable in soil restoration and soil improvement, and they add plant-available nitrogen to the soil, potentially benefiting neighboring plants (6, 7, 10, 14, 15). These qualities make the species valuable to communities in the West, where they could be used in low-water landscapes and survive with minimal inputs of water and nitrogen.

Some information is available on breaking dormancy and germinating seeds of these species for habitat restoration. Zeidler and John (24) developed protocols for seed germination of roundleaf buffaloberry by planting seeds in a thin layer of sand on mulched beds. Seeds are allowed to stratify over winter and naturally germinate in the spring. It has also been suggested by some that a sulfuric acid soak can break seed dormancy in this species. Similar techniques have also been suggested for silver buffaloberry (2, 8, 23). Many of these methods are impractical for commercial ornamental production, wherein it is economically crucial for maximum seed germination to occur over a relatively short time.

Little attention has also been given to characteristics of growing substrates that can maximize germination of species native to arid regions. It has been noted that substrates used to produce plants should be customized towards a particular plant's growing requirements (11). However, most commercial substrates are designed to have organic matter content and water-holding characteristics that are optimal for production of conventional landscape plants adapted to wetter environments. These characteristics may be detrimental to germination of species that are endemic to arid climates with rocky or sandy soils low in organic matter (7, 14). Some Intermountain West growers formulate and use their own growing substrates in an effort to improve germination and growth of native plant species, but these substrates have not been tested against conventional substrates for their efficacy. The purpose of our work was to determine germination success of these two underutilized western native species in substrates with diverse properties. Our results provide insight into substrate qualities that may optimize germination of drought-tolerant western native plant species.

Materials and Methods

Germination. Seeds of silver buffaloberry were purchased from a commercial vendor (Granite Seed, Lehi, Utah). Seeds of roundleaf buffaloberry were hand-collected from plants located 20 km (10 mi.) west of Escalante, UT on June 6 2005. On April 23 2006, seeds of the two species were placed into cold-stratification (3° C) for 16 weeks in sealed plastic 100 mm x 10 mm petri dishes between two #3-weight 90-mm filter papers, wetted with 2 ml distilled water. On Aug. 13 2006, 48 stratified seeds of each of the two species were sown into flats, each filled with 1 of 3 substrates. Each flat contained 16 seeds of each species, and there were 3 flats of each substrate, for a total of 9 flats. Each set of 16 seeds in each flat was an experimental unit (N = 3).

Germination was monitored daily for 40 days. Flats were hand-watered twice daily to saturation and then allowed to drain to field capacity. Greenhouse temperatures during the experiment averaged 25.5° C day/20° C night, with an overall mean temperature of 22° C. Mean daytime photosynthetically active radiation during the experiment was 646 $\mu mol \cdot m^{-2} \cdot s^{-1}$. After 40 days, a tetrazolium (TZ) test was performed on ungerminated seeds recovered from the substrates to ensure homogeneity of distribution of viable nondormant seeds. Tetrazolium testing involved nicking each seed with a razor blade and then soaking the seed for 24 hours at 40 ° C in a 0.1% TZ. At the end of 24 hours, seeds were cut in a manner so that staining could be visualized (12).

Substrates. We used Turface MVP™ (Profile Products, Buffalo Grove, IL) a calcined montmorillonite clay product (hereafter referred to as ‘calcined clay’), Sunshine Mix #3 (hereafter referred to as ‘germination mix’) (Sun Gro Horticulture, Bellevue, WA) and a self-prepared, locally popular substrate (hereafter referred to as ‘native mix’) containing 2 peat moss : 2 perlite : 1 calcined clay : 1 sand (by volume).

We calculated total porosity, aeration porosity, and water-holding porosity for each substrate using methods easily duplicated by the nursery industry (4, 21). Substrate characteristics were measured by using plastic 500-ml water bottles with the bottom cut off so the new bottle volume was 400 ml. 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) × 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 field 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) ×100]. Water-holding porosity was determined by using the following equation: [(total porosity – aeration porosity)]. Substrate measurements were replicated three times to insure accuracy of technique.

Statistics. Statistical analysis was performed using the SAS/STAT software version 9.1 (18), using the general linear models procedure of SAS. Means separation was performed using Fisher's least significant difference (LSD) test, and Levene's test was used for evaluation of homogeneity of variance. Correlation between parameters was measured using the PROC CORR procedure of SAS.

Results

Germination of roundleaf buffaloberry was at least three times higher in calcined clay than in either of the other two substrates (Table 2-1). A negative correlation was found between germination of seed of this species and substrate water-holding porosity ($r^2 = -0.72$, $P = 0.0301$). Silver buffaloberry seed had the highest rate of germination of the two species over all substrates (Table 2-2), and germination was not different statistically among the three substrates ($P = 0.8363$) (Table 2-1).

Table 2-1. Uniformity of germination and germination success in three substrates

Substrate	Silver Buffaloberry			Roundleaf Buffaloberry		
	$T_{30\text{-}90}$[z]	**T_{50}[y]**	**Germination %**	**$T_{30\text{-}90}$**	**T_{50}**	**Germination %**
Germination Mix	12	13	53.7 a[x]	18	16	12.7 b
Native Mix	28	13	50.1 a	25	4	21.8 b
Calcined Clay	34	5	42.3 a	23	18	66.2 a

[z] Number of days from 30-90% of the measured germination rate
[y] Number of days to reach 50% of the measured germination rate
[x] Means within the germination columns followed by the same letter are not statistically different at the $P < 0.05$ according to Fisher's least significant difference test. Germination is calculated as a percentage of viable seed.

Table 2-2. Results of tetrazolium testing

Species	Seed Quality			
	Germination (%)[z]	**Dormant (%)**	**Non-Viable (%)**	**Empty (%)**
Silver buffaloberry	44.0 a[y]	21.5 a	1.4 a	32.6 a
Roundleaf buffaloberry	25.7 a	31.2 a	13.2 a	30.0 a

[z] Germination is expressed as a percentage of all seed of each species.
[y] Means within the germination columns followed by the same letter are not statistically different at the $P < 0.05$ according to Fisher's least significant difference test.

