Methods for Improving Re-Vegetation Success in the Sagebrush Steppe using Solid Matrix Priming and Seed Extrusion Technology

A Thesis
Presented in Partial Fulfillment of the Requirements for the
Degree of Master of Science
with a
Major in Natural Resources
in the
College of Graduate Studies
University of Idaho
by
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May 2019

Authorization to Submit Thesis

This thesis of Samuel J. Price submitted for the degree of Master of Science with a Major in Natural Resources and titled "Methods for Improving Re-Vegetation Success in the Sagebrush Steppe using Solid Matrix Priming and Seed Extrusion Technology" has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

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Abstract

There is a need to develop cost-effective techniques for re-establishing native vegetation on degraded sagebrush (*Artemesia* spp. L.) steppe ecosystems. In this study, we evaluate the use of solid matrix priming (SMP) and extruded seed pellets as technologies for improving re-vegetation success within the sagebrush steppe for three native bunchgrass species: bluebunch wheatgrass (*Pseudoroegneria spicata, PSSP6*), Idaho fescue (*Festuca idahoensis, FEID*), and prairie junegrass (*Koeleria macrantha, KOMA*). This involved: 1) the formulation of seed priming medium, 2) the incorporation of priming medium and native seed into pellets designed for transportation and subsequent planting for re-vegetation purposes, 3) the determination of optimal seed priming durations at two water potentials (-0.7 & -1.0 MPa), and 4) a comparison of total emergence and time to 10, 25, and 50% emergence for primed seeds vs. non-primed seeds. Primed pelleted treatments for FEID and PSSP6 showed increases in total emergence within the first seven days compared to non-primed pellets. By day seven, FEID and PSSP6 primed pelleted emergence was 8.4–22 and 31.6–58.5 seedlings, and non-primed pellet emergence was 0.2–6.8 and 19.3–31 seedlings ($P<0.05$). KOMA showed mixed results concerning emergence in the first seven days between primed treatments and non-primed pellets. Further experimentation showed that KOMA total germination, not emergence, was increased by day seven with priming (primed germination = 12.1–16.3 seeds, bare seed germination = 3.1–5.7 seeds; $P<0.05$). Further comparison between primed treatments and non-primed pellets showed a reduction in time to 10% and 25% emergence (T_{10}, T_{25}) for PSSP6 only (T_{10}: primed emergence = 2.0–4.1 days, non-primed pellet emergence = 4.4–6.1 days; T_{25}: primed emergence = 2.9–5.4 days, non-primed pellet emergence = 6–7.5; $P<0.05$). Three out of six PSSP6 primed treatments also showed a reduction in time to 50% emergence (T_{50}: primed emergence = 6.9, 7.1, 8.4 days; non-primed pellet emergence = 19.7 days, $P<0.05$). T_{10} and T_{25} was not affected by priming in FEID or KOMA. FEID and KOMA bare seed also never reached T_{25} within the 21-day study period and so comparisons were not possible. The pellet materials provided an increase in total emergence over 21 days of growth for FEID and KOMA (emergence at day 21: FEID bare seed emergence = 10 seedlings, non-primed pellet emergence = 20 seedlings; KOMA bare seed emergence = 2 seedlings, non-primed pellet emergence = 26 seedlings, $P<0.05$). PSSP6 emergence results showed little positive effect coming from the pellet materials throughout the 21-day study period. In general, solid matrix priming and extruded seed pellets allowed for an increase in total emergence within the first seven days of growth for FEID, KOMA and PSSP6, and a reduction in T_{10}, T_{25}, and T_{50} emergence for PSSP6. These increases in emergence within the first seven days of growth may provide an increase in the number of wet-thermal days available for emerged seedlings prior to the onset of adverse environmental conditions (i.e. winter/summer). Implications of these results would

be a potential increase in the survival and establishment for a greater percentage of wildland seedings for these three species.

Acknowledgements

This work was supported by the USDA National Institute of Food and Agriculture, McIntire Stennis project 1009836.

Two representatives from KALO inc. worked with us to identify three rates of the soil surfactant Tournament-Ready® that would be safe for direct contact with seed.

