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Novel Techniques to Improve Restoration of Native Rangeland Species

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Master of Science

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ABSTRACT

Novel Techniques to Improve Restoration of Native Rangeland Species

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The sagebrush steppe is a particularly sensitive ecosystem that is easily disturbed by fires, oil and gas extraction, woody-plant encroachment, and overgrazing. The natural regeneration of native species following a disturbance within this system is typically slow and sporadic, which allows invasive grasses to occupy the landscape. Attempts to assist the recovery of these landscapes through direct seeding is commonly met with poor success rates, particularly in lower elevation, drier sites. Novel seed enhancement technologies and planting techniques that mitigate limiting factors impairing restoration efforts may improve the likelihood of restoring these degraded areas. For chapter 1, we evaluated a solid-matrix priming technique, where bluebunch wheatgrass (*Pseudoroegneria spicata*) and Lewis flax (*Linum lewisii*) were primed and then the priming matrix and seed were pelleted together. We evaluated primed seed that had been incorporated into pellets at two field sites against seed that was pelleted but been left unprimed, and untreated seed (control). These three seed treatments were planted in the spring (mid-march) in shallow (2-cm) and deep (15-cm) furrows, in a complete factorial design. We found that primed seeds generally produced higher plant densities than control seed at the beginning of the growing season; however, its influence diminished towards the end of the growing season. We also found that deep furrows increased plant density throughout the growing season and even into the following year. The combination of priming and deep furrows outperformed control seed in shallow furrows in all measured metrics. For chapter 2, we evaluated a seed conglomeration technique for improving Wyoming big sagebrush (*Artemisia tridentata* ssp. *Wyomingensis*) emergence and survival under fall and winter plantings. The trial was implemented at five sites across Utah and Nevada in a randomized complete block-split-split plot design, with site, and planting season, comprising the split-plot factors. Each site and season combination was seeded with conglomerated and control seed. We found that in most cases, a fall seeding of Wyoming big sagebrush was either the same or more successful compared to planting on the snow in the winter, which is the current suggested practice. Our results also demonstrated that seed conglomeration produced higher plant densities compared to control seed throughout the growing season. The higher density of plants produced from conglomerates combined with the improved seed delivery provided by the conglomeration technique was estimated to offset the cost in producing conglomerates and reduce overall restoration costs by 41%.

Keywords: priming, seed enhancement, microsite manipulation, bluebunch wheatgrass, Lewis flax, Wyoming big sagebrush, broadcast seeding, low purity seed, planting season

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CHAPTER 1

Improving Seeding Success in the Sagebrush Steppe with Seed Priming and Deep Furrow Plantings

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ABSTRACT

Seeding of native perennial species commonly fails in the presence of invasive annual weeds. The ability of weeds to deplete soil moisture by early germination and rapid growth gives them a competitive advantage. A possible solution to help native species compete with weeds is to enhance their germination rate and growth. We primed seeds of bluebunch wheatgrass (*Pseudoroegneria spicata*) and Lewis flax (*Linum lewisii*) in a matrix of compost, clay, and biostimulants for 4-7 days. For the priming duration that had the quickest germination, we used an extrusion technique to incorporate the seed and priming matrix into pellets. We evaluated primed seeds in pellets at two field sites against seeds in pellets that were not primed and untreated (control) seed. Seed treatments were planted in shallow (2-cm) and deep (15-cm) furrows, in a complete factorial design, with the expectation that the deep furrow treatment would provide an enhanced microsite to improve plant growth. In the first month after planting, primed seeds in deep furrows increased seedling emergence of bluebunch wheatgrass and Lewis flax by 128% and 303%, respectively, compared to control seed in shallow furrows. The following year, primed bluebunch wheatgrass and Lewis flax seeds in deep furrows increased plant biomass by 158 and 110%, respectively, compared to control seed in shallow furrows. Overall, this study indicates that the rapid germination of primed seeds and the use of deep

furrows may assist seedlings in establishing, which may allow them to better compete with invasive weeds.

INTRODUCTION

The degradation of ecosystems across the world is a growing biological and economic issue (Merritt and Dixon, 2011; James et al., 2013). It is estimated that globally \$1.6 trillion y^{-1} is spent to restore these degraded sites and that restoration costs are expected to increase (Merritt and Dixon, 2011). For example, in the western United States, fires, overgrazing, drought, and various other natural and anthropogenic disturbances have damaged rangelands in the sagebrush-steppe biome (Perrings and Walker, 1997; James et al., 2013). These disturbances have allowed for invasive annual grass species to move into formerly native perennial grass and shrub areas (Dantonio and Vitousek, 1992; Booth et al., 2003; Bradley et al., 2018). To assist in the recovery of disturbed sagebrush steppe sites that have decreased in native species abundance and diversity, land managers will commonly seek to restore these sites through direct seeding. Unfortunately the success of these seeding efforts is highly variable due to high mortality during the early stages of plant development (Lysne and Pellant, 2004; James et al., 2013; Germino et al., 2018; Shriver et al., 2018).

Seed enhancement technologies have the potential to improve the likelihood of restoration success through the application of materials and treatments that enhance germination, emergence, and early seedling growth (Madsen et al., 2016). Invasive weed species that are capable of rapidly colonizing dryland systems could help guide the development of new seed enhancement technologies. Cheatgrass (*Bromus tectorum* L.) is a common invasive annual weed in the sagebrush-steppe biome, whose rapid seed germination is thought to provide it with an advantage over slower germinating perennial species (Wilson et al., 1974; Roundy et al., 2007). Seed priming is a technique commonly used on agriculture crops to accelerate and synchronize germination time (Paparella et al., 2015). Priming is done by hydrating a seed to initiate the seeds metabolic activity and progress towards germination, but the seed is dried before radicle emergence occurs (Paparella et al., 2015). Priming has the potential to improve plant establishment by providing native species with similar germination characteristics as invasive weed species, such as cheatgrass. Research has shown that priming can decrease seed germination time of cool-season grass species commonly seeded in the sagebrush steppe (Hardegree, 1994; 1996; Hardegree and Van Vactor, 2000; Hardegree et al., 2002). It is predicted that this earlier germination may give seedlings an advantage by giving them exposure to a longer period of available resources and protection from disease and predators (Mercer et al., 2011).

Solid-matrix priming is an approach that has been shown to increase germination timing in agriculture (Taylor et al., 1988; Pandita et al., 2010; Farooq et al., 2019), and native dryland seeds (Rogis et al., 2004; Madsen et al., 2018). Through this approach, seeds are mixed with a solid-matrix carrier that is moistened with water to achieve a desired water potential for priming. Madsen et al. (2018), demonstrated that seeds incorporated in a solid priming matrix could be extruded into pellets after priming was complete. Through this approach, the seed does not need to be extracted from the priming matrix material prior to planting and the priming matrix can aid in improving the microsite of the seed by increasing moisture and nutrient conditions.

In addition to getting seeds to germinate within an optimal time period, direct seeding success rates are higher if the seed is deposited within a "safe-site," which has increased shade (Eckert et al., 1986), higher humidity (Harper et al., 1965), elevated and prolonged moisture (Winkel and Roundy, 1991), and more moderate temperatures (Winkel et al., 1991). The availability of natural safe sites are often low in degraded areas (Elmarsdottir et al., 2003).

Traditional rangeland drills create safe-sites for seeds, which enhances seeding success (Asher and Eckert, 1973; Haferkamp et al., 1987; Ott et al., 2016). In one fluid motion, soil is pushed outside a small V-shaped furrow and the seed is deposited below the soil surface of the furrow. Rangeland drill furrows are generally 3-6 cm deep, depending on the soil texture (Clary, 1989). This depth provides seed with moderate temperatures and typically higher and more consistent soil moisture for a greater period of time (Chambers, 2000).

The objectives of this study were to 1) determine how germination timing is influenced by the priming duration within the matrix used to form extruded pellets, 2) compare seedling emergence timing and plant growth of untreated seed, pelleted seed, and primed and pelleted seed, and 3) evaluate how these different seed treatments perform within shallow and deep furrows. We hypothesized that 1) primed seeds would have faster seed germination rates, which would lead to greater seedling emergence and plant growth, 2) deep furrows would improve seedling growth and survival, and 3) the combination of primed seeds and deep furrows would be the highest performing treatment.

MATERIALS AND METHODS

Laboratory Trials

Laboratory research was performed at Brigham Young University on "Anatone" bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) Á. Löve) and Lewis flax (*Linum lewisii* (Pursh)). Bluebunch wheatgrass is a palatable forage for a wide variety of wildlife and livestock and is considered a drought tolerant species that is adapted to stabilize disturbed soils (USDA 2018). Lewis flax provides desirable forage for wildlife while also being of value for erosion control and a fire suppressant in a greenstrip planting (USDA 2018).

Seeds were primed in a matrix consisting of 91 g of calcium bentonite powder (American Colloid Company, Hoffman Estates, IL), 288 g of ground compost, and 25 g of Azomite® (Azomite Mineral Products, Inc., Nephi, UT). The matrix was hydrated to a water potential of -1.5 MPa with a mixture of 690 ml of water, 0.027 g of Captan® fungicide (Southern Agricultural Insecticides, Inc., Palmetto, FL), and 0.107 g of ASET-4001 surfactant (Aquatrols Corporation of America, Paulsboro, NJ). Water potential was found by using a WP4C Dewpoint Potential Meter (METER, Pullman, WA) and adjusting the moisture content until the desired water potential was met. Priming was performed at 20°C in a Precision Plant Growth Chambers (Thermo Fischer Scientific, Waltham, MA) set to a 12-h light period in a 24-h cycle. Bluebunch wheatgrass and Lewis flax seeds were primed at five different daily intervals ranging from 1-5, and 3-7 d, respectively. The range of days seeds were primed was determined from preliminary trials indicating that germination occurred after 5 and 7 d for bluebunch wheatgrass and Lewis flax, respectively. After priming was completed, seeds were sieved from the solid matrix and air-dried.

The study was installed as a randomized complete block split-plot design. Temperature comprised the split-plot factor. Seeds were germinated at 5, 10, 15, 20, and 25°C in the same plant growth chambers used for priming, with the same light period. At each temperature, seeds treated with the five priming durations and untreated control seed for each species (12 treatments), were incubated separately in 9-cm diameter Petri dishes (25 seeds dish^{-1}) that contained a single layer of blue blotter paper that was moistened with water as needed throughout the study. Each temperature contained five replicate treatments that were arranged in blocks. Blocks were enclosed in plastic bags to prevent seeds from drying out. Germination was recorded every 1-3 d. Seeds were considered germinated once the radicle reached 2 mm in length.

Laboratory Statistical Analysis

From the germination data, the following indices were calculated: mean germination time (MGT), time to 50% germination (T_{50}), and final germination percentage (FGP) in the program AutoGerm (Richardson et al. 2018). MGT was calculated by using the following equation:

$$\bar{t} = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i}$$

where: $\bar{t}$ = mean germination time, t_i = time from the start of the experiment to the i^{th} observation, n_i = number of seeds germinated in the i^{th} time, k = last time of germination. Time to reach T_{50} was calculated as follows:

$$\mathrm{T} = \left[\left(\frac{t_a - t_b}{n_a - n_b}\right)(N - n_b)\right] + t_b$$

where: T = time (d) to subpopulation germination, t_a = incubation day when subpopulation germination was reached, t_b = incubation day before subpopulation germination was reached, n_a = number of germinated seeds on day that subpopulation germination was reached, n_b = number of germinated seeds on day before subpopulation germination was reached, N = number of germinated seeds equal to 50% of the total population.

MGT, T_{50}, and final germination were analyzed using a standard least-squares analysis in JMP® version 13 (SAS Institute Inc., Cary, NC). Temperature, species, and treatment were considered fixed factors and block was random in a full factorial analysis. MGT and T_{50} data underwent a log transformation to obtain a normal distribution, while final germination data did not require a transformation. Student t-tests were used to compare the difference between treatments for each of the indices measured at the five temperatures. Differences were considered significant when $P < 0.05$. In the text and figures, mean density values are reported with unique letters to denote significant differences.

Field Trials

Field research was conducted at two degraded Wyoming big sagebrush sites in Utah. The Lookout Pass site (40.139003, -112.507367), is located near the historic Pony Express Trail, east of the Onaqui Mountains, approximately 8.4 km from Vernon, UT. This site was seeded to "Hycrest" *Agropyron cristatum* (L.) Gaertn. (crested wheatgrass) in the fall of 1996, following the Aqueduct fire. The Lookout Pass site is characterized as a semi-desert gravelly loam ecological site that receives an average of 287 mm of precipitation per year. The Santaquin site (39.907287, -111.816306) is located at the southern end of Utah County, approximately 16 km south of Santaquin, UT within the Santaquin Wildlife Management Area. The area is primarily dominated by bulbous bluegrass (*Poa bulbosa* L.), field bindweed (*Convolvulus arvensis* L.), jointed goatgrass (*Aegilops cylindrical* Host) and cheatgrass. The area is classified as an Upland Stony Loam ecological site that receives an average of 481 mm of precipitation per year.