Tests of substrate properties revealed that germination mix had the greatest total porosity; its total porosity was 16% greater than the total porosity of native mix and 30% greater than the total porosity of calcined clay (Table 2-3). Water holding porosity was also greatest in germination mix. Aeration porosity was greater in the native mix as compared to the other substrates. Organic matter content was greatest in the germination mix and more than double that of the native mix. The calcined clay contained no organic matter. Levene's test for homogeneity of variance revealed homogeneity of distribution across substrate samples for measurements of total porosity, aeration porosity, and water-holding porosity (P = 0.460, P = 0.1873, P = 0.1323, respectively). Organic matter content was obtained from manufacturers' ingredient lists.

Tetrazolium tests of recovered seed revealed that seed lots of each species were of similar quality (Table 2-2). Levene's test of homogeneity of variance revealed homogeneity of distribution across substrates among dormant, nonviable, and empty

Table 2-3. Substrate properties

	Substrate Properties			
Growing substrate	**Total porosity (%)**	**Aeration porosity (%)**	**Water-holding porosity (%)**	**Organic Matter ($g \cdot kg^{-1}$)**
Native Mix	61.6	19.3	42.3	290.0
Germination Mix	71.3	16.3	55.0	710.0
Calcined Clay	55.0	15.5	39.5	0.0

seeds of each 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).

Discussion

Our work shows that substrate properties can affect germination of roundleaf buffaloberry, and that commercial production of silver buffaloberry is possible with multiple substrate types.

The reason roundleaf buffaloberry germination was maximized in the calcined clay is most likely due to the nature of how water is held within the substrate at field capacity. Due to the relatively large particle size (between 1 and 2 mm), water contained within in the calcined clay is held in capillary pore spaces inside particles themselves leaving noncapillary pore space largely filled by air (11, 22). This resulted in a situation that allowed for excellent drainage of water from the substrate, while water held within the calcined clay particles created a 100% humidity atmosphere for seed germination. These conditions, in which maximal germination of roundleaf buffaloberry occurred, are similar to those that roundleaf buffaloberry seed would be exposed to in its native environment. Fairchild and Brotherson (7) analyzed soil samples from several locations where roundleaf buffaloberry is native, and found that average content of sand, silt, and clay was 83, 12, and 5%, respectively. The high sand content suggests that the soil was well drained. No organic matter is mentioned in their analysis, and the authors state that soil composition varied little between locations. We sampled soil from beneath roundleaf buffaloberry growing in its native habit and found that it was a sandy loam with organic matter content at 13 $g \cdot kg^{-1}$. Soils in the arid west are characterized by low organic matter content (7), in the range of 10-30 $g \cdot kg^{-1}$. We realize that the organic matter contents of native mix and germination mix are out of the range of physiological relevance for plants endemic to western soils.

Others 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 introduced plants growing in southern California salt marshes found that germination of species native to that habitat was optimized when variable conditions from the natural environment were met under artificial growing conditions (13). In an experiment involving plains cottonwood (*Populus deltoides* Bartr. Ex. Marsh [Salicaceae]), Shafroth and others (19), examined environmental characteristics that affected germination of the species and showed that greatest germination rates occurred under moist conditions in direct sunlight, conditions that closely replicate those of their natural habitat. Brumback and others (3) found that Robbins' cinquefoil (*Potentilla robbinsiana* ex. Rydb. [Rosaceae]) produced within a greenhouse environment experienced high mortality rates, but once surviving plants were transplanted back into their native environment, a 90% survival rate occurred after the first year of transplanting.

When comparing the water holding porosities of the substrates, it must be taken into account how the incorporated sphagnum peat moss in the native mix and germination mix impacted water availability within the substrate. These substrates contain 33 to 75% peat moss (by volume), creating a situation in which more liquid water comes in direct contact with the seed. Drzal and others (5) studied characteristics of various substrate components and found the particle size of their peat moss varied from 0.5 to 2 mm. They also found that particles of this size creates a substrate with a high water holding capacity, and the pores within this grade of peat moss are capable of storing water that is readily accessible to plants. Argo (1) also states that peat moss can retain water that is easily accessible to plants as compared to other substrates.

In addition, we noted that ingredient separation occurred when water was poured over the native mix too quickly during measurement of substrate properties. This created a perched water table in the columns and could potentially do the same in nursery containers. This could adversely affect germination of many species by decreasing drainage to a point that seeds could rot. We have noticed that buffaloberry seeds are prone to fungal infestation when cold-stratified under very wet conditions (data not shown).

Our findings that silver buffaloberry can be readily germinated in multiple substrates suggests that this species is relatively insensitive to substrate properties. Indeed, Canadian researchers were able to grow over 16,000 units to an outplantable size in one year for the purpose of land reclamation (16). Commercial propagation is also possible in an outdoor setting for the purposes of bare-root production (8).

Conclusions

Our research has shown germination of roundleaf buffaloberry can be maximized in a substrate low in organic matter and with good drainage properties. Because of the shortage of drought-adapted native shrubs in the industry (17), we recommend further study of conditions that enhance production of these species for use in water-conserving landscapes.

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CHAPTER 3

NODULATION OF SEASIDE ALDER TOPDRESSED WITH *CONTROLLED RELEASE FER*TILIZER[2]

Abstract. *Alnus maritima* (Marsh.) Muhl. ex Nutt. (seaside alder) is a rare species adapt*ed for ornamental use.* It can be produced easily by commercial growers, and when soil-inoculated in nursery containers, will form root nodules containing nitrogen (N_2)-fixing *Frankia* bacteria. Nodulated N_2-fixing plants may better adapt to landscape situations as a result of their enhanced ability to grow in N-deficient soils. It has been demonstrated that nodulation within this species can be controlled by the amount of N plants receive from a liquid source. However, controlled release fertilizers (CRF) also are commonly used by commercial growers, and nutrient-release rates from these products can be highly variable. Our objectives were to determine if greenhouse-grown inoculated plants treated with CRF will form nodules, and what level of CRF maximizes nodule formation while sustaining acceptable plant health and growth. Inoculated containerized plants were topdressed with CRF at 0, 0.5, 1, 2, 4, 8, 16, and 32 g. A group of uninoculated plants received the manufacturer's prescribed rate of 6 g CRF. We found that treatment with 2 g CRF maximized root nodule formation (26 nodules; 110 mg dry weight). Plant growth parameters and leaf N content of 2-g treated plants were similar ($P < 0.10$) to those of uninoculated plants receiving the prescribed rate of CRF. We conclude that inoculated CRF-treated plants of seaside alder can form nodules, and that these plants maintain acceptable growth and N-status at CRF levels lower than that prescribed.

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[2] Co-authored by Taun Beddis and Heidi A. Kratsch.