- Bill Cosdon: Northwest Business Manager at KALO inc. Overland Park, Kansas
- Mike Edens: Western Regional Sales Manager at KALO inc. Overland Park, Kansas

Don Regan: Manager at the Franklin H. Pitkin Forest Nursery, Moscow, Idaho

Jason Karl, Professor of Rangeland Ecology and Harold F. and Ruth M. Heady Endowed Chair of Rangeland Ecology at the University of Idaho, Moscow, Idaho

Robert Heinse: Associate Professor & Interim Director of Water Resources in the Department of Soil and Water Systems at the University of Idaho, Moscow, Idaho

Shaun Bushman: Research geneticists, USDA-ARS Forage & Range Research Lab, Logan, Utah

Todd Harris: President of Western Reclamation, Inc., Eltopia, Washington

Tom Jones: Research geneticist, USDA-ARS Forage & Range Research Lab, Logan, Utah

Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

Catastrophic wildfires occur more frequently and at larger scales than in previous decades in the American west, causing mass degradation to the sagebrush (*Artemesia* spp. L.) steppe and other rangeland ecosystems (Connelly et al. 2004; Eiswerth et al. 2009; Abatzoglou & Kolden 2011). Wildfires, among other disturbances, act as vectors for exotic annual plant invasion (e.g., cheatgrass (*Bromus tectorum* (L.)) & medusahead (*Taeniatherum caput-medusae* (L.) Nevski) into sagebrush steppe ecosystems (Epanchin-Niell et al. 2009), destroy greater sage-grouse (*Centrocercus urophasianus*) habitat (Connelly et al. 2004), and limit forage available for livestock and wildlife grazing (Madsen et al. 2016). Community recovery following disturbances can be slow (MacGillivray et al. 1995), and without intervention can remain or shift towards degraded states (Larson et al. 2015). And so, reseeding rangelands (i.e., re-vegetating rangelands) following disturbance has become a key tool that land managers use to stabilize soils, inhibit the spread of invasive plants, and restore ecosystem function (Eiswerth et al. 2009; Larson et al. 2015). Reseeding efforts, however, are an expensive practice that often fails to meet desired outcomes (Knutson et al. 2014). Slow germination at low temperatures from native species in combination with other abiotic factors, such as variable precipitation, topography and soil conditions common to rangelands may be accredited for the low success rates following seeding efforts (James et al. 2011). Until recently, research has primarily been focused on the mechanistic side of re-vegetating, such as improving seed drill technology (Madsen et al. 2016; Ott et al. 2016). This research has improved our ability to deliver seeds in rangeland systems, however, seedling establishment is still poor. As federal land management agencies increase their use of native plant species in their seed mixes (Thompson et al. 2006) it will be increasingly important to better understand how ecological processes and mechanisms are driving native seed recruitment on re-vegetation sites.

For seeded natives the transition between germination and emergence has often been identified as a critical life stage transition for most species (James et al. 2011; Larson et al. 2015; Svejcar at al. 2017). It is well known that only a small fraction of seeds sown for re-vegetation purposes go on to establish (Chambers 2000; Knutson et al 2014). In regard to germination, native perennial species can take 10-15 days to germinate (Rawlins et al. 2012). Once germinated, adverse soil and environmental conditions can lead to seedling mortality prior to emergence, when the radicle is especially vulnerable to pathogen attacks and drying out. In a review of dormant fall seedings (November – December), Svejcar et al. (2017) stated that upwards of 70% of seeds germinated, but never emerged, prior to the onset of winter, which resulted in these germinated seeds incurring high

rates of mortality due to frozen soils and other abiotic and biotic limitations. Chambers (2000) showed under a fall planting, there was as little as 2% emergence in the spring, and for seeds carried over from the fall to spring, Pyke et al. (1990) showed high rates of native seedling mortality in the first summer after seeding (≈ 85%). Larson et al. (2015) found that over 90% of mortality in native seeds was explained by the transition from germination to emergence. James et al. (2011) showed similar results finding that, in large, the death of seeds sown was after germination but before emergence. The fate for the bulk of seeds sown in the fall appears to be inevitable mortality. Is the time between germination and emergence simply too long for seeded natives? Could the total time needed to emerge be too long? Chambers (2000) noted that once seedlings had emerged, survival appeared to increase dramatically (of the < 2% of seeds sown that emerged, 54% survived on to the end of the 2-year study).Larson et al. (2015) also noted and that emerged seedlings appeared more resistant to a wider range of precipitation scenarios. If the transition from germination to emergence is a primary bottleneck preventing the establishment of seeded natives, and if seed survival increases once emerged, then more rapid and complete emergence may increase the likelihood of survival for greater percent of seeded natives.