In preparation for seeding, both sites were sprayed in April of the previous year and two weeks prior to planting, with 280 g ai · ha^{-1} of glyphosate (Accord Concentrate®, Dow AgroSciences, Indianapolis, IN). Plant material that was not killed by the herbicide was removed by hand on the day seeds were sown. Seeds were planted at Santaquin and Lookout Pass on the 17 and 18 March, 2017, respectively. Both sites were fenced to keep livestock and wildlife (including rodents) out of the enclosure.

Field Experimental Design

At each site, the study was set up as a randomized block split-plot design with 10 blocks. Furrow depth comprised the split-plot factor. The study incorporated a total of three seed treatments for bluebunch wheatgrass and Lewis flax, including: primed seeds within a pellet (primed), pelleted seeds that were not primed (pelleted), and seeds where no treatment was applied (control). At both sites, the three treatments for each of the two species, were sown in deep-wide furrows (15 cm deep, 25 cm wide, and 1.5 m long) and shallow furrows (1 cm deep, 25 cm wide, 1.5 m long), for a total of 12 treatments. Each furrow contained ~ 125 pure live seeds, buried ~5 mm deep.

The priming duration that had the quickest germination timing from the laboratory trial was used to prime seeds for the field trial, which was 4 and 7 d for bluebunch wheatgrass and Lewis flax, respectively. Once priming was completed, 18.7 g of Stockabsorb 660 (Evonik Stockhausen, Greensboro, NC) and 690 ml of liquid (same ingredients aforementioned) was applied to the matrix to assist flow through the 5 mm extruder. Pellets were then cut to a length of ~1.5 cm. Once pellets were formed they were dried using a forced-air dryer at 43°C (Brace Works Automation and Electric, Lloydminster, SK, CAN). Pelleted seeds were created using the same methods as used for the primed pellets, only the seed was not primed. For each of the pellet treatments, the number of seeds per gram of pellet material was calculated from 7 replicate samples of approximately 3.15 g to estimate the weight of pellets that were needed to plant the targeted seeding rate.

Seed-bed soil moisture and temperature conditions were measured using MPS-6 water potential sensors (METER, Pullman, WA) that were randomly buried 2 cm below the surface, with five replicate sensors each in deep and shallow furrows at each site. Long term and monthly precipitation measurements during the period of the study were derived from models developed by PRISM's (Parameter-elevation Regressions on Independent Slopes Model) Oregon Climate Service (PRISM Climate Group 2018). Annual average precipitation and temperature were estimated from 1981–2010.

Emerged seedlings were marked monthly with plastic toothpicks to track emergence and seedling survival from 20 April – 14 July and 11 April – 5 June at Lookout Pass and Santaquin, respectively. Total tillers and stems in a row were counted on 25 May during the second growing season at Lookout Pass. On 26 June, during the second growing season, plant density was recorded and biomass was collected by clipping the entire row of established plants 2.5 cm from the soil surface. Clipped biomass was then dried in a plant drier at 60°C for one week and weighed.

Field Statistical Analysis

Monthly average water potential values were calculated for each block. Mean differences in water potential within deep and shallow furrows were analyzed separately for each month and site using a two-sample t-test. A log transformation was performed on the absolute value of the data to meet the assumptions of normality. Differences were considered significant when $P < 0.05$.

Seedling density data were analyzed in JMP® version 13 (SAS Institute Inc., Cary, NC) using a repeated measures mixed-model analysis. In the model, site, species, furrow depth and treatment were considered fixed factors, sampling period was designated as a repeated measure, and block was random. All fixed factors were included in a full factorial comparison. A log transformation was used on the data to meet the assumption of normality. All variables were included in the initial model as a full factorial; however, to simplify models all insignificant four and three-way interactions were removed from further models (Table 2). Monthly mean estimates and second growing season metrics (i.e. plant density, tillers, and biomass) were analyzed separately by site and season using the least square means differences Student t-test method. For all statistical field comparisons, a significance level of $P < 0.1$ was used. Second growing season metrics were not done for the Santaquin site due to a grasshopper infestation removing all of the above-ground biomass.

RESULTS

Laboratory Trial

In general, the speed that seeds germinated increased with the duration seeds were primed, and treatment response was greatest at colder temperatures (Fig. 1). Across all temperatures, priming durations from 4 - 5 d with bluebunch wheatgrass were higher than the control. At the coldest temperature tested (5°C), bluebunch wheatgrass primed for 4 and 5 d decreased T_{50} by 7.8 and 8.6 d, respectively (Fig. 1). Additionally, at 5°C, priming for 4 and 5 d lowered the MGT by 9.1 and 9.7 d, respectively (Fig. S1). Final germination of bluebunch wheatgrass was not influenced by priming (Fig. S2).

Lewis flax responded similarly to priming as bluebunch wheatgrass with priming for 7 d exhibiting the quickest germination response. Seeds primed at 7 d showed a decrease in T_{50} at 5°C by 11.8 d (Fig. 1) and a decrease in MGT by 10.9 d (Fig. S1). Final germination was not influenced by priming except with a 7 d priming duration where it decreased values by 15% at 5 and 15°C; however, this priming treatment improved germination by 17% at 25°C (Fig. S2).

Field Trial

Annual precipitation was below the 30 yr average at Lookout Pass (287 mm) and Santaquin (481 mm). However, precipitation from January to April was above average (Fig. 2). In March of 2017, when the study was planted, there was 94 and 24% more precipitation than average at Lookout Pass and Santaquin, respectively. The average annual temperature was relatively consistent with the 30 yr average (Fig. 2).

Water potential in deep furrows was consistently higher than shallow furrows throughout the growing season (Fig. 3). In the first growing season, deep furrows had 566 and 470 additional hours above -1.5 MPa at Lookout Pass and Santaquin, respectively, which was the water potential we considered the threshold for germination (Fig. S3). The average hourly temperature in deep furrows was more moderate than shallow furrows, especially during the hottest months of the year, July and August (Fig. 3).

The repeated measures mixed-model analysis indicated several three-way interactions. Two of the three-way interactions included site combined with species and month ($P < 0.01$) and species and treatment ($P = 0.04$). Another three-way interaction included species, furrow, and treatment ($P = 0.03$). To better understand these interactions, models were examined where sites and species were separated. At Lookout Pass, in the first month, primed seed of bluebunch wheatgrass and Lewis flax sowed in deep furrows improved emergence by 46 and 127%, respectively, compared to control seed sown in deep furrows (Fig. 4). The priming treatment effect was amplified when combined with deep furrows. For example, in the first month, primed seed of bluebunch wheatgrass and Lewis flax sown in deep furrows improved emergence by 128 and 303% compared to shallow furrows sown with control seed (Fig. 4). At the end of the growing season, emergence from primed seed was similar to emergence from control seed in their respective furrow depths. However, primed and control seed of bluebunch in deep furrows had 113 and 105 % more emergence than control seed in shallow furrows. Lewis flax demonstrated a similar response with primed and control seeds in deep furrows increasing emergence by 184 and 112 %, respectively, compared to control seeds in shallow furrows.

The following year, at Lookout Pass (433 d post-planting), primed seed in deep furrows continued to demonstrate improvement for bluebunch wheatgrass and Lewis flax by increasing plant establishment by 64 and 100 %, respectively, compared to control seed in shallow furrows (Fig. 5A). Primed bluebunch wheatgrass seed in deep furrows produced 158 % more tillers, respectively, compared to control seed in shallow furrows (Fig. 5B). Additionally, primed and control bluebunch wheatgrass seed in deep furrows increased plant biomass by 195 and 124 %, respectively, compared to control seed in shallow furrows (Fig. 5C). Primed and Pelleted Lewis flax demonstrated a similar response by improving the number of stems by 56 and 68 %, respectively, compared to control seed in shallow furrows. Also, primed and control Lewis flax seed planted in deep furrows exhibited a 110 and 100% increase in plant biomass compared to control seed in shallow furrows (Fig. 5C).

Similar to treatments planted at Lookout pass, primed seed of bluebunch wheatgrass and Lewis flax planted in deep furrows improved emergence in the first month following seeding by 169 and 233 %, respectively, compared to the control seed in shallow furrows at Santaquin (Fig. 4). In May, three months after planting, primed bluebunch wheatgrass seed in deep furrows improved seedling emergence by 33% compared to control seed planted in shallow furrows, while primed seed of Lewis flax was similar to the control (Fig. 4). We were not able to measure treatment responses in the second year of the study for the Santaquin site due to a grasshopper invasion that removed all of the above-ground biomass in the study.

DISCUSSION

Seedling survival is a major developmental bottleneck in the progression from seed to an established plant (James et al., 2019). Leger et al., (2019) found that seed populations that were more successful in overcoming this bottleneck were generally characterized by accelerated germination rates and fast-growing roots. It is possible that priming and deep furrows may artificially provide seeds with these important characteristics. Priming is able to stimulate early germination while deep furrows provide a microsite that can substitute for fast-growing roots. Our results generally support our first hypothesis, where we found that primed seeds would have faster seed germination rates, which in some instances lead to greater seedling emergence and plant growth. Our findings also generally support our second hypothesis that deep furrows would improve seedling emergence, growth and plant survival. Additionally, our results partially support our third hypothesis that the combination of primed seed and deep furrows would be the highest performing treatment.

Accelerating Germination and Emergence Through Seed Priming

This study demonstrated through laboratory trials and generally through field trials that primed seeds have the potential to influence rangeland restoration efforts, specifically for a spring planting. (Fig. 1 and 4). In the laboratory, priming accelerated seed germination for both species used in the trial with the greatest treatment response at colder temperatures. Priming's ability to accelerate seed germination under cold temperatures (Fig. 1) may be of value for seeding in cold desert regions, particularly in years and sites when soil moisture and temperature are marginal or inadequate for germination (Richardson et al., 2018; Leger et al., 2019).

Field research provides some indication that primed seeds can improve seedling emergence during the initial part of the growing season, but a treatment effect was generally only significant when primed seeds were combined with a deep furrow treatment (Fig. 4). In all scenarios with deep furrows, except for Lewis flax at the Santaquin site, primed seeds exhibited higher emergence than control seeds in the first month after planting (Fig 4). Presumably, in this study, seedlings are emerging faster from the soil because of accelerated seed germination (Fig. 1). Priming is likely to have its greatest utility when adequate moisture is just sufficient to complete the germination process for primed seed but lacking for unprimed seed. Additionally, in regions where summer precipitation is minimal, such as our study area, early emergence may improve seedling survival by allowing the plant to have an extended period of growth (Goldberg et al., 1999; Mangla et al., 2011) and possibly to produce longer roots that can assist with depleting moisture as it declines from the soil surface during the summer (Peek et al., 2005; Leger et al., 2019). In our study, priming exemplified its greatest treatment effect during the first three months of the growing season (Fig. 4), which may indicate as did our laboratory trials that priming can decrease germination time (Fig. 1) and subsequently lead to earlier emergence. Earlier germination and emergence may address the high bottleneck that occurs during the transition of germinated seeds to seedlings (James et al., 2011; James et al., 2019). Extended rain events throughout the spring in our study likely promoted control seeds to have similar final emergence by the end of the season as primed seeds (Fig. 4). If this study had been sown on a year when precipitation was not as consistent during the spring we might have seen a larger treatment effect in seedling emergence. Future research should be done to repeat this trial on additional years and sites, to understand the full utility of the technology.

Future research should also be done to evaluate primed seeds in the presence of invasive annual grasses. Vaughn and Young (2015) researched short-term priority effects between exotic annual grasses and perennial grasses. They found that if perennial grasses were planted two-weeks earlier than exotic annual grasses, this short-term priority effect increased the establishment of native perennial grasses. Had our study been done in the presence of invasive annual grasses, early emergence produced by primed seeds could have made the seeded species more competitive. This priming technique may also assist other native species in their ability to compete with invasive species in early demographic stages.