Introduction

Seaside alder is a threatened species native to the United States in Georgia, Oklahoma, the Delmarva Peninsula in Delaware, and Maryland (Schrader et al., 2005). Efforts have been made to encourage its use by the nursery industry because it has been found to be adapted to landscape use (Kratsch, 2008; Schrader and Graves, 2004) and can be propagated readily using both sexual and vegetative means (Schrader and Graves, 2000). *Alnus maritima* has the ability to fix atmospheric N_2 by way of a relationship with *Frankia* bacteria colonizing root nodules (Hennessey et al., 1985). Such N_2-fixing species have the capacity to survive in N-poor soils in the wild (Huguet et al., 2001; Paschke, 1997) and in cultivated situations (Hansen and Dawson, 1982) where they add significant N to the soil environment. This capacity makes them useful in the home landscape where the use of N-fertilizer can be reduced.

It has been demonstrated with various species of alder that nodulation can be limited when plants receive high concentrations of N (Berry and Torrey, 1985; Gentili and Huss-Danell, 2003; Kohls and Baker, 1989; Martin et al., 2004). Laws and Graves (2005) investigated the dynamics of this response in seaside alder and demonstrated that N-induced inhibition of nodulation is reversible.

No literature exists on effects of CRF on nodule formation, and CRF are widely used in the green industry. We performed an experiment to determine whether N contained in CRF is inhibiting to nodulation in seaside alder. Our objectives were to determine if greenhouse-grown inoculated plants treated with CRF would form nodules, and what level of CRF maximizes nodule formation while sustaining acceptable plant health and growth.

Materials and Methods

Seeds of *A. maritima* from a population in Oklahoma were cold-stratified at 4°C for 8 weeks and germinated in a flat (height = 6 cm, width = 27 cm, length = 53 cm) filled with germination mix (Sun Gro Horticulture, Bellevue, WA) on a greenhouse misting bench. Seedlings were transplanted into round plastic pots (height = 12 cm, volume = 235 cm^3) containing an unpasteurized growing substrates composed of 1 Sphagnum peat moss : 1 perlite (by volume). Plants were watered daily to field capacity and treated three times weekly with Peters Cal-Mag™ fertilizer (15N–2.9P–12.5K) (Scotts Sierra Horticultural Products, Marysville, OH) adjusted to pH 5.5 and containing 21 mM N to prevent nodulation.

On 1 Aug 2007, fertilization was halted. Forty-four three-month-old seedlings of consistent size were selected and the growing substrate was washed from the roots to confirm that roots had not formed nodules. The seedlings were transplanted into square plastic pots (height = 13 cm, volume = 2200 cm^3) containing the same growing substrate as used previously.

On 14 Aug 2007, seedlings were assigned randomly to one of eleven groups, and each plant was treated as an experimental unit (n = 4). Plants were arranged on a greenhouse bench in a randomized complete block design. Group one was destructively harvested at the start of the experiment for baseline measurements of leaf area and leaf, shoot, and root dry weights. Each of nine groups was topdressed with either 0, 0.5, 1, 2, 4, 6, 8, 16, or 32 g Osmocote Plus™ (15N–3.9P–10K) (Scotts Sierra Horticultural Products, Marysville, OH) (CRF) formulated to have a 3-4 month release-rate (and containing micronutrients Mg, S, B, Cu, Fe, chelated Fe, Mo, and Zn). Group 11 received no CRF, but was fertigated twice daily with 250 ml house nutrient solution modified to contain 8 mM NH_4NO_3 and adjusted to a pH of 5.5 (Bugbee, 2004). Group 12 remained uninoculated and unfertilized and was used to confirm that uninoculated plants did not form nodules. No further testing or analysis was performed using these plants. All treatments, with the exception of plants receiving 6 g CRF and group 12, were inoculated at the base of the stem with 30 g field soil from under roots of *Alnus incana* (L.) Moench ssp. *tenuifolia* (Nutt.) Breitung (thinleaf alder) located in Logan Canyon, UT at the Utah State University Forestry field station. The manufacturer's recommended application rate for the 6-in. nursery pots used in our research is 6 g CRF; this 6-g CRF uninoculated treatment was used for comparison of growth of inoculated plants with that of uninoculated plants fertilized at the recommended rate.

For the duration of the experiment, all plants except those receiving liquid NH_4NO_3 were irrigated twice daily with approximately 250 ml tap water (pH 7.3) by using an automated drip system to maintain the growing substrate near container capacity. Nitrate-N concentrations of leachate were measured on days 1, 14, 28, and 42 of the experiment on all repetitions from the 0, 2, 6, 8, 32 g and liquid NH_4NO_3 treatments by using an NO_3-N colorimeter test kit (#3649 SC; Lamotte Company, Chestertown, MD) . Leachate was collected from substrate by suspending plants in their pots above empty collection dishes just before the second daily irrigation. Approximately 50 ml leachate was collected per sample.

Greenhouse temperatures during the experiment were 25.5° C day/20° C night, with an overall mean temperature of 22° C. Average temperature of substrates in pots was 27º C day /22º C night. Mean photosynthetically active radiation during the experiment was 646 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

All plants were destructively harvested after 45 days, and leaf area was measured along with dry weights of leaves, shoots, roots and nodules. Leaf N content was determined by using the Kjehdahl method (Bradstreet, 1965).

Statistical analysis was performed using SAS/STAT software version 9.1 (SAS Institute Inc. Cary, NC) and the general linear models procedure. Means separation was performed using Fisher's least significant difference (LSD) test. Levene's test was used for determining homogeneity of variances. Based on these results, a cubed-root transformation was used to achieve homogeneity of variance on nodule and root dry weights, and nodule count for further analysis. Analysis of NO_3-N contained in leachate was performed using Proc Mixed repeated measures analysis (SAS Institute Inc. Cary NC, 2006). Repeated measures analysis showed an interaction effect of a week; therefore, analyses were performed on each week separately. Regression analyses were used to test linear and polynomial effects of fertilizer level on the dependent variables. Regression analyses were also used to determine approximately when NO_3-N would have been depleted from the leachate of CRF treated plants. Measurements from the first test date were excluded to allow nutrient release from the CRF to normalize.