Cheatgrass, has proven to be a troublesome weed across the American west largely due to its exceptional emergence characteristics at low temperatures in the spring. Cheatgrass seems to do best when it can germinate and emerge in the fall, overwinter in a dormant state, and resume growth with the onset of warmer conditions in the spring (Klemmedson and Smith 1964). Mack and Pyke (1983) found that death among cheatgrass seedlings emerging in the fall (late September – October) was extremely low, and that seedlings which emerged in the fall and survived through to the spring often produced the highest abundance of seeds. Cheatgrasses ability to emerge after the onset of fall rains, produce biomass, and survive through the winter and into spring has made it a tough competitor on rangelands across the American west. Is it possible then, to mimic this kind of growth cycle for native seedings? It may be likely that rapid emergence after the onset of fall rains would allow for native seeds to accrue the biomass necessary to survive the winter, and, like cheatgrass, emerge in the spring with the onset of warmer conditions.

Prior to emergence, however, there are many germination processes that must occur. The maturation drying of seeds, causes damage to seed cell membranes and organelles. Upon rehydration, these membranes and organelles must be repaired prior to germination. Once imbibed, metabolic activity resumes, and repair begins. Early stages of germination consist of mitochondrial repair and protein synthesis before DNA repair and cell division can take place (Bewley 1997). The time it takes for these processes to occur is variable from species to species and can take several days to weeks to

complete themselves before radicle extension and seedling emergence (Nonogaki et al. 2010). For exotic annual species the rate of germination is often higher than that of native perennial species (Hardegree et al. 2010; Rawlins et al. 2012; Wainwright & Cleland 2013). Wainwright and Cleland (2013) looked at germination rate from a resource capture standpoint and showed that the increased germination rate in exotic annual species, in combination with higher germination plasticity, provided them with a competitive advantage over native perennial species in the early spring. Seed priming is one potential solution that allows early germination processes in seeds to take place prior to sowing, and possibly increase germination rate in seeded native perennial species (Taylor et al. 1988).

Osmopriming and solid matrix priming (SMP) are two seed priming methods which have been identified to increase germination rate (Taylor et al. 1988). Osmopriming involves soaking the seed in an aerated solution containing either inorganic salts, or polyethylene glycol (PEG). The seed and solution are then kept at a specific water potential and temperature for a defined amount of time (Hardegree 1996). This method effectively allows the seed to complete the early phases of germination (i.e. imbibition, mitochondrial repair, protein synthesis, DNA repair, and DNA synthesis; Bewley 1997), which result in a more rapid germination (Wu et al. 2001). Osmopriming, however, comes with several practical and technical limitations. The priming solution can easily become too concentrated, negatively affecting seed germination. For this reason, priming solution measurements must be precise. There are also considerable differences between priming osmoticum. Inorganic salts are much smaller compared to PEG and can penetrate through the seed coat adversely affecting seed germination. PEG is larger and incapable of penetrating the seed coat, however, it is associated with negative effects relating to seed germination if not completely removed from the seed post-priming. Costs are increased for the use of PEG through the safe disposal of the product after priming (Taylor et al. 1988). As costs and complexity increase, the potential for osmopriming seeds on a commercial scale decrease. Hence, a method is needed that can be completed on a commercial scale that is both cost-efficient and practical.

One other possible method for increasing germination rate is SMP. SMP involves mixing the seed into a solid matrix at a defined water potential. This mixture is then held at a specific temperature for a pre-determined duration so that the seed can complete the early phases of germination (i.e., is primed). SMP has several advantages over osmotic priming including ease of handling, increased aeration, and the possible inclusion of biological agents along with other beneficial additives (Wu et al. 2001). The major limitation concerning SMP involves the mechanical separation of the solid matrix material from the seed without harming them, after priming is complete. This limitation may be alleviated if the seeds could be effectively planted within the SMP medium.

Furthermore, if the SMP material were to enhance germination and seedling growth through the creation of an optimal microenvironment, seeding efforts could be further improved. Madsen & Svejcar (2016) showed that seeds could be placed together in extruded pellets using machinery for making pastas and pastries. Pellets are formed by creating a "dough" containing seed, various clay filler materials, absorbent materials, bio-stimulants, plant protectants, water, and other desired ingredients. The mixture is then passed through an extruder that forms and cuts the extruded material into desired shapes. Seed pellets are designed for broadcast application or drill seeding, improving seed coverage and enhancing conditions for seed germination and growth (Madsen et al. 2016).

SMP and extruded seed pellets represent two distinct seed technologies. Primed seed pellets are then a combination of the two. The purpose of this study is to develop methods for increasing re-vegetation success of three bunchgrass species native to the sagebrush steppe using SMP and seed extrusion technologies. Specific objectives include:

1. Create a SMP material which can be combined with seed and used to form extruded seed pellets.
2. Define optimal priming conditions for three species of bunchgrass native to the sagebrush steppe at two water potentials.
3. Compare seedling emergence and time to 10, 25, and 50% emergence between primed seeds within pellets and unprimed seeds within pellets in rangeland soils.
4. Compare seedling emergence and time to 10, 25, and 50% emergence between non-primed seeds within pellets and bare seed (untreated control) in rangeland soils.