Primed seeds may have benefited from the pelleting material as well as through priming. Pelleted seed coupled with deep furrows did not demonstrate early seedling emergence, but for all of the other measured plant metrics (i.e. plant density, tillers/stem density, and biomass) pellets outperformed control seed in shallow furrows (Fig. 5). It is possible that the compost, surfactant, fungicide, and Stockosorb 660 powder used in the pelleting matrix benefited plant establishment and growth. Compost was initially applied to make the pellets more friable; however, compost may have provided additional nutrients to assist with plant establishment. Fungicides may help seeds and seedlings in their early demographic stages when they are most vulnerable to pathogen attack (Gilbert, 2002). Surfactants used in seed coatings have been shown to improve seedling drought tolerance, particularly in the presence of water repellent soils (Madsen et al., 2012; Madsen et al., 2013; Madsen et al., 2014). Additionally, Stockosorb 660 may have assisted seeds and seedlings in their ability to absorb and retain moisture, which could have diminished the effects of summer drought. The ability of these ingredients to improve conditions for seedling growth and establishment may have been one of the drivers for improved plant growth in the second year.

One of the deficiencies in this study is the lack of a fall (dormant) planting. While this study demonstrates the potential success priming can have for a spring planting, it does not indicate priming would be successful for a fall planting. Potentially, for some species, seeds may naturally prime over the winter so germination occurs rapidly in the spring. For species with relatively quicker germination times, priming may cause premature germination before or during the winter period when conditions are not suitable for plant survival (Boyd and Lemos, 2013). However, for slow germinating species, priming may provide some benefit in years with comparatively low spring precipitation by increasing the time for root development before entering a prolonged dry period (Boyd and Lemos, 2015). Further research needs to be done to determine how effective priming could be for a fall planting.

Improving Microsite Conditions by Deep Furrow Implementation

Our results support previous research that suggests a microsite can improve seedling survival and establishment (Asher and Eckert, 1973; Haferkamp et al., 1987; Clary, 1989). This study demonstrated that deep furrows can produce earlier seedling emergence (Fig. 4) and in most instances, higher plant densities (Fig. 5A), tiller/stem densities (Fig. 5B), and above-ground biomass (Fig. 5C). Improvements in these metrics may be due in part to the furrow's having higher soil moisture availability and moderated soil temperatures (Fig. 3). Increased soil moisture and moderate soil temperatures provided by the furrow would directly assist seeds in progressing towards germination (Hardegree and Van Vactor, 1999; Hardegree et al., 2008), promote seedling emergence, and enhance plant growth and survival (James et al., 2019). This was evident at Lookout Pass, where seedlings in deep furrows more than doubled the amount of seedlings in shallow furrows (Fig. 4). This trend persisted into the second growing season by producing two times the amount of biomass in deep furrows than shallow furrows (Fig. 5C). The ability for furrows to provide more moisture and moderate temperatures should be evaluated across various soil types and plant species to determine their full utility.

Deep furrows may be an increasingly useful tool for improving restoration efforts in the face of climate change, which is manifested by warmer ambient and soil temperatures (Karl et al., 2009; Barros et al., 2014). Specifically, summer soils have become dryer and hotter, resulting in less favorable conditions for germination and seedling development (Terry et al., In Preparation). Deep furrows are a potential solution to counteract dry soils and provide seeds with a more favorable environment for germination and plant survival.

Furrows have the potential to not only give an advantage to native seeded species but also reduce the competitiveness of weed species (Kettler et al., 2000) by sidecasting weed seeds from the furrow and burying them at a depth that restrains their emergence (Young et al., 2014). Additionally, a rough surface, such as furrows, may trap weed seeds near the edge of a restoration site, which limits their spatial distribution and propagule pressure (Johnston, 2019).

Priming and Deep Furrows

This study demonstrated that the combination of priming and deep furrows can greatly improve plant establishment. This technique provides a two-prong benefit by stimulating early germination (Fig. 4) when adequate moisture is available, and providing germinated seeds and seedlings with prolonged moisture within furrows (Fig. 3). In most cases, control seed in deep furrows improved emergence, density, tillers/stems, and biomass compared to control seed planted in shallow furrows (Fig. 4 and 5). However, the combination of priming with deep furrows consistently outperformed control seed in deep furrows. The improvement that priming in deep furrows provided at the beginning of the study was likely diminished due to the high number of seedlings, which led to intraspecific competition. The high amount of early seedling emergence this technique provides may allow for land managers to lower their seeding rate, which could prevent intraspecific competition and save costs while still reaching management objectives.

MANAGEMENT IMPLICATIONS

This study contributes to a growing body of knowledge that seed enhancement technologies and improved planting techniques can advance restoration efforts (Madsen et al., 2016; Erickson et al., 2018; Hoose et al., 2019). The seed priming and deep furrow treatments evaluated in this study show promise for improving seeding success of sagebrush steppe areas and may also have an application in other dryland systems. Specifically, this research demonstrates two areas for improving seeding efforts: 1) seed priming accelerates germination, which in some cases improves emergence, establishment and plant biomass, and 2) deep furrows provide longer periods of moisture and moderate near surface soil temperature, which directly promotes emergence and plant growth. Additionally, this study indicates that the combination of priming and deep furrows may allow land managers to have the highest degree of success in their restoration efforts. Future research is merited for the continued evaluation of priming and deep furrow planting techniques to determine if these technologies should be adopted by land managers.

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FIGURES

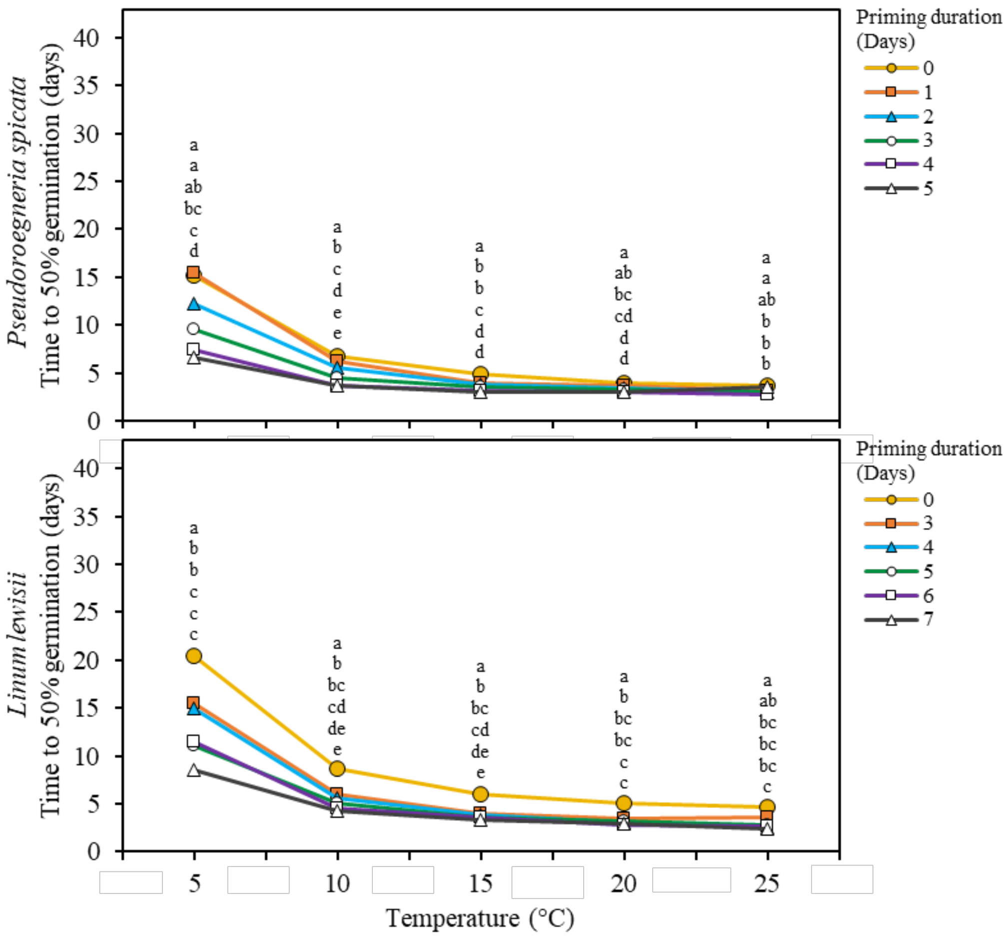

Figure 1-1. Time to 50% germination of "Anatone" bluebunch wheatgrass (Pseudoroegneria spicata (Pursh) Á. Löve) and Lewis flax (Linum lewisii (Pursh)) at priming durations from 0-7 d at a range of temperatures from 5-25 C.

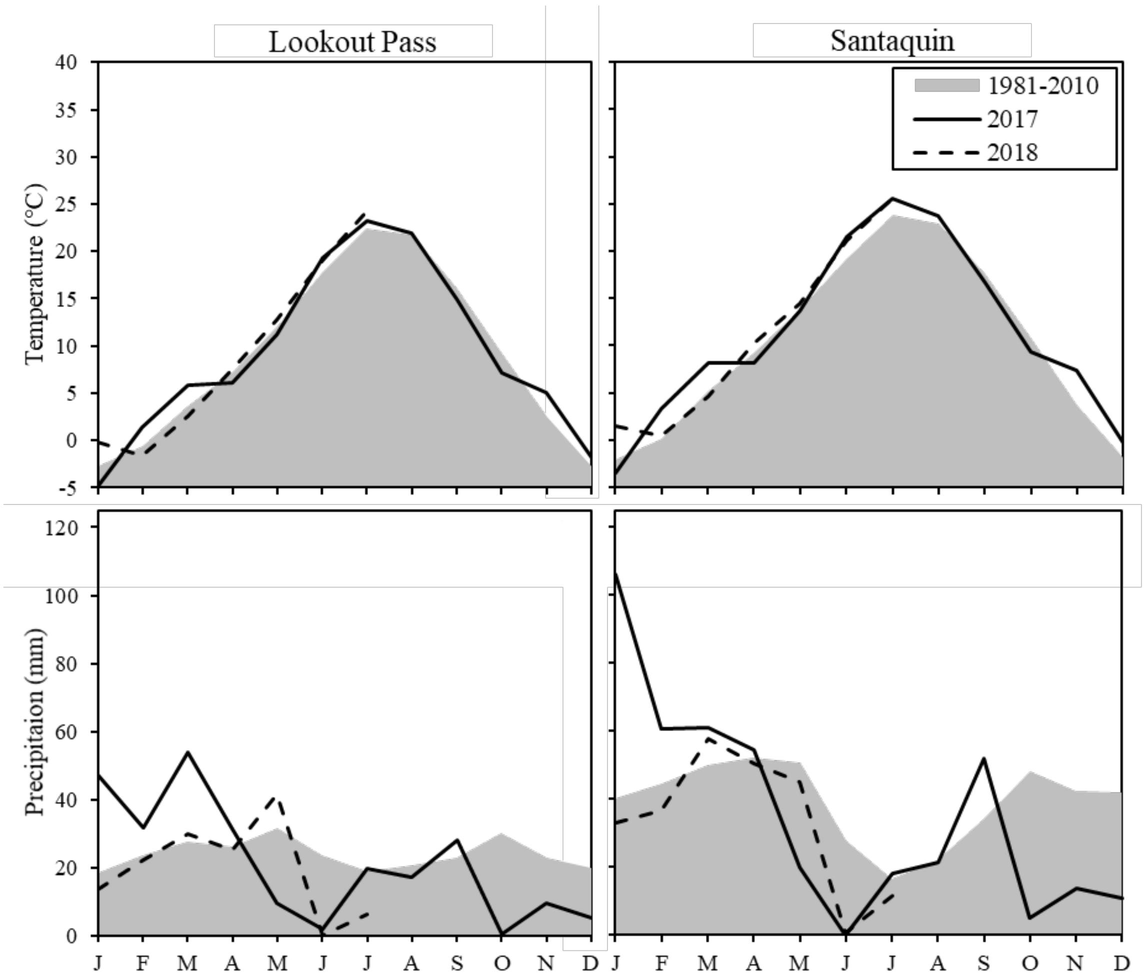

Figure 1-2. A comparison of 2017-2018 monthly temperature and precipitation levels at Lookout Pass and Santaquin study sites against the 30 yr average (1981-2010).