Results

After 45 days, mean growth measurements of inoculated plants that received 2-g CRF were significantly different from those of plants at initial harvest but not different from those of uninoculated plants that received 6 g CRF (Table 3-1). Plants that received less than 2 g CRF had not grown significantly during that time. Mean leaf nitrogen content and leaf area also were similar between the two CRF treatments. Mean nodule count and dry weight were greatest in plants that received 2 g CRF. Mean nodule count of plants that received 2g CRF was nearly double that of plants receiving the next highest level of CRF, although differences from plants in other treatments that formed nodules were not significant (Table 3-1). Nodule dry weight for the 2-g treatment was 20 mg greater than for plants in the 1-g treatment and more than double that of any other nodulated plants.

Mean nodule count and nodule dry weight sharply decreased in plants that received more than 2 g CRF, and nodule formation was inhibited at the 8-g-CRF level. No differences existed in root-to-shoot ratios between plants in any treatment and initial measurements taken from plants destructively harvested at the beginning of the experiment ($P < 0.05$) (data not shown). Quadratic regression functions best describe the influence of CRF level on nodule dry weight (Fig. 3-1A) and nodule count (Fig. 3-1B), and they show that these parameters were maximal in plants that received 2-g CRF. A quadratic regression function also best describes the influence of CRF level on leaf N content, with maximal leaf N concentration at 16 g CRF (Fig. 3-1C). Note that the mean leaf N content of the 2

Table 3-1. Means separation of parameters used to evaluate effects of various fertilizer amounts on seaside alder plants grown for 45 days in a greenhouse.

		Nodule		Leaf			
Treatment	**Whole plant dry weight (g)**	**Count**	**Dry Weight (mg)**	**Nitrogen Content (%)**	**Area (cm^2)**	**Shoot Dry Weight (g)**	**Root Dry Weight (g)**
Initial Harvest	15.15 c[y]	0.00 c	0.00 b	2.03 bcd	1094.56 c	9.37 d	5.78 cd
0.0 g CRF[z]	24.11 abc	0.33 abc	30.10 ab	1.61 e	1033.74 c	12.14 bcd	11.97 ab
0.5 g CRF	20.11 bc	12.00 ab	40.20 a	1.71 de	948.83 c	12.13 bcd	7.99 abcd
1.0 g CRF	22.97 bc	14.00 ab	81.90 a	1.72 cde	1301.60 abc	15.19 bcd	7.79 abcd
2.0 g CRF	28.35 ab	26.30 a	103.10 a	2.13 bc	1177.52 abc	16.02 abc	12.33 ab
4.0 g CRF	14.49 c	11.50 abc	6.70 ab	2.00 bcde	1239.33 abc	9.55 d	4.95 d
8.0 g CRF	29.03 ab	0.00 c	0.00 b	2.20 b	1783.86 ab	18.17 ab	10.86 abc
16.0 g CRF	22.18 bc	0.00 c	0.00 b	2.30 b	1147.65 bc	15.15 bcd	7.03 bcd
32.0 g CRF	25.50 abc	0.00 c	0.00 b	2.40 b	1638.84 abc	16.86 ab	8.64 abcd
8 mM NH_4NO_3	25.11 abc	0.00 c	0.00 b	3.00 a	1868.35 a	18.32 ab	6.79 bcd
6.0 g CRF[x]	34.93 a	0.00 c	0.00 b	2.36 b	1826.60 ab	21.55 a	13.39 a

[z] CRF = controlled release fertilizer.
[y] Means within each column followed by the same letter are not statistically different at $P < 0.10$ according to Fisher's least significant difference test.
[x] Uninoculated treatment.

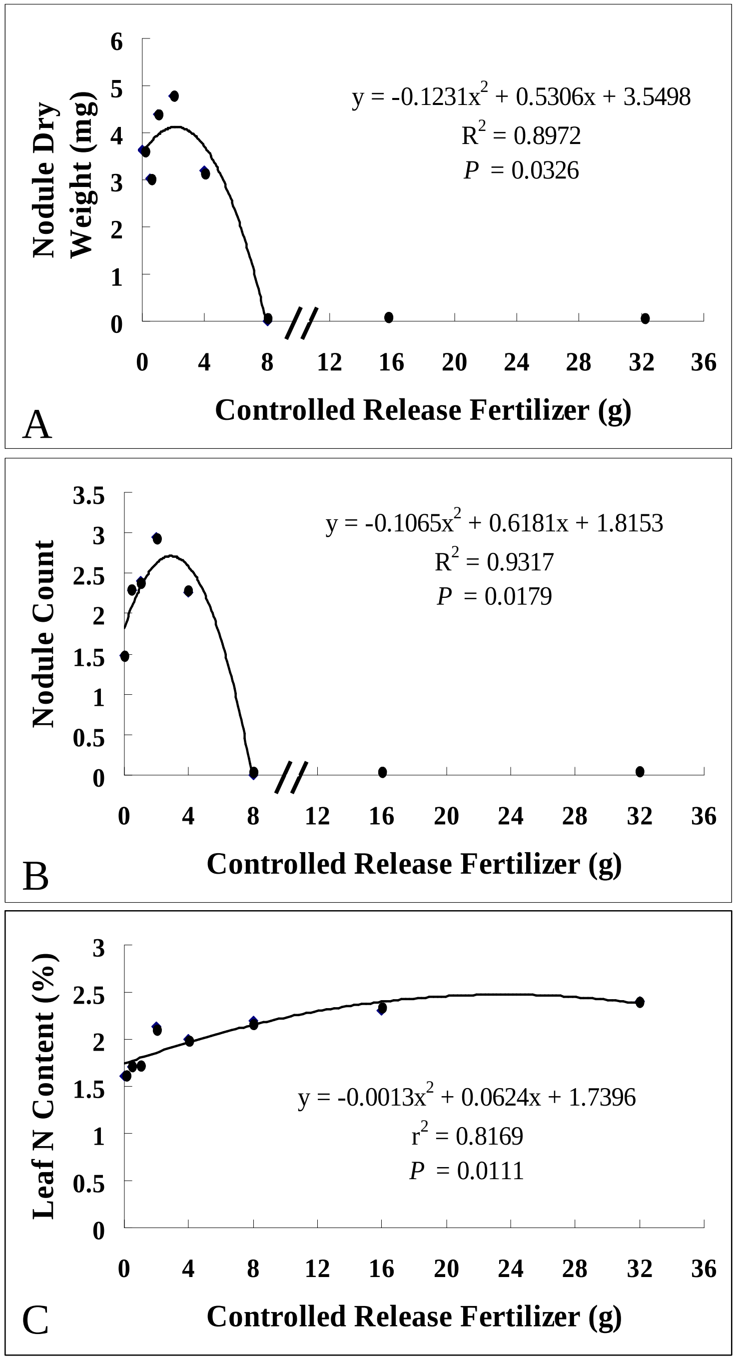

Fig. 3-1. Regression analysis of controlled release fertilizer levels (from 1 to 8 g) as the independent variable and the means of nodule dry weight (A), nodule count (B) and leaf N content (C) as dependent variables (*N*=4). A cubed-root transformation was performed on the raw data of nodule dry weight and nodule count to meet assumptions of homogeneity of variance. The data is shown in its transformed state.