We compared primed pellets to non-primed pellets, and non-primed pellets to bare seed separately to look at the individual effects coming from the priming process and pellet materials themselves. Conclusions will be made as to possible additive effects of combining the two technologies.

Specific hypotheses include:

H_1: The time necessary to emerge to 10%, 25% and 50% total emergence will be less for primed-pelleted seed than for non-primed pelleted seed.

H_2: Total emergence from day to day throughout the first seven days of growth will be higher for primed-pelleted seed than for non-primed pelleted seed.

H_3: The time necessary to emerge to 10%, 25% and 50% total emergence will be less for non-primed pelleted seeds than for non-pelleted bare seed.

H_4: Non-primed pelleted seeds will have greater total emergence than bare seed over 21 days of growth.

Chapter 2: Methods and Materials

Species

The species presented below are all components of a healthy rangeland ecosystem within the Snake River Plains or greater Great Basin. Their adaptation to various site conditions, as well as their tolerance to fire and drought make them ideal species for re-vegetating degraded rangelands.

Bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Love, PSSP6) is a long lived cool-season perennial bunchgrass common to the northern Great Plains and Intermountain regions of the western United States. It is adapted to a wide range of soil and site conditions receiving 25-50 cm of annual precipitation. It is cold tolerant, drought tolerant, moderately shade tolerant and fire tolerant (Ogle 2002). PSSP6 has been identified by the USDA NRCS as palatable for all classes of livestock and wildlife, as well as, preferred forage at various times of the year. Due to its long-lived nature and extensive root structure it is also adapted extremely well for soil stabilization (Ogle 2002). PSSP6 used for this study was collected from Sheepshead Mountain, Oregon, and purchased from BFI Native Seeds in Moses Lake, Washington (Lot #: BFI-17-24189757).

Idaho fescue (*Festuca idahoensis* Elmer, FEID) is a long lived cool-season perennial bunchgrass common to the western United States, however, is rare or absent in the American southwest. FEID is adapted to a wide range of soil and site conditions receiving 35-50 cm of annual precipitation. It is tolerant of cold, drought, shade and fire (Ogle et al. 2007). The USDA-ARS has identified it is fair to good forage for all types of livestock, and good year around forage for wildlife. It's long-lived nature and extensive root structure also make it well adapted to soil stabilization (Ogle et al. 2007). FEID used for this study was collected from Winchester, Idaho, and purchased from BFI Native Seeds in Moses Lake, Washington (Lot #: Bfi-15-11147661).

Prairie junegrass (*Koeleria macrantha* (Ledeb.) J.A. Schultes, KOMA) is a medium lived cool-season perennial bunchgrass commonly found on rangelands at elevations above 1200 m (Ogle et al. 2006). The USDA-NRCS has identified it as fair forage for both livestock and wildlife in the spring and early summer. KOMA is a drought and fire tolerant species (Ogle et al. 2006). KOMA used for this study was collected from the Ochoco National Forest, Oregon, and purchased from BFI Native Seeds in Moses Lake, Washington (Lot #: BFI-17-121575611).

Germination Trials

Germination test were conducted for each species to determine the germination percentage, which dictates pure live seed (PLS) for a particular seedlot. For the germination test, twenty seeds

were placed into 15 cm petri dishes containing a single layer of white blotter paper soaked in deionized water. Seeds were spaced evenly across the bottom of the petri dishes, labeled, and then placed into the growth-chamber where they were incubated at 15°C with a 12 hour/day light cycle. For each species, germination tests were replicated 5 times (for a total of 100 seeds). The number of seeds germinated were counted once daily for 21 days total. Seeds were considered germinated once the radicle had extended at least 2.0 mm out of the seed. Petri dishes were rotated to different shelves within the growth chamber throughout the trial. Blotter paper was re-moistened as needed using a pipette to drop water around the edges of the blotter paper.