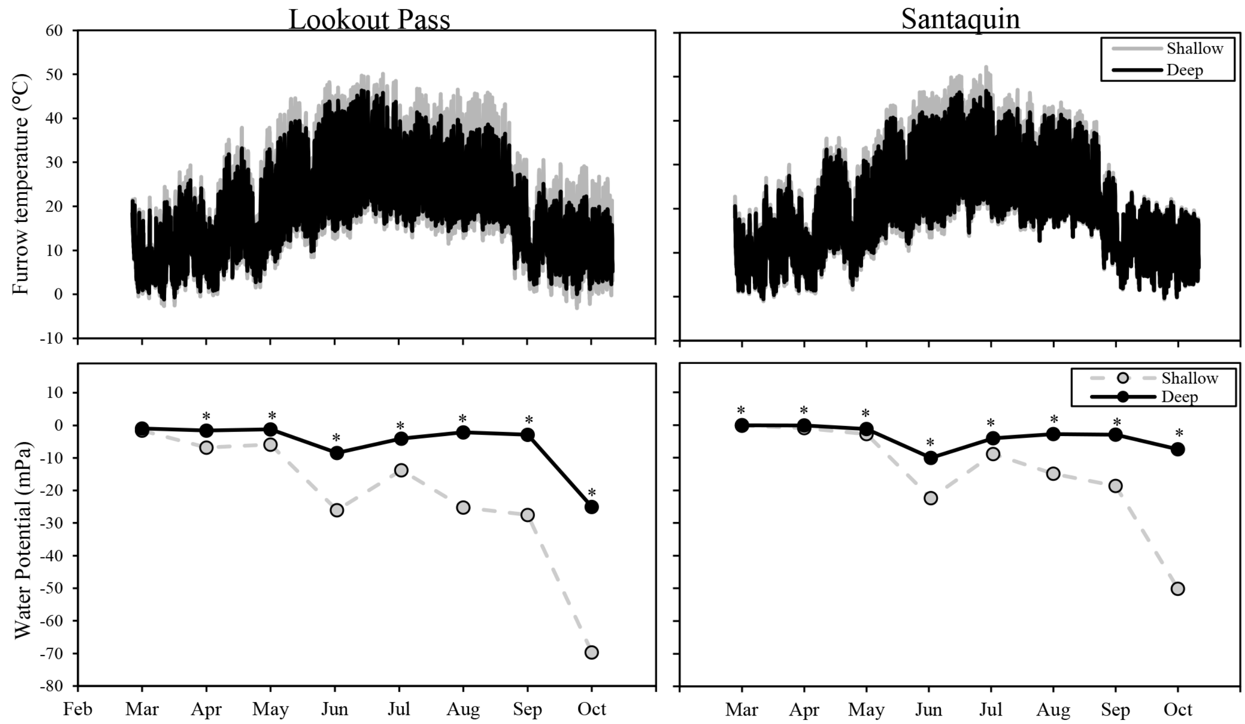

Figure 1-3. Monthly average water potential and hourly averaged temperature at 2 cm below the soil surface in deep and shallow furrows at Lookout Pass and Santaquin study sites. Statistical difference at $P < 0.05$ are indicated by asterisk marks (*).

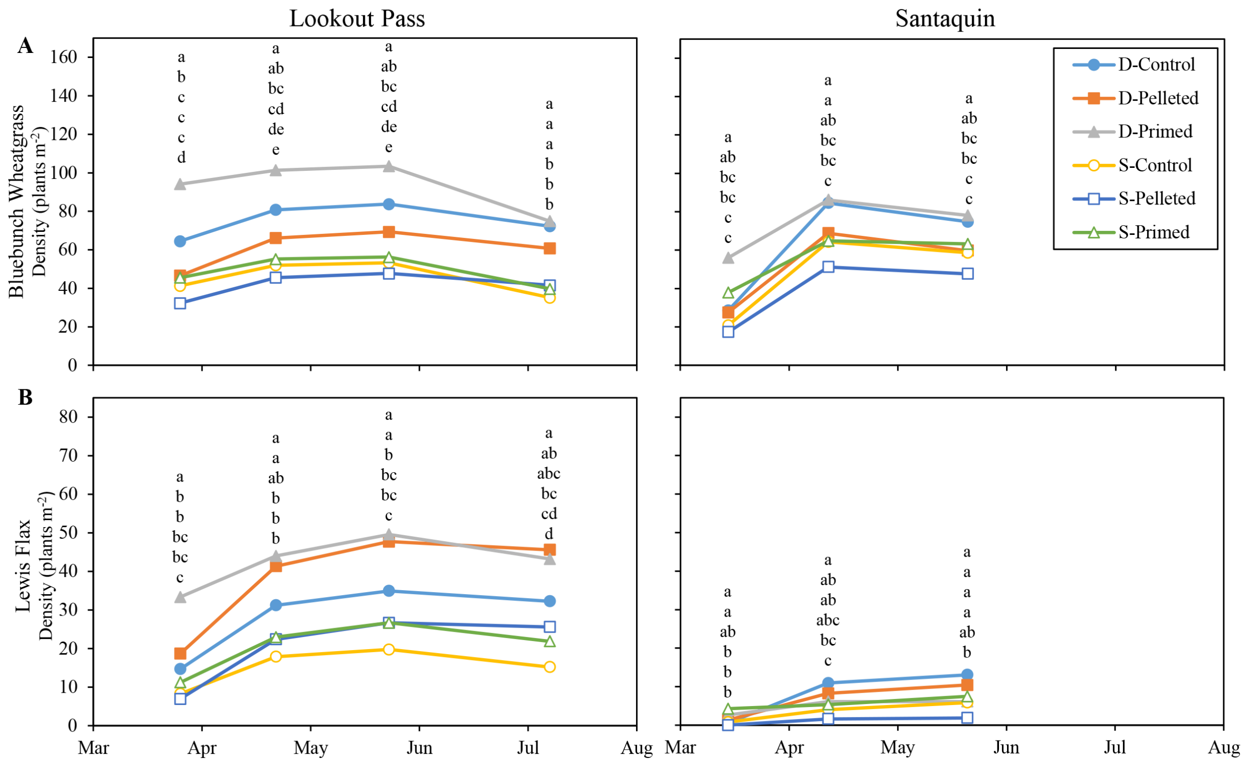

Figure 1-4. Plant density of bluebunch wheatgrass (A) and Lewis flax (B) at Lookout Pass and Santaquin study sites, produced from primed, pelleted and un-treated seed (control) in deep (D) and shallow (S) furrows throughout the first growing season. Emergence counting stopped at the end of May at Santaquin due to a grasshopper invasion that removed all above-ground biomass. Unique letters denote significant differences ($P < 0.1$).

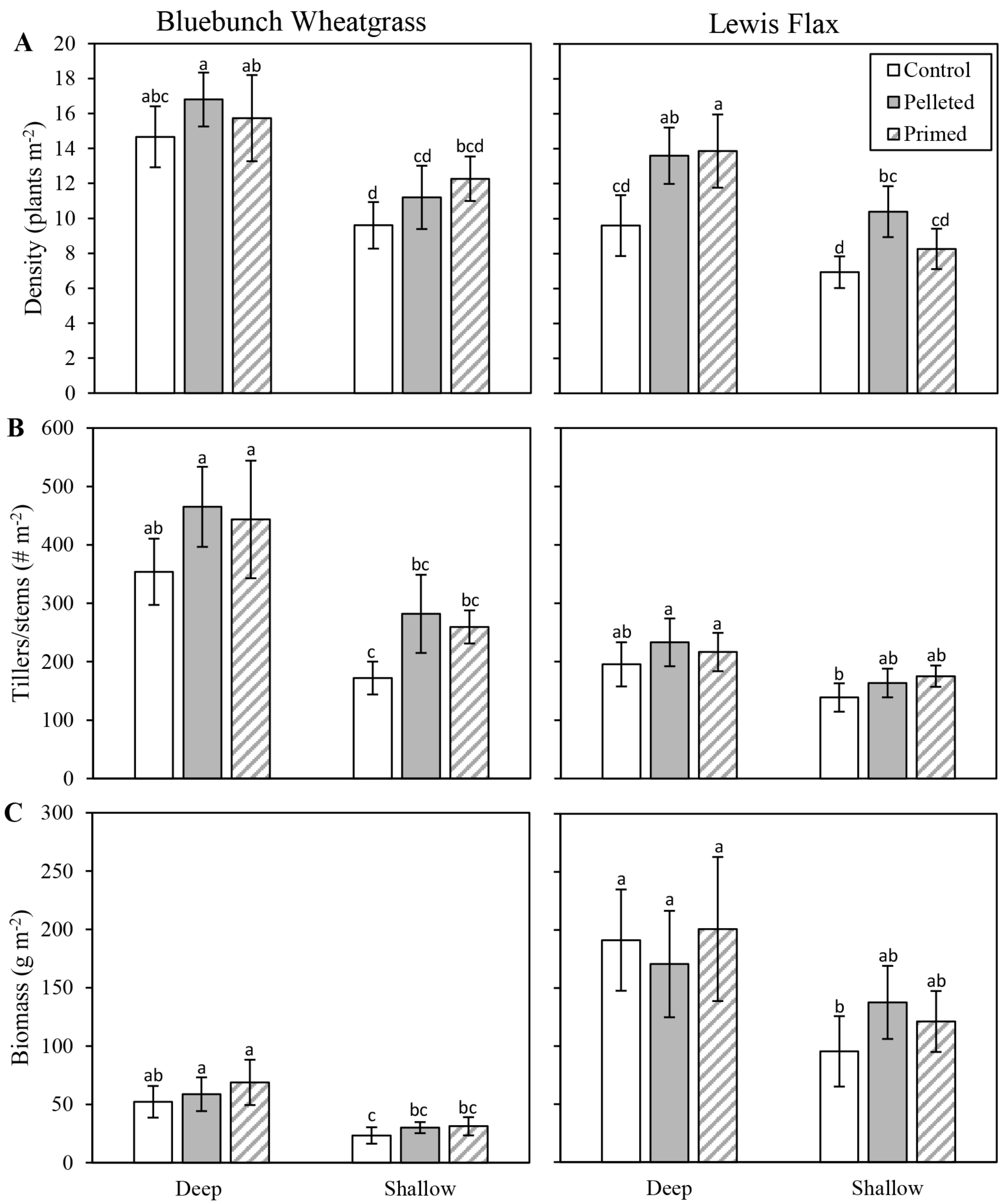

Figure 1-5. Plant density, tillers/stems, and biomass of bluebunch wheatgrass and Lewis flax produced from primed, pelleted, and un-treated seed (control) in deep and shallow furrows at Lookout Pass study site. Bars reflect means with standard errors. Unique letters denote significant differences ($P < 0.1$).

SUPPLIMENTARY MATERIAL

Table 1-S1. Ingredients used in the solid matrix priming medium with their respective amounts.

Trade Name	Product	Supplier	wt. (g)
Pelbon	Calcium Bentonite	American Colloid Company (Hoffman Estates, IL)	91.3
	Compost	Brigham Young University (Provo, UT)	287.9
Azomite	Hydrated sodium calcium aluminosilicate	Azomite Mineral Products, Inc (Nephi, UT)	25.0
Captan	Fungicide	Arysta LifeScience (Cary, N.C.)	0.027
Stockabsorb 660	SAP	Evonik Stockhausen (Greensboro, NC)	18.7
ASET-4001	Surfactant	Aquatrols Corporation (Paulsboro, NJ)	0.107

Table 1-S2. Results from a repeated measures mixed-model analysis showing effects on emergence throughout the growing season for sites (Lookout Pass and Santaquin), species (bluebunch wheatgrass and Lewis flax), treatment (primed, pelleted, and control), furrow depth (deep and shallow) and month emergence was recorded.