g CRF treatment falls above the regression line and is similar to that of plants treated with CRF levels of 8 g or above. Regression analyses between CRF level and shoot dry weight (P = 0.1000, r^2=0.7856), root dry weight (P = 0.6697, r^2=0.0324) and plant dry weight (P = 0.9808, r^2=0.0395) were not significant.

Analysis of NO_3-N contained in the leachate revealed that date of testing, treatment level, and their interaction were statistically significant. Nitrate-N concentrations of the leachate from tested containers were greatest during week 2 for all CRF-treated plants. Linear regression revealed that levels of NO_3-N contained in the leachate would have been depleted from the leachate for the 2-, 6-, 8-, and 32-g treatments on days 51.50 (P = 0.0065, r^2 = 0.4460), 48.19 (P = 0.0065, r^2 = 0.4700), 53.80 (P = 0.0127, r^2 = 0.4780), and 60.79 (P = 0.0011, r^2 = 0.5587), respectively, with a mean depletion time of 53.57 days. These same regressions also revealed that after day 14, NO_3-N levels in the CRF-treated plants decreased in a linear fashion until the end of the experiment (Appendix III). No differences in leachate NO_3-N existed between the 0- and 2-g treatment on any test date (Fig 3-2A-D) (P < 0.10). At all other CRF levels, NO_3-N concentration of the leachates were different statistically from one another accept for week 1 within each test day (Fig 3-2A-D) (P < 0.10). Plants receiving 8 mM NH_4NO_3 had statistically greater leaf N content than all other treatments (data not shown). Nitrate-N in leachate of plants in this treatment also increased on each successive test date wherein the greatest levels were measured on day 42 of the experiment.

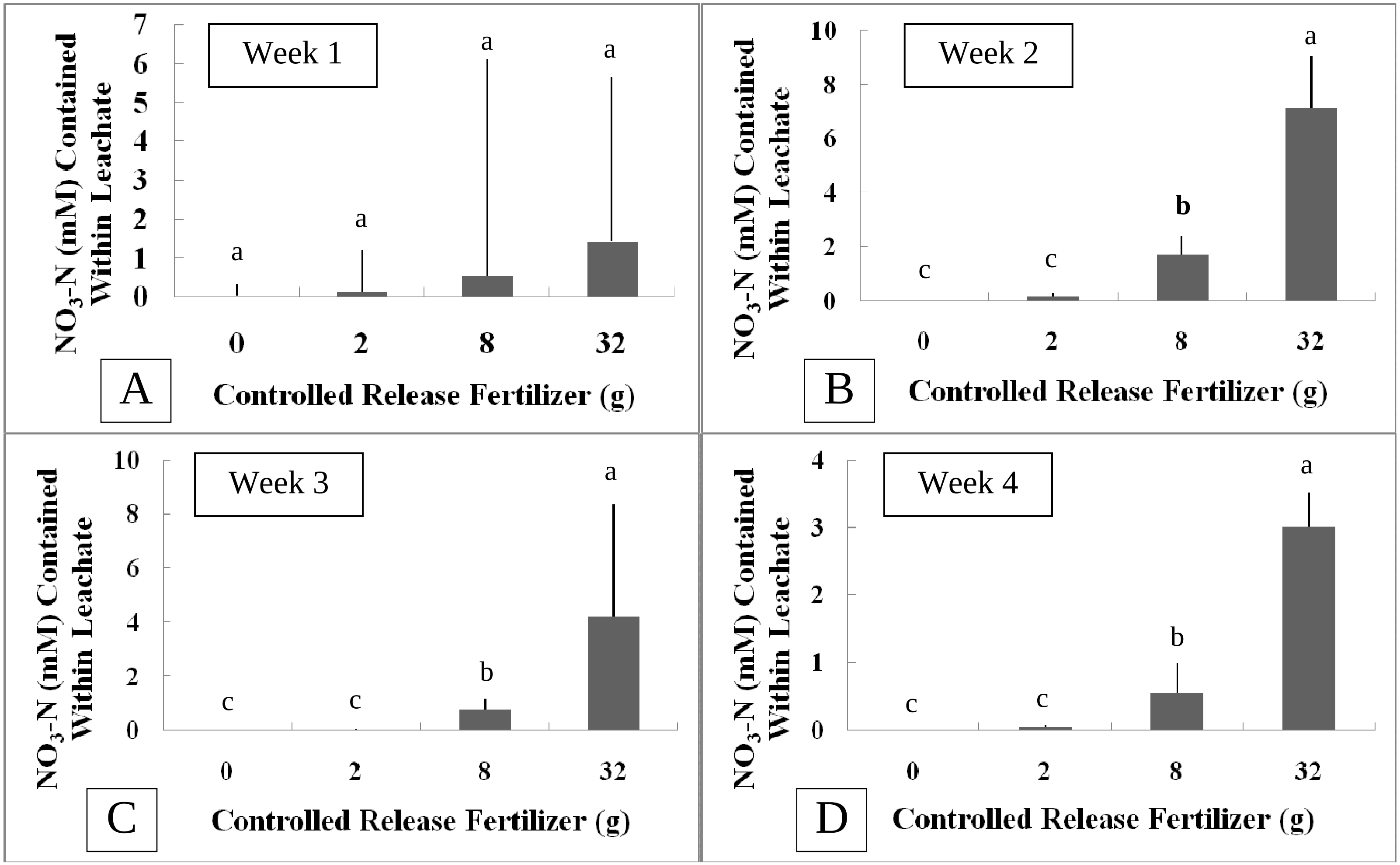

Fig 3-2. Means of NO_3-N amounts contained in leachate from CRF-treated seaside alder plants analyzed by week. Week 1 (A), Week 2 (B), Week 3 (C), Week 4 (D), ($N = 4$). Means of NO_3-N levels with the same letter are not statistically different at $P < 0.10$ according to Fisher's least significant difference test. Error bars indicate standard deviation.