Pellet Formulation

Many commercial operations already use SMP techniques for priming seeds. Of those contacted (a list of operators contacted is found in Appendix A), the primary components of their priming mixture consisted of vermiculite and peat/sphagnum moss. A minor third component (often <10%) in these priming mixtures was a wetting agent (i.e., surfactant). One such example is the priming media used by the University of Idaho Franklin H. Pitkin Forest Nursery, which consists of 45% vermiculite, 45% peat/sphagnum moss, and 10% wetting agent by weight. Nurseries and seed production companies that did not prime used similar soilless media for planting. This mixture of ingredients is inexpensive, easy to source, and already used to grow seedlings for commercial purposes. By using these same materials that are already being used on a commercial scale for our seed priming research, methods developed are more cost effective and practical for commercial production. Aside from being readily available, once dispersed in the field, vermiculite and peat/sphagnum moss may increase soil porosity, nutrient availability (via high cation exchange capacitates), and water holding capacity within a microsite (Yong & Warkentin 1975; Hudson 1994).

The ideal pellet needs to be rigid enough to withstand transportation and planting, have high moisture holding capacity, and readily dissolve in the environment once planted. The priming media for our SMP study consisted of a 1:1 ratio of vermiculite to peat/sphagnum moss by weight, bentonite clay, fine sand, powder fungicide (CapTan) and a liquid surfactant (Tournament-Ready®) (Table 1). Peat moss and vermiculite were ground to <2mm prior to inclusion in the mixture. No previous SMP research using the soil surfactant Tournament-Ready® from KALO, Inc. Overland Park, Kansas had been done prior to this study. Tournament-Ready is a long-lasting ionic surfactant for use in controlling dry spots, improving infiltration/drainage in hydrophobic soils, and enhancing soil microbes. Tournament-Ready is typically used to treat golf courses and lawns, however, has been shown to be highly effective at treating soil water repellency and increasing water infiltration (Madsen et al. 2013). Experimentation with and without surfactant showed that a rate of 350 µL

surfactant for every 120 g of dry priming media had no effect on germination, and positively influenced both the pellets ability to absorb moisture, as well as readily dissolve. The above ingredients and their amounts were determined through an iterative process of trial and error. Recipes were created, formed into pellets, tested, and ranked to achieve desired pellet attributes.

The pH of this priming media and triple distilled water was tested using a Fisher Scientific AB15 pH meter and found to be on average 5.07. Triple distilled water is recommended for use with the Fisher Scientific AB15 pH meter. Although the effect of this pH on seed priming is unknown, the use of comparable media with similar pH values is used by several commercial scale operators. The low pH of this priming media may serve as a pH buffer when distributed on rangeland sites where the soil pH is high (> 7).

Pellets were created using a hard-plastic form measuring 15 x 15 cm (Fig. 1). The form consisted of two parts, a bottom form 2 cm thick with twenty 1.25 cm diameter holes drilled into it, and a top form with twenty 1.25 cm diameter pegs measuring 4 cm long. Media and seed were spread evenly across the bottom form filling the holes, and the top form was set on top. Approximately 20 psi was then applied to the top form for 1 minute to achieve desired compression. The product was a 1.25 x 1.25 cm pellet (diameter x height; Fig. 2). Pellets were formed and dried in an oven at 25°C for 2 hours before they were stored in a humidity-controlled fridge at 5°C in paper bags. Pellets for all studies were stored for 7 days before use.

The rigidity of the pellet is a critical aspect of a recipe, so only recipes that maintained greater than 90% of their structure after the rigidity test were tested for their ability to absorb moisture and dissolve in the environment. To test the recipes rigidity, twenty pellets were placed in a paper bag and shaken for one minute. Before and after weights using only whole, intact pellets were used. This was done 5 times for each recipe, averaged, and ranked. For the shake test, effort was made to simulate the same conditions for each recipe. To test the pellets ability to absorb moisture, a water drop test was used. Water droplets were dropped onto 5 pellets of equal size for each recipe, the number of water droplets absorbed were recorded for each pellet and an average for each recipe was ranked. Pellets of equal size were then placed into weigh boats with 5, 10, and 15 mL of water. To test the abilities to dissolve, using a stop watch, the amount of time required for 50% of the pellet to dissolve was recorded. This was repeated five times for each recipe. The average time for each recipe was then ranked. A total of 35 recipes were evaluated using a combination of all three test described above to determine the most suitable recipe.