Effect	*F*	*P*
Site	37.85	**<0.01**
Species	293.06	**<0.01**
Seed Treatment (ST)	11.27	**<0.01**
Furrow	24.77	**<0.01**
Month	160.52	**<0.01**
Site x Species	43.56	**<0.01**
Site x Furrow	2.24	0.15
Species x Furrow	0.05	0.81
Site x Month	2.92	0.07
Site x ST	0.56	0.58
Species x ST	1.79	0.17
Species x Month	0.93	0.40
Furrow x ST	1.06	0.35
Furrow x Month	0.85	0.43
ST x Month	5.38	**<0.01**
Site x Species x Month	30.97	**<0.01**
Species x Furrow x ST	3.66	**0.03**
Site x Species x ST	3.15	**0.04**

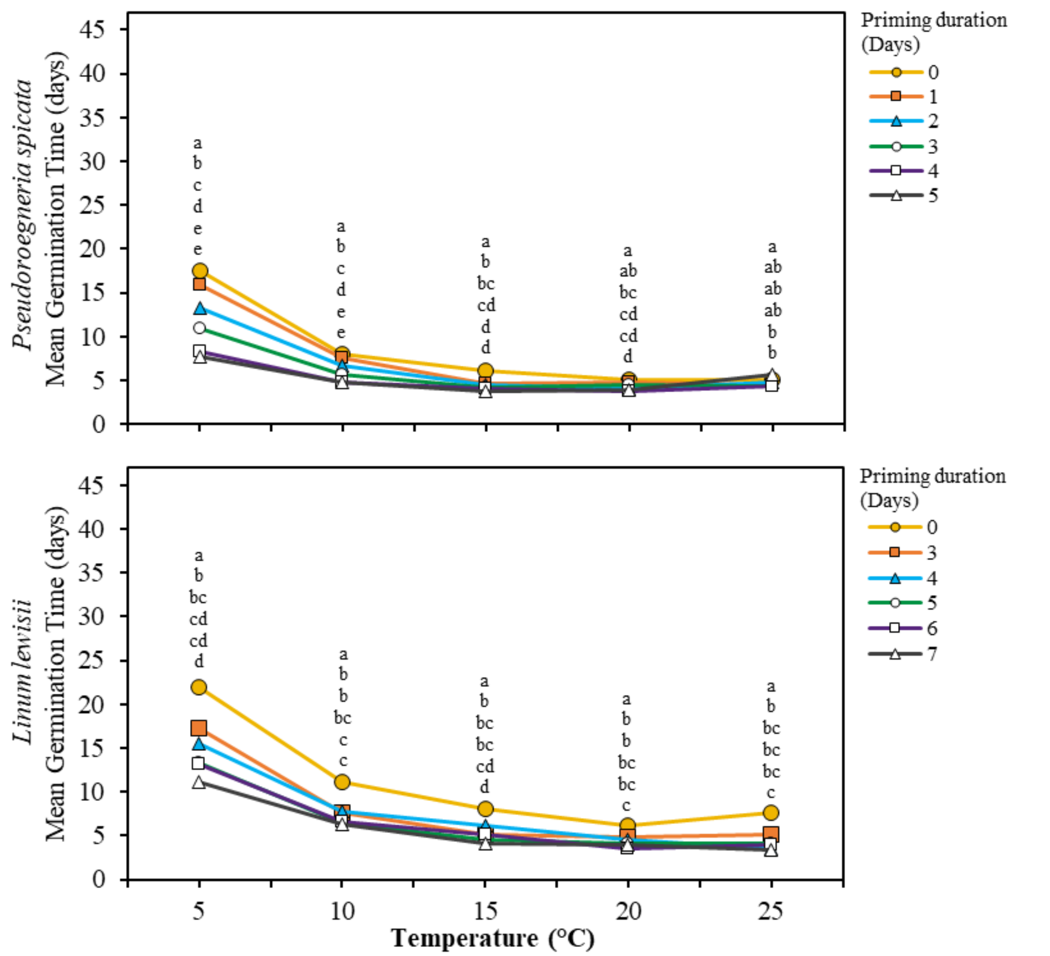

Figure 1-S1. Mean germination time of “Anatone” bluebunch wheatgrass (Pseudoroegneria spicata (Pursh) Á. Löve) and Lewis flax (Linum lewisii (Pursh)) at priming durations from 0-7 d at temperatures ranging from 5-25°C.

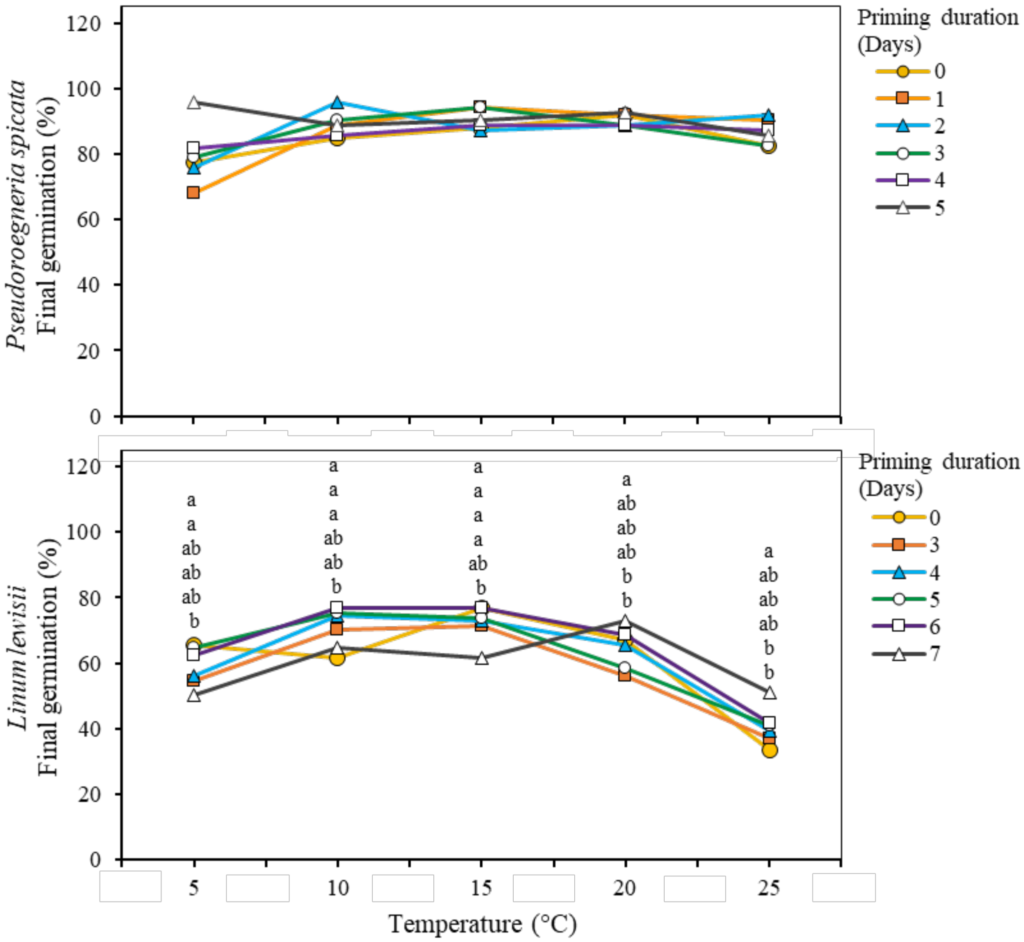

Figure 1-S2. Final germination of bluebunch wheatgrass and Lewis flax at priming durations from 0-7 d at temperatures ranging from 5-25°C.

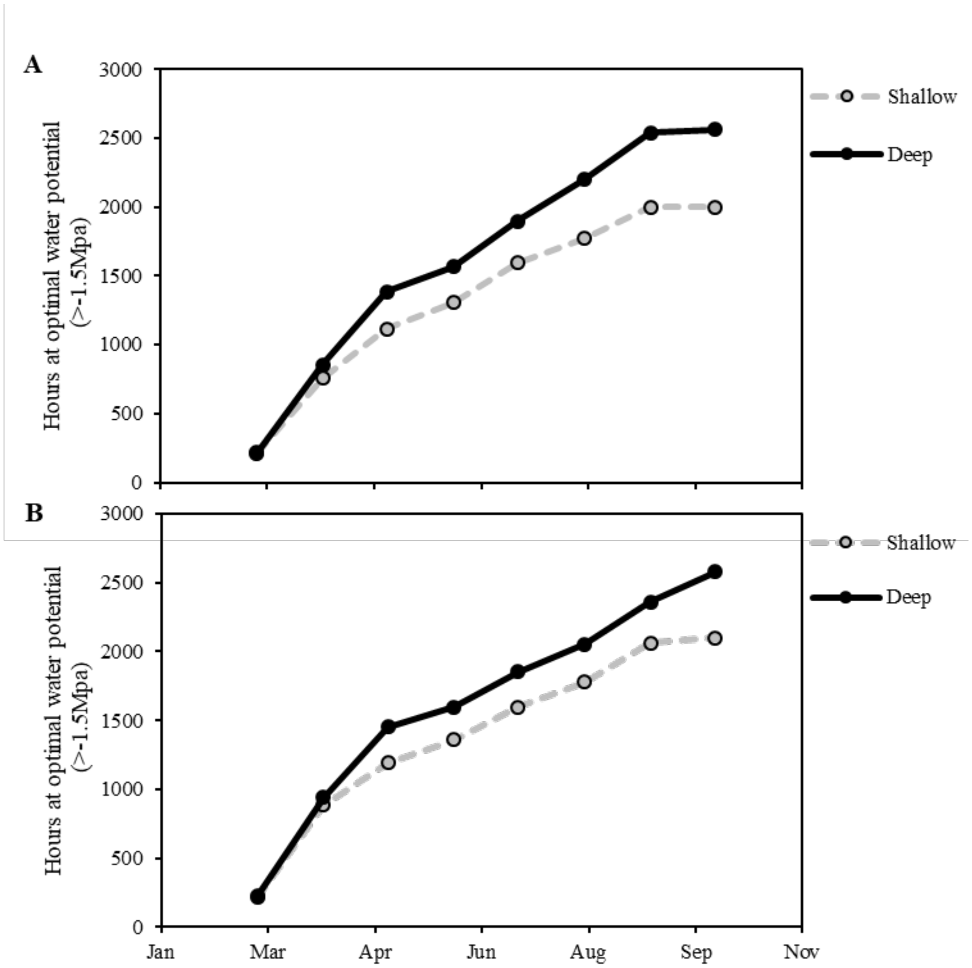

Figure 1-S3. Accumulated hours where water potential was above -1.5 MPa at 2 cm below the soil surface in deep and shallow furrows at Lookout Pass (**A**) and Santaquin (**B**).

CHAPTER 2

The Influence of Seed Conglomeration Technology and Planting Season on Wyoming Big Sagebrush Restoration

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ABSTRACT

Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis* [Beetle & A. Young] S. L. Welsh) is a species of great concern due to its massive reduction in the last century. This species is challenging to incorporate into restoration projects due to its small seed and accompanying non-seed parts, which impedes flow and broadcast distance. Additionally, seeding efforts commonly fail to produce plant densities that are sufficient to meet management objectives. Seed conglomeration is a novel technique that improves seed delivery and may also enhance plant establishment. We evaluated how seed conglomeration influences Wyoming big sagebrush plant emergence and establishment, for seed sown in the fall and winter, at five sites throughout Utah and Nevada. We found that seed conglomeration improved emergence by 60% and plant establishment by 26% in comparison to untreated seed. We estimate that this improvement in seeding success reduced the cost to produce established plants by 6%, which comes on top of savings that are already provided by the technology through decreased time and labor required to sow the seed. We also found that the influence of planting season varied by site, with a fall planting out performing a winter planting at two sites, a winter planting out performing a fall planting at one site, and at the other two sites planting season had no effect. This finding goes contrary to traditional seeding recommendations for Wyoming big sagebrush,

which suggest seeding success will be higher with a winter planting. Based on the results of this research, further evaluations are merited to continue the development and assessment of seed conglomeration technology. Land managers should also consider moving their winter plantings of Wyoming big sagebrush to fall.

INTRODUCTION

Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis* [Beetle & A. Young] S. L. Welsh), is a keystone species within the dryer, lower elevation sites of the sagebrush (*Artemisia* L.) steppe (Chambers, 2000; Brabec et al., 2015). Populations of this sagebrush subspecies have been reduced by processes such as altered fire regimes, invasive species, over-grazing, energy development, and urbanization (Dantonio and Vitousek, 1992; Braun et al., 2002; Davies et al., 2011). Following these disturbances, natural recovery is typically slow and often requires artificial seeding (Shaw et al., 2005). However, this sub-species seed characteristics makes it challenging to sow using mechanical equipment. Additionally, seeding success with Wyoming big sagebrush is commonly not high enough to produce stand densities that meet management objectives (Lysne and Pellant, 2004; Arkle et al., 2014). Subsequently, technologies that lower seeding costs and improve seeding success are needed for Wyoming big sagebrush restoration efforts to be economically feasible (Taylor et al., 2013; Madsen et al., 2016).

Delivery of Wyoming big sagebrush is challenging due to its small, low weight seeds and the high amount of associated non-seed parts (~80%) (Jorgensen and Stevens, 2004; Shaw et al., 2005). Unlike many other native species, Wyoming big sagebrush seeds cannot efficiently be cleaned to a high purity, which is likely due to the seeds and non-seed parts being similar in size and weight. These characteristics decrease Wyoming big sagebrush's flow through seeding equipment (Shaw et al., 2005) and limit the distance the seed can be spread through a broadcast seeder (Hoose et al., 2019).