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Discussion

Our results demonstrate that N from CRF influences nodule formation in seaside alder, and that healthy nodulated seaside alder plants can be produced by using fertilization rates below the CRF manufacturer's recommendations. The data suggest thatnodulated 2-g-CRF treated plants used applied N more efficiently than did plants treated with higher levels of CRF.

Plants receiving 8 mM NH_4NO_3 had statistically greater leaf N content than all other treatments. We suspect that the 3.0% N contained in the leaves of these plants was near the maximum amount of N that can be stored by this species (Kratsch and Graves, 2004, 2005). This is further verified by NO_3-N monitoring, which showed that NO_3-N in leachate of plants in this treatment increased over time and were at their highest levels on day 44 of the experiment (data not shown), suggesting that plants in this treatment had slowed their rate of N-uptake.

Nodulation patterns of plants in this experiment are generally consistent with previous work with N_2-fixing species. Laws and Graves (2005) state that both nodule formation and plant vigor was maintained in seaside alder plants receiving between 0.5 and 2 mM NH_4NO_3 from modified Hoagland's solutions, and inhibited at levels above 4mM. Similar patterns have been observed among other actinorhizal species: nodulation was prevented by application of 6 mM NH_4NO_3 in both Mexican cliffrose (*Purshia mexicana*) and bitterbrush (*Purshia tridentata*) in a greenhouse setting (Righetti et al., 1986). Arnone et al. (1994) showed that nodule formation was inhibited in *Casuarina* *cunninghamiana* plant root systems when exposed to 3.00 mM nitrate, but nodules were observed on all root systems exposed to 0.05 mM nitrate.

Plants in our 0-g-CRF treatment formed fewer nodules than plants in the 2 g treatment. In contrast, Laws and Graves (2005) showed that inoculated plants receiving N-free modified Hoagland's solution formed the greatest number of nodules. They did not recommend this as an acceptable way to encourage nodulation though, because they found that stems of plants receiving no N were deformed, and leaves were not as bright green as in plants receiving higher levels of N. Because plants in their experiments were fertigated daily with a modified Hoagland's solution containing optimal levels of all essential plant nutrients except N, nodulation was supported. Plants in our experiment were given no supplemental nutrients apart from what was provided in the CRF. It has been shown by others that nutrients in addition to N influence nodule formation in actinorhizal species. For example, Valverde et al. (2002) showed that nodule dry weight was maximized when *Discaria trinervis* plants were treated with P at rates between 100 and 1,000 µM as compared to plants receiving reduced amounts of P. It also has been suggested that other micronutrients such as Fe and Mo may influence nodule function (Huss-Dannell, 1997). In our study, regression analysis revealed that nodule dry weight and nodule count were maximized in the 2-g CRF treatment. It is likely that the 2 g CRF applied in our study provided just enough of all nutrients to support nodule function without levels of N that would inhibit nodule formation (Fig. 3-1 A and B).

Although N_2-fixation assays were not performed, we are reasonably certain that N_2-fixation was occurring because of the similarities in leaf N content and other measured growth parameters between nodulated 2-g CRF-treated plants and uninoculated plants receiving 6 gCRF (Table 3-1). Evidence of this can be seen in Fig. 3-1C wherein leaf N content of the 2-g treatment is well above the regression line and is similar to N content of plants that received 8 g CRF and more.

Conclusions

Seaside alder has potential for use in sustainable landscapes and can be produced readily in a nodulated form by growers. Satisfactory growth and health was observed, and nodule formation occurred when quantities lower than manufacturer's recommendations were used. Future work should test the hypothesis that nodulated plants will have increased survival upon installation in the landscape. Nitrate leaching from nodulated plants in the landscape also should be tested, since no data is available on optimal levels of N fertilization of nodulated landscape plants.

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CHAPTER 4

SUMMARY AND CONCLUSIONS

Sustainability has become increasingly important to the green industry for several reasons. Many government agencies require the use of conservation methods to reduce water use and pollution from over-fertilization. The public is also becoming increasingly interested in sustainability, and an aspect of this is utilization of landscape plants needing fewer inputs than those currently produced by growers.

Unfortunately, limited research exists concerning commercial production and propagation protocols for many drought-tolerant native plants. In response to this, we chose four plants that prospectively could be used within the local landscape that would require fewer inputs in production and few to no inputs once established. They include Mexican cliffrose (*Purshia mexicana*), silver buffaloberry (*Shepherdia argentea*), roundleaf buffaloberry (*Shepherdia rotundifolia*), and seaside alder (*Alnus maritima*). Mexican cliffrose and both species of buffaloberry are locally native, and seaside alder is native to Oklahoma, Georgia, and the Delmarva Peninsula. Although not indigenous to the Intermountain West, seaside alder is potentially adapted to IMW landscapes in locations that have occasionally water-logged soils, where it shows no potential for invasiveness.

Many within the local green industry expressed concern about a lack of information concerning maximizing germination rates in commercial settings of many local native plants showing potential for use in the landscape. I also suspected that these species are detrimentally impacted by traditional growing substrates where water-holding porosity is impacted by high amounts of organic matter. To determine the validity of this assumption, I sowed previously stratified seeds of roundleaf buffaloberry and silver buffaloberry in three substrates with differences in water-holding porosities and water release characteristics primarily due to varying amounts of organic matter within each substrate. I found that roundleaf buffaloberry germination was maximized in a calcined clay containing no organic matter and greater amounts of air-filled pore space. This species had low germination rates in the substrates containing organic matter. Silver buffaloberry germination rates varied, and were not statistically different across substrates. This shows that the influence of organic matter content on water-holding porosity can be detrimental to germination of some species native to arid environments, but that others are more adaptable to the various substrates that may be used in commercial production.

It is also known that nodulation of actinorhizal species can be impacted by various concentrations of nutrients received from a liquid source, especially N, but little to no information exists on effects of a controlled release fertilizer (CRF) on nodule formation, and CRF are widely used in the green industry. I investigated impacts of a CRF on nodule formation in seaside alder and specifically sought to determine if previously inoculated plants treated with CRF would form nodules, and what level of CRF maximizes nodule formation. I found that 2-g CRF maximized root nodule formation. Plant growth parameters and leaf N content of 2-g treated plants were similar to plants receiving greater amounts of CRF. Although methods to determine if *Frankia* bacteria within root nodules were fixing atmospheric N_2 were not included, evidence such as similarities in leaf N content between the inoculated 2-g treatment and treatments receiving greater amounts of CRF and similarities in other growth characteristics such as leaf dry weight and root dry weight support that nodulated plants with the 2-g CRF treatment were fixing atmospheric N_2.