Moisture Release Curve & SMP

To achieve the desired water potentials within the priming media, a moisture release curve was created for values between -0.6 MPa to -1.6 MPa using a Decagon dew-point potentiometer WP4-C. The WP4-C uses a chilled mirror dew point technique to measure water potential in MPa. The Decagon WP4-C has difficulty reading samples when they are too wet, so our curve was stopped at -0.6 MPa when variation in readings began to increase and our curve flattened out. The WP4-C was calibrated using potassium chloride verification standards purchased from Decagon after every 5th sample. To create the curve, dry priming media was mixed and portioned into 5 g samples. Different percentages of DI water were added to the media based on dry weight ranging from 15% to 60%, and then capped and sealed with parafilm for a period of twenty-four hours to acclimate to laboratory temperatures. After the 24-hour period, a sample was removed and placed into a WP4-C sample cup. Each moisture content was repeated five times. The percent moisture and measured water potentials were then be used to plot a regression line and determine the moisture content necessary to achieve the desired water potential within the priming media (Fig. 3). For this study, the preferred water potentials were identified as -0.7 MPa and -1.0 MPa.

Optimal Priming Duration

Optimal priming duration for this study was defined as the time necessary, at a specific water potential, for a species to reach ≈ 10% germination within the priming medium. All three species were primed in plastic containers measuring 25 x 14 x 7.5 cm (length x width x depth) at water potentials of -0.7 and -1.0 MPa. Each priming container had a lid which was sealed with parafilm once priming began. Priming containers were weighed daily to ensure that moisture loss was negligible. Priming containers were kept sealed in a growth chamber at 15°C with 12-hour light intervals for the priming duration.

Starting on day three of the priming process, a 30g subsample of the priming material was removed and all seeds within the subsample examined for coleorhizae protrusion. Priming media for each subsample was discarded after the seeds were extracted. The total number of seeds and the number of seeds with coleorhizae protrusion were recorded to determine percent germination at that point in time. This process was done once daily to determine the optimal priming duration that seeds needed to achieve ≈ 10% germination (Fig. 4). The observed optimal priming durations varied for each species/water potential combination. In an effort to assure accuracy, optimal priming durations ± 12 hours were used in the emergence study.

Comparison of Seedling Emergence in a Growth Chamber

Once optimal priming durations were chosen, each species/water potential combination were tested in a growth chamber to discern differences with respect to time to 10, 25 and 50% emergence (T_{10}, T_{25}, T_{50}) and total emergence at daily intervals. Primed treatments were tested against non-primed pellets, and non-primed pellets were tested against bare seed for each species. Planting trays measuring 24 cm X 50 cm were filled with 5 cm of autoclaved field soil (hand-textured as "loamy"). Field soil was collected from a Wyoming big sagebrush (*Artemisia tridentata* ssp. *Wyomingensis* Beetle & Young) and Great Basin wild rye (*Leymus cinereus* (Scribn. & Merr.) Á. Löve) plant community south of Kuna, Idaho (43.37382, -116.40616). Field capacity for this soil was determined to be 20% moisture content ((wet wt. – dry wt.)/dry wt.). Trays were watered to field capacity before treatments were sown into rows at 1.25 cm depths in a randomized block design with 6 replicates or blocks (Fig. 5). The "=RAND()" statement was used in Excel (Excel version 1902, updated 2018) to generate a list of random number between 0 and 1 for each block. Numbers and their corresponding treatments were sorted from smallest to largest and placed into their corresponding rows within each block (Table 2). Rows included 8 pellets with ≈ 11, 10, and 7 (PSSP6, FEID, KOMA) PLS seeds/pellet for each treatment. Bare seed rows received the same number of PLS/row depending on species. Every 4th day, trays were watered to field capacity. Once trays were sown, they were incubated within a growth chamber at 20°C with 12-hour light intervals. Trays were moved daily throughout the growth chamber during this experiment from top to bottom. This experiment ran for 21 days.

Total emergence was a cumulative count of daily emergence. T_{10}, T_{25}, and T_{50} emergence was calculated from daily emergence counts using the following equation:

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

where:

T = time (days) to subpopulation emergence

t_a = day when subpopulation emergence was reached (e.g. 10, 25 or 50%)

t_b = day before subpopulation emergence was reached (e.g. 10, 25 or 50%)

n_a = number of emerged seeds on day that subpopulation emergence was reached

n_b = number of emerged seeds on day before subpopulation emergence was reached

N = number of emerged seeds equal to 10, 25, or 50% of the total population

Comparison of KOMA germination inside of a growth chamber

A separate experiment was conducted for KOMA alone prompted by the results of the seedling emergence experiment. This experiment was designed to assess the effect of priming on KOMA germination. Seeds were primed at two water potentials (-0.7 & -1.0 MPa) for two durations (7.5 & 10 days) as they were before, dried, and stored at 5°C for one week. Seeds were later separated from the media and placed in petri dishes lined with moistened blotter paper (20 seeds/petri dish; 5 petri dishes/treatment; 3 treatments total). Pellets were not used for this experiment. This experiment was conducted inside of a growth chamber at 15°C with 12-hour light intervals alongside unprimed seeds and ran for a total of 21 days.