Seed enhancement technologies are a potentially cost-effective solution to overcome the challenges associated with Wyoming big sagebrush seed delivery. A recent US patent application (16262864) describes a novel technique that allows for the coating of sagebrush seeds with non-seed parts into relatively small spherical-shaped conglomerates ~ 2 – 4 mm in diameter (Madsen et al., 2019). Conglomerates are formed by mixing seed, clay, and an aeration medium such as compost in a rotary seed coater, and adding water and binder in separate steps. This simple technique improves the flowability and broadcast distance of seeds through a broadcast seeder, which in some cases may reduce restoration costs as fewer passes are required to cover a given area (Hoose et al., 2019). Seed conglomeration technology has not been evaluated extensively in the field. Before this seed enhancement technology can be recommend as a treatment, it should be evaluated across a broad range of site conditions.

Recent research also indicates that seeding success of Wyoming big sagebrush may be improved by altering the timing of planting (Ott et al., 2017; Gunnell and Summers, 2018; Richardson et al. 2018). Wyoming big sagebrush recruitment and establishment, from direct seeding, is sporadic and highly variable (Lysne and Pellant, 2004), which is likely due to high inter-annual and intra-seasonal weather variability (Hardegree et al., 2016; Hardegree et al., 2018; Shriver et al., 2018). It is possible that the influence of weather variability may be diminished by adjusting the planting season. For example, research by Richardson et al. (2018) found that the time it takes for sagebrush seed to germinate at 5 °C was between 18 – 35 days longer than 9 other species evaluated in the study. In contrast, at warmer temperatures 15-25 °C, Wyoming big sagebrush was among the quickest germinating species. Wyoming big sagebrush seeds are commonly planted during the winter months on snow (Monsen and Stevens, 2004; Lysne, 2005; Jacobs et al., 2011). Planting seeds on the snow is an attempt to mimic natural seed dispersal from mother plants, which generally occurs in early winter (Meyer, 1994; Schlaepfer et al., 2014). It is possible that seeds planted on the snow do not accumulate the necessary time to germinate or germinate late in the spring, which could leave them more susceptible to summer drought. Despite early winter being the preferred season for planting, there is some research that suggests a fall planting may be more advantageous for Wyoming big sagebrush seeding success (Ott et al., 2017; Gunnell and Summers, 2018). These recent findings provide justification to evaluate the influence conglomerates have on sagebrush establishment for a fall (pre-snow) and winter (post-snow) planting.

The objective of our research was to determine how seed conglomeration technology influences the emergence, survival, and cost associated with seeding Wyoming big sagebrush, in both fall and winter plantings. We hypothesized that 1) conglomerated seeds would have higher initial emergence and subsequently lead to greater plant recruitment compared to untreated seed, 2) improved efficiency in seed delivery and seeding success would outweigh costs associated with applying the conglomeration technology, and 3) fall plantings would be most successful in restoring Wyoming big sagebrush.

METHODS AND MATERIALS

Study Sites

Field research was conducted on 3 sites in Utah, near Lookout Pass, Santaquin, and Fountain Green; and 2 locations in Nevada near Tuscarora and Paradise Valley. All five research sites were degraded Wyoming big sagebrush sites. The sites had a wide range of soil pH (7.2-8.2), mean precipitation (30 year average, 271-481 mm), slope (1.9-6.5 %), and elevation (1620-1730 m) (Table 1). Lookout Pass (40.139003, -112.507367) is located in Tooele County, to the east of the Onaqui Mountains approximately 8.4 km from Vernon, UT. This study location is characterized as a semi-desert gravelly loam ecological site, which was seeded in the fall of 1996, following the Aqueduct fire and is primarily dominated by crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.) (Hulet et al., 2010). The Santaquin site (39.907287, -111.816306) is located at the northern end of Juab County, approximately 16 km south of Santaquin, UT, within the Utah Division of Wildlife Resources Santaquin Wildlife Management Area. This area is an Upland Stony Loam site that is currently dominated by bulbous bluegrass (*Poa bulbosa* L.), field bindweed (*Convolvulus arvensis* L.), jointed goatgrass (*Aegilops cylindrical* Host) and cheatgrass (*Bromus tectorum* L.). The Fountain Green site (39.609189, -111.616850) is located in Sanpete County, approximately 2.8 km to the southeast of Fountain Green, UT. This area is classified as an Upland Stony Loam site that was previously seeded with oats (*Avena sativa* L.) and is currently heavily invaded by annual kochia (*Kochia scoparia* (L.) Roth). The Tuscarora site (41.696060, -116.534200) is located in Elko County, approximately 59 km Northwest of Tuscarora, NV, and is classified as a Dry Floodplain site. The area was burned in early July of 2018, approximately five months prior to the implementation of this study, in the Martin fire. This fire was a largescale high-intensity fire that burned 176,270 ha and was classified as the largest fire in the history of Nevada. The Paradise Valley site (41.635251, -117.277930) is a loamy site that is located in Humboldt County, approximately 27 km Northeast of Paradise Valley, NV. Similar to the Tuscarora site, this location was also burned in the Martin fire.

Experimental Design

The study was installed as a randomized complete block-split-split plot design with eight replicate blocks at five sites. Site and planting season (fall or winter) comprised the split-plot factors. At each site, conglomerated and untreated Wyoming big sagebrush seed was sown separately on the soil surface in the bottom of shallow-wide furrows (5 cm deep, 40 cm wide, and 1.5 m long) for both fall and winter plantings for a total of 4 combined treatments (2 planting seasons x 2 treatments). Furrows were implemented to sidecast weedy seeds and to keep seeds from drifting from one row to another. Fall seed treatments were planted on the 24, 25, and 26 October, and the 8 and 9 November, at Fountain Green, Lookout Pass, Santaquin, Tuscarora, and Paradise Valley, respectively. Winter seed treatments were installed on the 12 of December for Santaquin and Lookout Pass, 13 December at Fountain Green, and 18 and 19 December at Paradise Valley and Tuscarora, respectively.

Seed Coating

Seed coating was performed at Brigham Young University Seed Enhancement Laboratory on Wyoming big sagebrush seed acquired from the Utah Division of Wildlife Resources Great Basin Research Center (Ephraim, UT), that was collected in Elko County, NV at an elevation of 1700 m. Seeds were conglomerated in a 31 cm diameter rotary drum seed coater (Universal

Coating Systems, Independence, OR). Conglomerates were created by adding 31 g of seed, 290 g of Azomite® (Azomite Mineral Products, Inc., Nephi, UT), 101 g of Swell Clay (Redmond Agriculture, Redmond, UT), and 60 g of ground vermiculite (Therm-O-Rock West, Inc., Chandler, AZ) into the spinning rotary drum. Vermiculite was used as the aeration medium in place of the compost used by Hoose et al. (2019). Prior to the conglomeration process, vermiculite was ground and sieved to < 0.5 mm. After the dry materials were mixed, 220 ml of water was slowly added to the mixture over a span of 1.5 min. After all the 220 ml of liquid was added we waited (~30 - 60 s) until the majority of the conglomerates were ~2 mm in diameter. At this point, another burst of Azomite (291 g) and Swell Clay (101 g) was added to the mixture and an additional 30 ml of water was then applied. Following the addition of water, 20 ml of Agrimer 15 (Ashland Inc., Covington, KY) was applied to the conglomerates to provide a thin protective coat, to decrease dust if the seed were to be applied commercially. Agrimer-15 was previously produced by slowly mixing 400 g of product into 1,000 ml of water, producing a binder with a 40% solid content. After the addition of Agrimer-15, the conglomerates were spun for 30 s and then dried for 15 min on a forced air dryer at 36°C (Brace Works Automation and Electric, Lloydminster, SK, CAN). After drying, the majority of conglomerates were between 2 - 3 mm in diameter, however conglomerates were passed through a 3.5 mm sieve to eliminate larger masses.

We determined the average number of seeds per gram for the conglomerated and untreated seed by weighing out ~1.023 g and ~0.046 g of seed, respectively and placed the sample on a No. 140 sieve (106 μm), which was then rinsed and lightly rubbed against the sieve. This technique was implemented to remove the conglomerated material from the seed and was repeated on untreated seed to not introduce bias. Following the rinsing, the samples were air-dried and the total number of seeds was counted. This procedure was repeated 8 times for both treatments and seed was mixed thoroughly between each sample measurement.

Soil moisture and temperature conditions were measured using two MPS-6 water potential sensors (METER, Pullman, WA) that were buried 1 cm below the soil surface at each site. Daily average soil temperature and water potential was calculated to compare relative differences between sites. Long-term and monthly averages of precipitation and ambient temperatures were derived from models produced by PRISM's (Parameter-elevation Regressions on Independent Slopes Model) Oregon Climate Service (PRISM Climate Group 2020). The long-term averages were taken from 1981-2010.

Data Collection

Plant density was counted three times throughout the growing season to determine the number of seedlings that 1) emerged in spring, 2) survived to summer, and 3) survived to produce an established plant in fall. Seedling emergence was counted between 23 April and 1 May, seedling survival in summer was counted between 6 Aug and 21 Aug, and plant establishment at the end of the growing season was counted between 26 September and 11 October. We determined if a plant was alive by the presence of green photosynthetically active plant tissue.

Analysis of Seeding Cost

Our cost-benefit analysis assessed separately seed costs and delivery costs with respect to the number of plants that were produced from untreated and conglomerated seed. We used plant density values recorded in this study from our April and October measurements. When determining seed costs for untreated seed, we divided the cost of seed by the number of seedlings produced. For conglomerated seed, we added the cost to conglomerate the seed (Table 2) to the cost of the seed (\$125.31 PLS kg^{-1}, personal communication, Kevin Gunnell, Great Basin Research Center) and then divided the total by the number of seedlings produced. Our estimates to produce conglomerates were based on what it would cost if the treatment was applied within a commercial seed coating facility (\$0.77 kg^{-1} to coat grass seed, personal communication, Steve Luttrell, Summitt Seed Coatings). Conglomeration costs were a sum of the materials used (Table 2), plus an estimate of what it would cost to make the conglomerates within a commercial seed coating facility. We assumed the cost to make the conglomerates would be approximately four times higher than what a typical seed coating cost, given that the unique nature of this patent-pending (16262864) technology and the challenges in working with low purity seed.

Delivery costs followed the same approach as was used to assess seed costs by dividing the cost to deliver the seed by the number of seedlings produced. We assumed the seed was sown using a broadcast spreader attached to the back of a tractor at a rate of 0.22 kg PLS ha^{-1}. The cost to deliver seed included labor costs to operate the tractor at \$25 $hour^{-1}$, and the cost of the tractor at \$70 hr^{-1}. We assumed the tractor would operate at a speed of 2.5 m s^{-1}. Broadcast distances of untreated seed (3.09 ± 0.18 m) and conglomerated seed (6.91 ± 0.27 m) were taken from data published in Hoose et al. (2019). This cost analysis excludes the cost of filler materials (i.e. rice hulls), which only would be needed to deliver untreated seed and the potential shipping cost that may be required to send seeds to a commercial seed coater. Additionally, seed costs and delivery costs were calculated to demonstrate the total costs associated with planting untreated and conglomerated seed. Due to the low cost to produce an individual plant, the cost to produce 1,000 plants are shared to display differences in cost to the nearest cent.

Statistical Analysis

We evaluated the effect of conglomeration and planting season on the odds of seedling emergence (April count), summer seedling establishment (August count), and fall plant establishment (October count) using generalized linear mixed-effects models with a binomial response distribution (Sileshi, 2012). Treatment and planting season were treated as fixed effects and site, block, and row were treated as random effects. Seasons were implicitly nested in blocks and blocks were implicitly nested in sites. We tested for significant two-way interactions between treatment, season, and site by comparing models with and without interaction terms using likelihood ratio tests. Due to a significant interaction between site and season, we also conducted separate analyses for each season using the same modeling structure.

RESULTS

Environmental Conditions

In general, ambient air temperature in relation to the long-term mean was slightly warmer in fall at the time of planting, and somewhat colder in late winter (particularly for Fountain Green, Tuscarora, and Paradise Valley). Air temperature was similar to the long-term mean in spring and summer (with the exception of Fountain Green, which was cooler), and cooler in the fall one year after planting (Fig. 1). Precipitation was higher than the long-term mean in the fall at the time of planting for Santaquin and Fountain Green. For all sites, precipitation tended to be higher in winter and spring, and dryer in the summer and fall of the following year. The exception of this was in Paradise Valley and Tuscarora, which had more precipitation in September (Fig. 1).