I also found a lack of seed testing within the local native plant industry to be problematic. Twice, I purchased seeds for experimental use and was only able to get enough seeds for the actual experiments and not for seed testing due to limited availability. Seeds did not include basic seed quality tests as required by law, and two experiments were detrimentally affected because of this (Appendices I and II). Specifically, Mexican cliffrose was initially included in the germination experiment involving roundleaf buffaloberry and silver buffaloberry, but was eventually excluded due to uncharacteristically poor germination rates. However, trends in the data suggest that its germination was maximized in substrates containing little to no organic matter. The second experiment involved roundleaf buffaloberry germination. I desired to further refine seed stratification protocols of this species. To do so, I used combinations of either heat- or acid-scarification combined with cold-stratification. Again, due to poor seed quality, results were inconclusive.

It is realized that this work is not comprehensive when considering the various aspects involved in determining propagation and production protocols of species that could be used in a sustainable manner. However, I hope that research continues and that more species better adapted to the local environment will become more available for use.

APPENDICES

Appendix I

Germination of Roundleaf Buffaloberry in a calcined clay using combinations of cold stratification, acid scarification, and heat scarification

In a continuation of the experiment involving germination of roundleaf buffaloberry, and silver buffaloberry in three substrates, I further wanted to refine techniques in breaking seed dormancy to maximize roundleaf buffaloberry germination. In the previously mentioned experiment, I used 16 weeks of cold-stratification to break seed dormancy in both species. This is a standard amount of time used by many local growers. However, some also have used alternative methods such as heat- and acid-scarification to do so. Beyond techniques local growers utilize, I located little formal research involving breaking seed dormancy of roundleaf buffaloberry in a nursery setting.

To further my knowledge in this area, I designed an experiment involving various periods of seed stratification in combination with heat-scarification or acid-scarification.

Materials and Methods

Seeds used in these trials were from the same accession and obtained from Maple Leaf Seed (Ephraim, UT 84627). Initially, I sought to determine a minimum amount of time seeds required to be soaked in sulfuric acid to breakdown the seed coat to a point at which water uptake was maximized but possible embryo damage was minimized. To do so, the following protocol was followed:

- Samples of 25 seeds were considered an experimental unit and were soaked in sulfuric acid (reagent 95-98% pure, EMD Chemicals, Gibbstown, New Jersey, 08027) for 0, 1, 5, 10, or 15 min with three repetitions per treatment (n=3).
- To do so, a seed group was placed in 15 ml sulfuric acid and vigorously stirred for 30 sec. After stirring, the seeds were allowed to soak for the remaining allotted time.
- Following the acid soak, seeds were vigorously rinsed in a colander under luke-warm tap water for 1 minute. Seeds were then allowed to soak in a container filled with 200 ml of tap water for an additional 5 min.
- After soaking, seeds were air-dried on paper towels for 14 hrs, and then weighed for initial weights.
- After weighing, each group was placed in 100 mm petri dishes containing 1 piece of 90 mm germination paper wetted with 4 ml deionized water.
- I then cold-stratified the seeds at 3°C for one week.
- When the week waiting period was finished, seed groups were again weighed to determine total water uptake. Water uptake was calculated using the following formula:

Percent increase = (final weight – initial weight)/initial weight) * 100%

After determining an appropriate amount of time seeds should be soaked in sulfuric acid to maximize water uptake, I next sought to determine an optimal treatment to maximize germination rates in a calcined clay.

- Groups of 50 seed were considered an experimental unit. Treatments included: groups of seed cold-stratified for 16, 8, 4, and 0 weeks; groups of seed scarified for 10 minutes in sulfuric acid using procedures listed in experiment 1, and then stratified for 8, 4, and 0 weeks; groups of seeds scarified by placement in 250 ml boiling tap water (98° C), allowed to cool to room temperature, and then stratified for 8, 4, and 0 weeks.
- Kord #1006 inserts (Kord Products Inc., Brampton, Ontario, Canada, L6T 1G8) (individual cell dimensions: L = 5.17 cm, W = 5.17 cm, H = 6.37 cm) were placed in Kord #1020 flats (Kord Products Inc., Ontario, Brampton, Canada, L6T 1G8) (L = 50.8 cm, W = 26.67 cm, H = 6.37 cm) and filled to the top with Turface MVP™ , a calcined clay product (Profile Products, Buffalo Grove IL, 60089). Seeds were sown approximately 2 cm deep within the calcined clay.
- Flats were placed into a randomized, complete block design (n=4), and seeds were overhead irrigated twice daily to container capacity. Germination rates were recorded daily for 45 days.
- Tetrazolium tests were performed on all ungerminated seeds from the first repetition at the end of the experiment to determine seed quality. Tests were not performed before the experiment due to a lack of seed.

Statistics. Statistical analysis was performed using the SAS/STAT software version 9.1 (SAS Institute Inc. Cary, NC), using the general linear models procedure of SAS. Statistical significance was considered at the 95% level. Means separation was performed using Fisher's least significant difference (LSD) test, and Levene's test was used to gauge homogeneity of variance.

Results

Water uptake was statistically equal and best in the 10 and 15 min treatments, but I opted to use the 10 min treatment to diminish possible damage to seed embryos by the acid. Seed germination was not found to be statistically different among treatments. However, the treatment receiving 8 weeks cold-stratification in combination with heat-scarification exhibited 7% germination: higher than any other treatment (Table A-1). Lowest germination rates were observed in the acid-scarified treatments.

Table A-1. Viable, nonviable and germination percentages of roundleaf buffaloberry.