Chapter 3: Analysis

All data was analyzed in R version 3.5.1. All percentages referenced in the results were calculated based on shortest/greatest distances from confidence interval edges from raw total cumulative emergence for a single day. For primed treatments, a linear mixed-effects model (LMER) and ANOVA analysis for significant effects was used to analyze differences in cumulative total emergence and T10, T25, and T50 between primed treatments and non-primed pellets from day to day (H1, H2). In the model, seed treatments were considered a fixed factor and blocks a random factor. When significant effects were found, least square means were separated using the EMMEANS procedure with P-values adjusted using a Tukey test ($P<0.05$). Only the non-primed pellet treatments and bare seed were used to determine whether the pellet itself influenced total cumulative emergence or T10, T25, T50. The analysis of T10, T25, T50 requires that at least three subpopulations of each treatment have positive values in order to generate means and confidence intervals. Several treatments did not meet these requirements within the 21-day study period and comparisons were not possible for those treatments. A linear mixed-effects model (LMER) and ANOVA analysis for significant effects was used to analyze differences. When significant effects were found, least square means were separated using the EMMEANS procedure with P-values adjusted using a Tukey test ($P<0.05$).

Chapter 4: Results

The Priming Effect: Primed Pellets vs. Non-primed Pellets (H1 & H2)

Time to T_{10} and T_{25} emergence was reduced for primed treatments of PSSP6 compared to non-primed pellets (Table 3). T10 and T25 for PSSP6 non-primed pellets was 7–67% and 10–61% slower than primed treatments, respectively (T10: primed = 2.0–4.1 days, non-primed pellet = 4.4–6.1 days; T25: primed = 2.9–5.4 days, non-primed pellet = 6–7.5 days; $P<0.05$; Table 3). T_{10} and T_{25} for all PSSP6 primed treatments and non-primed pellets occurred in the first seven days of growth. PSSP6 was the only species which reached T_{50} within the 21-day study period. Three out of six primed treatments of PSSP6 (-1.0 MPa 8.5 and 9 incubation days (id), -0.7 MPa 4.5 id; Table 3) reached T_{50} earlier than non-primed pellets (T_{50}: primed: 6.9, 7.1, 8.4 days; non-primed pellet: 19.7 days, $P<0.05$; Table 3). T_{10} and T_{25} for FEID were not less for primed treatments compared to non-primed pellets (ANOVA analysis, T_{10}: $P=0.69$, T_{25}: $P=0.87$). No FEID treatments reached T_{10} or T_{25} within the first seven days of growth (Table 3). T_{10} and T_{25} for KOMA were not less for primed treatments vs. non-primed pellets. Two primed treatments of KOMA (-1.0 MPa 10 id, and -0.7 MPa 7 id) reached T_{10} in less than 7 days (4.3 ± 2.1 days, 3.6 ± 1.8 days; Table 3).

By day four of growth, primed treatments of PSSP6 showed higher total emergence compared to non-primed pellets (primed emergence = 13.1–37.5 seedlings, non-primed pellet emergence = 0–6.9 seedlings; $P<0.05$; Table 4). PSSP6 non-primed pellet emergence was between 2–67% lower than primed treatments at day seven (primed emergence = 31.6–58.5 seedlings, non-primed pellet emergence = 19.3–31 seedlings; $P<0.05$; Table 4). This trend was similar, but less pronounced in FEID and KOMA. Significant differences in total emergence between FEID primed treatments and non-primed pellets did not occur until day five for four out of the six primed treatments (-1.0 MPa 6.5, 7, and 7.5 id, -0.7 MPa 6.5 id; Table 4). FEID non-primed pellet emergence was between 19–98% lower than these four primed treatments on day seven (primed emergence = 8.4–22 seedlings, non-primed pellet emergence = 0.2–6.8 seedlings; $P<0.05$; Table 4). Primed KOMA had a single treatment (-1.0 MPa 10 id; Table 4) which had higher total emergence on days 4 and 5, however, by day seven KOMA non-primed pellet emergence was not different from five out of six primed treatments, and had higher emergence than one primed treatment (-0.7 MPa 8 id emergence = 3.7 ± 3.8 seedlings, non-primed pellet emergence = 16 ± 3.9 seedlings; $P<0.05$; Table 4). Non-primed KOMA total emergence ranged from 12.1–19.9 seedlings by day seven, whereas primed treatments ranged from 0–16.9 seedlings (Table 4).