Soil temperatures during the period that would influence seed germination and seedling emergence (October – April), generally tended to be similar to each other (Fig. 2A), except for the period from early February to late March (Fig. 2B). During this time, soil temperatures began to increase above freezing for all sites except Fountain Green and Paradise Valley. At Fountain Green and Paradise Valley, soil temperatures remained at or near freezing for approximately 16 –20 additional days, respectively (Fig. 2B). For the remainder of the growing season, soil temperatures were relatively similar across the sites, except for Fountain Green, which was colder than the other sites (Fig. 2A). Measurements of soil water potential showed that the soil started to maintain relatively high moisture levels (water potential > -1.5 MPa) around the end of November to the end of April (Fig. 2C and D). Tuscarora did have some low moisture readings during this period, but this is likely due to the soil moisture sensors' inability to detect water under freezing conditions (Fig. 2C). Through the duration of the summer and into the fall, water potentials varied greatly between sites, with Santaquin, and to a slightly lesser extent Fountain Green, experiencing consistently higher water potentials compared to the other three sites (Fig. 2C).

Seed Conglomeration

There was no significant interaction between site and seed treatment (P = 0.695, P = 0.999, 0.999, for April, August, and October count dates, respectively) or season and seed treatment (P = 0.893, P = 0.671, P = 0.650, for April, August, and October count dates, respectively). For this reason, we analyzed treatment differences between untreated and conglomerated seed across all sites and planting seasons. Plant densities produced by conglomerated seed were consistently higher than plant densities of untreated seed, though differences between the treatments declined over the growing season (Fig. 3). Seedling emergence in spring (April), and the number of plants that survived through summer (August), and to fall (October) was higher in the conglomeration treatment by 60.4% ($P < 0.002$), 39.4% ($P = 0.012$), and 26.3% ($P = 0.049$), than untreated seed, respectively.

Cost Analysis

Assuming Wyoming big sagebrush seed costs are \$27.57 ha^{-1} (0.22 kg PLS ha^{-1}), conglomerating the seed will increase the total cost by 19 % (i.e., cost of seed + conglomeration = \$32.82; Table 3). However, our cost analysis demonstrated that the cost of conglomerates was offset by its higher plant densities (Table 3). Due to the increased plant densities of conglomerated seed, the cost to produce seedlings (plant density from the April count date) and established plants (plant density from the October count date) was reduced by 26 and 6 %, respectively.

Based on measurements of broadcast distance by Hoose et al. (2019), our analysis showed that conglomerates also decrease costs when seeded through a broadcast seeder (Table 3). We estimate that delivery costs (labor and equipment costs) to broadcast untreated seed would be \$38.82 ha^{-1}. Due to a reduction in delivery costs associated with wider broadcast seed distances of conglomerated seed, we estimate that the cost to deliver this treatment would be \$17.36 ha^{-1} (55 % reduction in delivery cost). Based on these estimates, it appears that the cost to broadcast conglomerated seed offsets the cost of the conglomeration material and saves \$16.11 ha^{-1}. The combined savings from the conglomeration technology that is associated with improved establishment and delivery of seed reduces overall costs to produce seedlings and established plants by 54 and 41%, respectively.

Planting Season

We identified a significant interaction between site and planting season ($P = 0.040$, $P < 0.001$, $P < 0.001$, for our April, August, and October count dates, respectively). In April, Fountain Green and Paradise Valley had 122 and 101 % higher plant densities for fall sown seeds than winter sown seeds, respectively (Fig. 4). At Lookout Pass, Santaquin, and Tuscarora seeds sown in the fall and winter had similar plant densities (Fig. 4). While seedling density declined, planting season treatment response between fall and winter plantings remained similar for August and October count dates for all sites except Santaquin. At Santaquin, fall densities increased slightly between April and October, while winter sown densities increased by 86% during this period (Fig. 4). The majority of the increase in plant density occurred between our April and August count dates (data not shown).

DISCUSSION

Species with small seeds and low purity are typically challenging to clean, deliver, and establish (Chambers, 2000; Shaw et al., 2005; Ott et al., 2017), which often results in them being represented at suboptimal rates or excluded altogether from seed mixes (Richards et al., 1998). Conglomeration is a technique that holds promise for addressing these challenges and may allow land managers to implement more diverse seed mixes (Hoose et al. 2019). We evaluated how seed conglomeration technology influences Wyoming big sagebrush seedling emergence and plant establishment, for seed sown in the fall and winter across multiple restoration sites. As hypothesized, the seed conglomeration treatment resulted in higher overall seedling emergence and plant establishment (Fig. 3). Additionally, our results support our hypothesis that the improved efficiency in delivery and higher plant establishment would outweigh the costs to implement the conglomeration technology. Lastly, our hypothesis that seeds sown in the fall would outperform a winter planting was partially supported, but variation in weather conditions between sites produced variable results.

Seed Conglomeration

Improved seedling emergence from the conglomeration treatment may be a result of enhanced seed germination. In general, seed imbibition can be constrained when seeds are broadcast on the soil surface and do not find a "safe site" that protects them from the drying effects of the wind and sun (Harper et al., 1965). Land managers can improve seed-soil contact following a seeding with the use of mechanical treatments such as a land imprinter (Clary, 1989), imprinter wheels (Ott et al., 2017), cultipacker (Ott et al., 2016), or anchor chain (Lysne and Pellant, 2004; Juran et al., 2008). However, these planting techniques are costly, time-intensive, and their use may be constrained by such factors as the site being too steep and/or rocky, high densities of tree skeletons, lack of financial or logistical resources, and cultural constraints (Vallentine, 1989; Bryan et al., 2011). In our study, the conglomeration material (i.e. clays and vermiculate) may have enhanced seed-soil contact, which improved water exchange from soil to seed (Wuest, 2002) and resulted in greater germination.

The reason we saw higher mortality from seedlings grown from conglomerates (Fig. 3) could be because the artificial microsite created by the conglomerate might not have been adequate to support seedling survival through the summer drought period. Schupp (1995) termed this effect where the microsite was capable of allowing for seed germination but not plant survival as "seed-seedling conflict" (Schupp, 1995; Chambers, 2000). In our study, the seed-seedling conflict effect may have been amplified by the below-average late season rainfall experienced during the research, from June to October (Fig. 1). It may be possible that the effects of summer drought, such as what was experienced in this study, could be mitigated by applying treatments within the conglomerate that would enhance seedling survival. The use of arbuscular mycorrhizal fungi is one such treatment that may improve the restoration efforts of Wyoming big sagebrush. Davidson et al. (2016) found that applying arbuscular mycorrhizal fungi to the soil increased Wyoming big sagebrush survival through the summer by 27 %. Future research could evaluate the use of arbuscular mycorrhizal fungi within conglomerates to improve the drought tolerance of seedlings and subsequently minimize seed-seedling conflict effects.

Cost Analysis

Seed conglomeration is a relatively simple and potentially cost-effective solution to address several limiting factors that often exclude Wyoming big sagebrush in restoration projects. Our results suggest that conglomerated seed is a practical treatment when considering the cost to produce individual plants. Due to the higher plant densities associated with the conglomeration treatment, our cost-benefit analysis indicated that it was less expensive to use this technology than to plant with untreated seed (Table 3).

We also found that conglomeration technology can further reduce the cost to produce Wyoming big sagebrush plants on the landscape by decreasing the cost to deliver the seed (Table 3). This level of cost savings could be significant for sagebrush restoration. For example, our research indicated that seed conglomeration can reduce seed delivery costs by \$16.11 ha^{-1}, which for a typical large scale seeding of 10,000 ha, this would result in a savings of over \$160,000. However, there are deficiencies in our cost analysis. Shipping costs were not included in our study due to the high variability that would be associated with where the seed is stored, where the seeds are treated, and the location of the products used to make the conglomerates. Additionally, our cost-analysis only determined the cost savings for a ground broadcast seeder, and not broadcasting from a fixed-wing aircraft or helicopter, which is commonly used for rangeland seeding projects. The additional weight and volume of conglomerated seed may increase their cost for this application. However, the increased weight provided by the conglomerate may compensate for this cost by decreasing seed drift from wind. Future research could identify the limitations of implementing this technology through an aerial broadcast seeder. We also excluded the cost of utilizing a filler material (e.g. rice hulls) with untreated sagebrush seed. It is likely that without a filler material the flow of untreated seed would not have been sufficient to deliver 0.22 kg ha^{-1} at the assumed tractor speed in our cost analysis. Had we included the cost of rice hulls or adjusted the tractor speed for untreated seed the difference in seeding costs would have increased. Hoose et al. (2019) found that conglomerated seed flowed more consistently at higher aperture settings on the seeder over rugged terrain. It is possible that the aperture setting could be opened more for conglomerated seed, which could allow for a hectare to be seeded even faster than our cost analysis predicted. The cost of conglomerates could be reduced further by using more economically friendly materials (Table 2). For example, crushed calcium carbonate could be used to replace some of the Azomite or Swell Clay in the recipe, which is more than three times less expensive. Future studies could focus on incorporating more affordable materials and evaluate their influence on seedling emergence and plant establishment.

Planting Season

The effect of planting season on seeding success varied by site and appears to be strongly associated with near-surface soil moisture and temperature (Fig. 4 and 2). The higher plant density from fall sown seeds at Fountain Green and Paradise Valley might be explained by the different soil temperatures experienced at these two sites compared to the other three sites. Soil temperatures from October to April were generally consistent across all sites, with the exception of early February to late March (Fig 2B). During this period, soil temperatures began to rise for all sites except Fountain green and Paradise Valley, where they maintained temperatures that were at or near freezing. These colder soil temperatures may have provided fall sown seed an advantage at Fountain Green and Paradise Valley by providing longer snow cover than was experienced at the other three sites, which has been shown to improve plant establishment (Maier et al., 2001). In addition to snow cover, the colder soil temperatures may have inhibited early germination at these two sites, which could have reduced seedling mortality from freeze-thaw events. It is also possible that this extended cold event may have negatively impacted the ability for seeds sown in the winter at these two sites to accumulate sufficient hydrothermal time, whereas seeds sown in the fall may have accumulated sufficient time in the fall so they were less affected by this cold event. Wyoming big sagebrush, compared to 10 other native species, requires a relatively long time to accumulate sufficient hydrothermal time to germinate (Richardson et al., 2018). Had soil temperatures from mid-February to mid-March at Fountain Green and Paradise Valley been comparable to soil temperatures experienced at the other sites, seeds sown in the winter may have accumulated sufficient hydrothermal time to have similar plant densities as seeds sown in the fall (Fig. 2). In contrast, precipitation was generally higher than long-term means from February to May (Fig. 1). If precipitation during this period was more characteristic of long-term means (Fig. 1), we may have seen a greater difference between fall and winter plantings across all sites, due to winter not accumulating the necessary time to germinate or germinate late in the spring.

As expected, plant densities decreased throughout the growing season (Fig. 3), with the exception of Santaquin (Fig. 4). At Santaquin, final plant densities (October) were higher than recorded densities in April (Fig. 4). It is possible that at Santaquin, germination could have occurred after our April count as a result of high precipitation during April and May (Fig. 1). Also, evidence that germination could have occurred after our April count date is shown in our soil water potential data, where Santaquin was consistently higher than the other four sites throughout the entirety of the study and had water potential values that appeared to be sufficient for germination (Fig. 2C). The extended spring precipitation events and consistently high water potential may have negated the advantage of a fall planting at Santaquin. In fact, when conditions are adequate for germination until late spring, like experienced at Santaquin, the additional time allotted to seeds in a fall planting could be a disadvantage. It is possible that the period between a fall planting (October) and winter (December) at Santaquin negatively impacted seed viability due to such processes as pathogens, freeze thaw-cycles, and lose of energy reserves. A fall planting is likely to have its greatest utility when spring conditions are not sufficient to produce resilient seedlings to survive summer drought. Despite the high variability in responses between a fall and winter planting, our findings are still important to guide management decisions. Wyoming big sagebrush is typically seeded in the winter while a majority of native species are sown in the fall (Monsen and Stevens, 2004). Our results suggest that Wyoming big sagebrush could be included in a fall seeding schedule and likely produce similar results or better than a winter seeding, which could reduce costs associated with another seeding event.