Treatment (Weeks ^{z})	Viable (%)	Non-Viable (%)	Total Germinated (%)
16	68.00	32.00	3.12
8	34.00	66.00	2.08
4	40.00	60.00	4.68
0	64.00	32.00	1.04
8 acid ^{x}	18.00	82.00	1.56
4 acid	26.00	74.00	1.04
0 acid	32.00	68.00	0.52
8 boiling ^{y}	74.00	26.00	7.29
4 boiling	60.00	40.00	2.60
0 boiling	74.00	26.00	3.65

^{z} Weeks of cold stratification
^{x} These treatments received cold stratification in combination with acid scarification
^{y} These treatments received cold stratification in combination with heat scarification

Tetrazolium tests. Tetrazolium tests from the 0 -week cold treatment receiving no heat- or acid-scarification revealed almost 60% of the seed to be viable and 36% to be non-viable. These results are considered baseline measurements for comparison to results from other treatments (Table 1-1). It also appears that acid-scarification for 10 min was damaging to seed embryos; in all cases, number of viable seed was far lower than the 60% seen on the 0 week cold stratification treatment and other treatments in general.

Discussion and Conclusions

If time allowed for repetition of this experiment, even though no statistical differences were found between treatments, results revealed that combinations of cold-stratification and heat-scarification should be focused on since germination was maximized in these treatments. Fewer seeds were also rendered nonviable by the heat scarification treatments as compared to acid (Table 6).

It is also unknown how old the seed was when we purchased it. The vendor thought it was less than 2 years old but was unsure. If the seed was of extreme age, this also could have accounted low germination rates, even though roughly 60% of the seed tested was viable.

It is also notable that the vendor apparently did not perform seed quality tests as required by law. No certificates of quality were included with our purchase. This is a problem within the local native plant industry that needs to be remedied. It inhibits growers from making judgments on how much seed to order for future needs.

Appendix II

Germination of *Purshia mexicana* in three substrates

Mexican cliffrose [*Purshia mexicana* (D. Don) Henrickson] was listed in the Potts (2002) study as being desired for use within the Colorado nursery industry. Because of this, it was initially included in the germination experiment entitled: Seed germination of *Shepherdia rotundifolia* and *Shepherdia argentea* in three substrates. Unfortunately, the purchased seed was of poor quality. Tetrazolium test after the end of the experiment revealed that 74% of the seed was empty and another 7% was nonviable.

Even though poor seed quality limited useful statistical analysis of the data pertaining to this species, trends suggest that its germination, like that of roundleaf buffaloberry, is maximized in a substrate containing little organic matter in which its germination rate in the germination, native and calcined clay substrates were: 11.1%, 6.3% and 19.1%, respectively, with a mean germination rate of 12.1%. Further research is needed to verify this and could be potentially useful to growers of drought-adapted native plants.

Personal experience has shown poor quality seed to be a problem in the local native plant seed industry despite existing government regulations pertaining to seed quality standards. These standards are all too often not followed. This is a especially a deterrent to commercial growers who often times must rely on seeds from wherever they can be found due to a limited supply.

Appendix III

Unpublished Data from Nodulation of Seaside Alder

Topdressed with Controlled Release Fertilizer

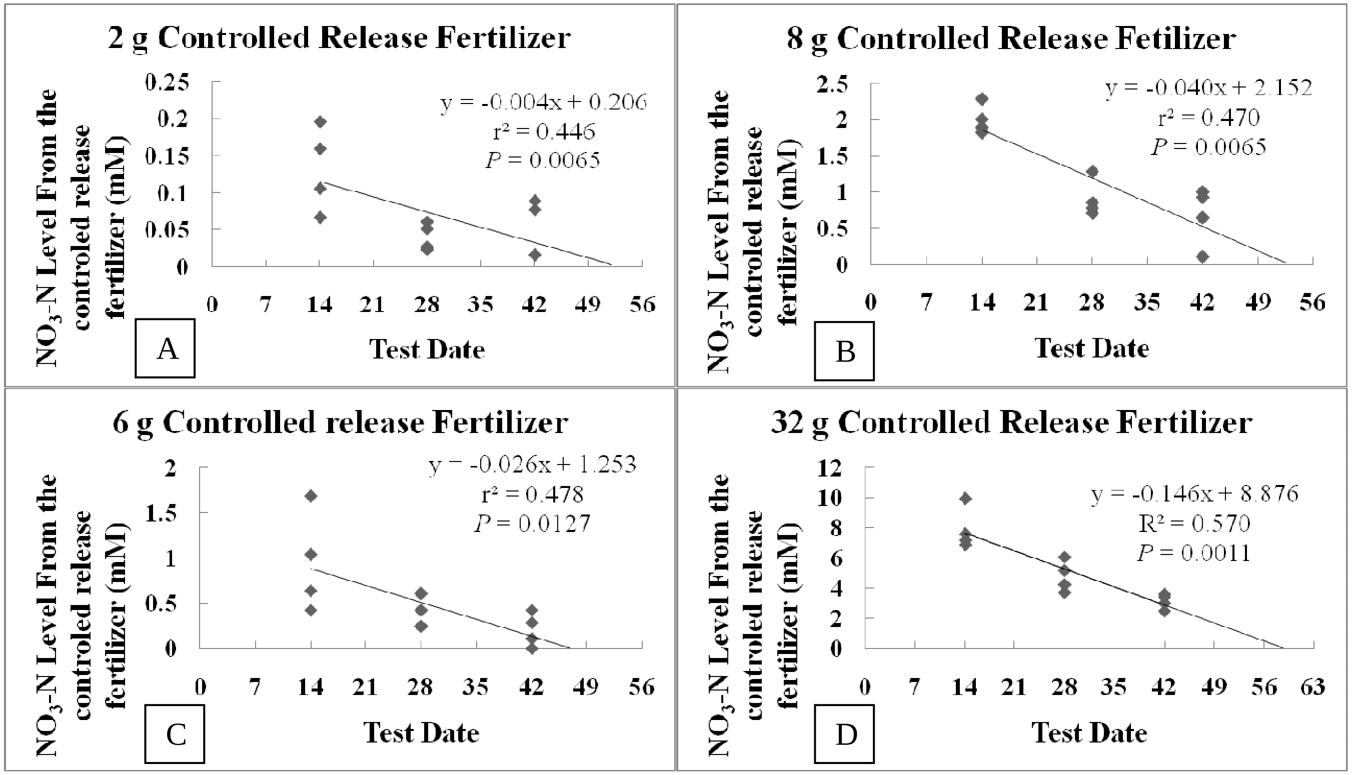

Fig. A-1 Regression analysis of controlled release fertilizer levels as the independent variable and NO_3-N levels contained in the leachate of 2 g CRF (controlled release fertilizer) (A), 6 g CRF (B), 8 g CRF (C) and 32 g CRF (D) as dependent variables (*N*=4