MANAGEMENT IMPLICATIONS

Seed conglomeration technology is a valuable tool for land managers to cost-effectively address limitations in seed cleaning, delivery, and plant establishment. The conglomeration technique evaluated in this study potentially addresses several limiting factors facing Wyoming big sagebrush restoration. For example, cleaning Wyoming big sagebrush can be a long and costly process, which rarely yields high purity seeds. This technique could reduce seed cleaning requirements by incorporating seed and non-seed parts together within a conglomerate. Cost savings can be found by the conglomeration technique allowing for consistent seed delivery over rugged terrain (Hoose et al., 2019) and greater seed broadcast distance. We also demonstrated that the cost to deliver and successfully produce seedlings is less expensive for conglomerates because they have higher plant establishment. This technology could potentially serve as a seed treatment for other low-purity native species such as, thickleaf penstemon (*Penstemon pachyphylus* [A. Gray]), mountain big sagebrush (*Artemisia tridentata* Nutt. spp. *vaseyana* [Beetle]), basin big sagebrush (*Artemisia tridentata* Nutt. spp. *tridentata),* black sagebrush (*Artemisia nova* [A. Nelson]), spiny hopsage (*Grayia spinose* [Hook]), and yellow rabbitbrush (*Chrysothamnus viscidiflorus* [Hook]). Our findings also suggest that in most cases, a fall seeding of Wyoming big sagebrush was either the same or more successful compared to planting on snow in the winter, which is the current suggested practice. Because most rangeland seedings with other species occur in the fall, seeding Wyoming big sagebrush also during this period could reduce the costs of another seeding event.

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TABLES

Table 2-1. Site characteristics of Lookout Pass, Santaquin, Fountain Green, Tuscarora, and Paradise Valley

Site	Soil Map	Soil Texture (%)			Soil pH	Avg. Precip.	Aspect	Slope	Elevation
		Clay	Sand	Silt		(mm)		(%)	(m)
Lookout Pass	Hiko Peak-Taylorsflat complex	14	44.8	41.2	8.2	287	SE	3.2	1665
Santaquin	Donnardo stony loam	20	42.1	37.9	7.6	481	NW	6.5	1620
Fountain Green	Mountainville cobbly fine sandy loam hardpan variant	12.5	70.9	16.6	7.6	332	S	4.0	1730
Tuscarora	Buffaran-Zevadez association	14	62	24	7.2	312	NE	1.9	1637
Paradise Valley	Hunnton-Goosel-Connel association	15	44.3	40.7	7.5	408	S	4.8	1720

Table 2-2. Estimates for producing sagebrush conglomerates broken down by the cost to purchase the materials and to produce conglomerates. Estimates are provided on a cost per kg of seed^{-1} and cost ha^{-1}, assuming a seeding rate of 0.22 kg ha^{-1}

Item	**Cost kg seed^{-1}**	**Cost ha^{-1}**
Azomite	$12.36	$2.77
Swell Clay	$1.78	$0.40
Vermiculite	$6.08	$1.36
Binder	$0.64	$0.14
Production Cost	$3.00	$0.67
Total	$23.86	$5.35

Table 2-3. Comparison of the cost in seed (and materials for conglomerates), delivery, and the combination of seed cost and delivery cost for untreated and conglomerated seed. Estimates were based on the amount of seed commonly used to plant one hectare. From these costs, we also estimated the price to produce 1,000 seedlings (April count date), and to produce 1,000 established plants (October count date).

	Seed Costs			Delivery Cost			Combined Cost (seed & delivery)		
Treatment	**(ha^{-1})**	**To produce 1,000 seedlings**	**To produce 1,000 established plants**	**(ha^{-1})**	**To produce 1,000 seedlings**	**To produce 1,000 established plants**	**(ha^{-1})**	**To produce 1,000 seedlings**	**To produce 1,000 Established plants**
Untreated	$27.57	$4.37	$4.74	$38.82	$6.76	$7.34	$63.88	$11.13	$12.08
Conglomerate	$32.82	$3.24	$4.47	$17.36	$1.89	$2.60	$47.19	$5.13	$7.07
Change	19%	-26%	-6%	-55%	-72%	-65%	-26%	-54%	-41%

FIGURES

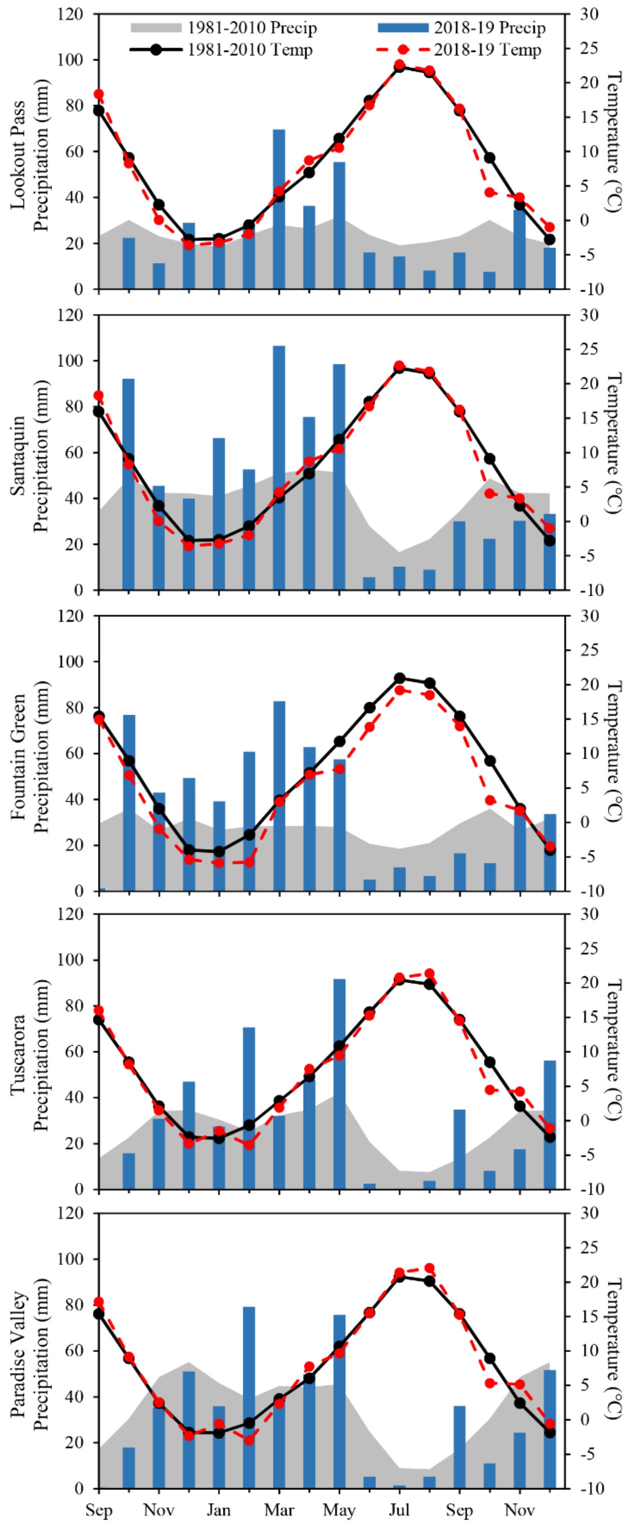

Figure 2-1. A comparison of precipitation and ambient temperatures experienced during the duration of the study (2018-2019) compared to long-term averages (1981-2010) at all five sites.

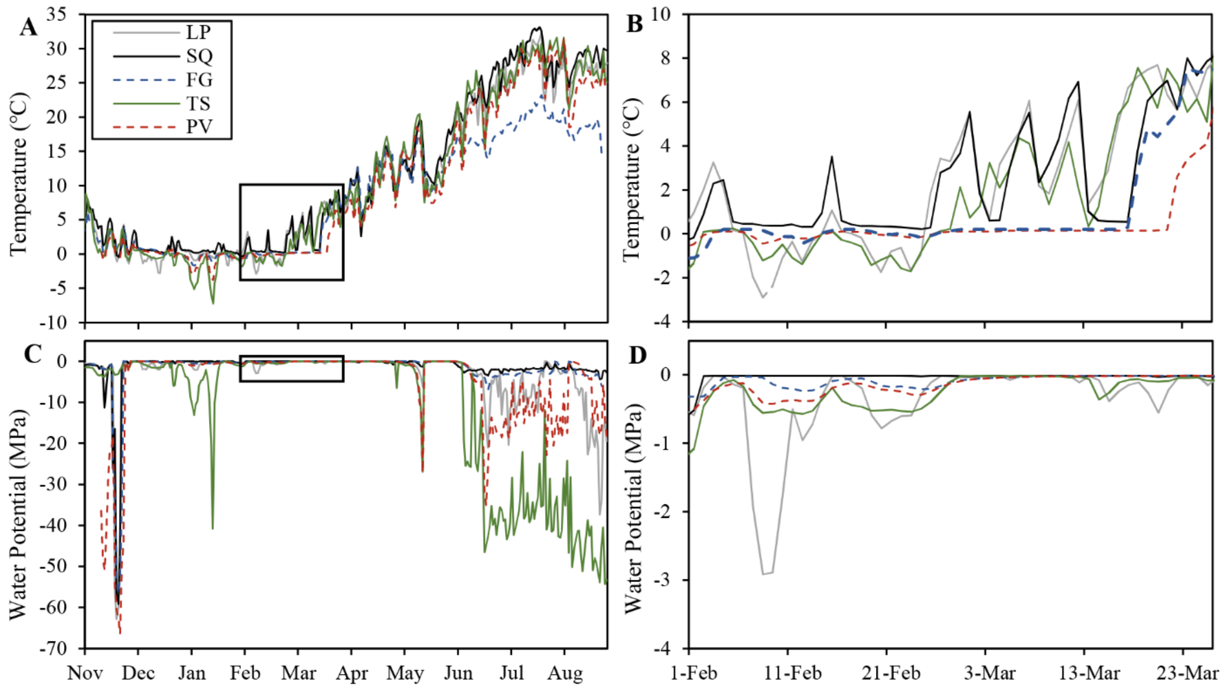

Figure 2-2. Average daily values of soil temperature and water potential at 1 cm below the soil surface at Lookout Pass (LP), Santaquin (SQ), Fountain Green (FG), Tuscarora (TS), and Paradise Valley (PV). Soil temperature and water potential values are displayed from November to August (A&C), and highlighted between February and March (B&D).

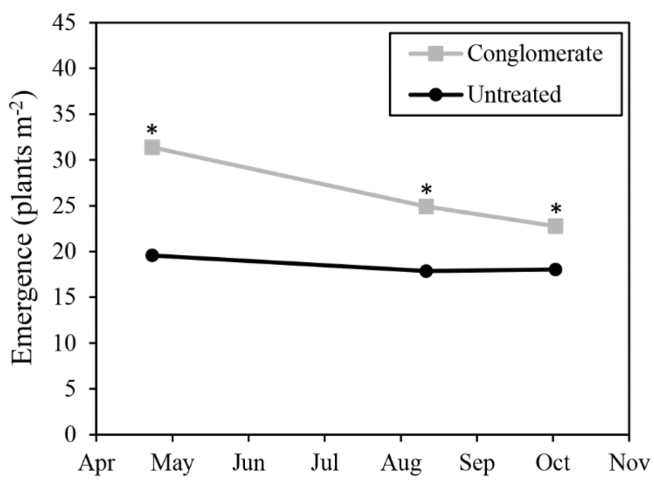

Figure 2-3. Plant density of untreated and conglomerated Wyoming big sagebrush seed averaged across both planting dates. Statistical differences ($P < 0.05$) are indicated by asterisk marks (*).

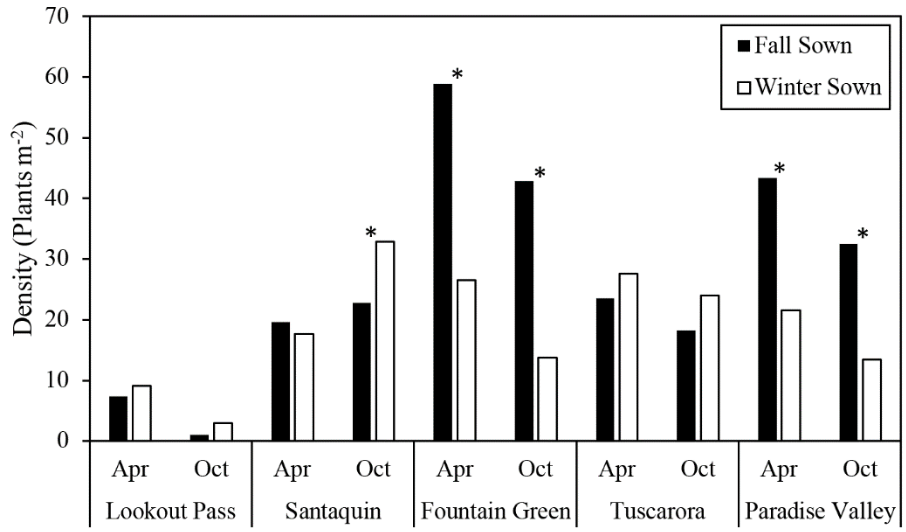

Figure 2-4. Plant density in April and October of seeds sown in the fall and winter across all five sites.