The Pellets Effect: Non-primed Pellet vs. Bare Seed (H3 & H4)

PSSP6 non-primed pellets showed a significant decrease in T_{10} emergence over bare seed (T_{10}: non-primed pellet = 4.4–6.1 days, bare seed = 7–8.6 days, $P<0.05$; Table 5). PSSP6 T_{25} was not different between non-primed pellets and bare seed. PSSP6 bare seed did not achieve T_{50} within the 21-day study period so a comparison was not possible (T_{50}: non-primed pellet = 13.9–25.5 days; Table 5). FEID non-primed pellets showed no decreases in T_{10} compared to bare seed (FEID T_{10}: non-primed pellet = 7.1–9.2 days, bare seed = 5.1–9.9 days; Table 5). FEID bare seed did not reach T_{25} within the 21-day study period and so a comparison was not possible (FIED T_{25}: non-primed pellet = 4–21.1 days; Table 5). KOMA non-primed pellets showed no decrease in T_{10} compared to bare seed (KOMA T_{10}: non-primed pellet = 4.5–7.9 days, bare seed = 5.8–12.4 days; Table 5).KOMA bare seed never achieved T_{25} and so a comparison between the two was not possible (KOMA T_{25}: non-primed pellet = 4.9–9.2 days; Table 5).

A mixed-effects model analysis of non-primed pellet vs. bare seed showed an increase in total emergence over 21 days for the non-primed pellets in both FEID and KOMA (Tables 6). FEID non-primed pellet emergence was greater from days 16–21 (emergence at day 16: bare seed = 11.2 seedlings, non-primed pellet = 11.5 seedlings, emergence at day 21: bare seed = 10 seedlings, non-primed pellet = 20 seedlings, $P<0.05$; Table 6). KOMA non-primed pellet emergence was greater from days 8–21 (emergence at day 8: bare seed= 3 seedlings, non-primed pellet = 24.7 seedlings, emergence at day 21: bare seed = 2 seedlings, non-primed pellet = 26 seedlings, $P<0.05$; Table 6). PSSP6 non-primed pellets showed intermittent increases in emergence compared to bare seed, however, the effect was short-lived (emergence at day 6: bare seed = 0–2.2 seedlings, non-primed pellet = 14.1–29.9 seedlings; emergence at day 21: bare seed = 46 seedlings, non-primed pellet = 40 seedlings; Table 6).

The Priming Effect on KOMA Germination

The results of the separate experiment for KOMA alone showed significant increases in total germination starting on day three and continuing to day seven (germination at day 3: primed = 8.1–12.5 seeds, bare seed = 0 seeds; germination at day 7: primed = 12.1–16.3 seeds, bare seed = 3.1–5.7 seeds; $P<0.05$; Table 7). The analysis also showed significant decreases in T10, T25, and T50 germination for primed treatments compared to bare seed (T10: primed = 1.9–2.3 days, bare seed = 4.9–5.3 days; T25: primed = 2–2.4 days, bare seed = 6.1–6.6 days; T50: primed = 2.3–3 days, bare seed = 7.7–8.2 days; $P<0.05$; Table 8).

Chapter 5: Discussion

In order for seeded natives to take advantage of the short time frames in which there is adequate surface soil moisture available for germination and emergence, they must germinate and emerge rather quickly. Chambers (2000) noted that sufficient soil moisture remained in the near surface soil layer (0-2 cm) for only a short period of time in which germination and initial root elongation could occur. Compared to depths below the surface layer (< 2cm), soil moisture is more stable and available for longer indicating a higher potential for growth and possibly survival (Donovan & Ehleringer 1994). Hence, emerged seedlings can begin accessing deeper, less variable, reservoirs of soil moisture making them more tolerant of a wider range of precipitation scenarios. These findings suggest that earlier and greater emergence provided by priming and seed extrusion technology could potentially increase the likelihood of survival for a greater percentage of seeded natives.

Primed Treatments vs. Non-primed Pellets

We emphasized the first seven days of growth in this study and assumed that seeds which germinated outside of the first seven days would be less fit to survive adverse environmental conditions typical of rangelands. Other studies have shown that SMP reduces the time to germination and increases germination velocity (Hardegree 1996; Madsen et al. 2018), this study showed that SMP also has a positive effect on total emergence within the first seven days of growth. Primed treatments, with the exception of KOMA, on average began emerging earlier and reached higher total emergence compared to non-primed pellets within the first seven days of growth (Table 5). KOMA primed treatments began emerging earlier than non-primed pellets, however, total emergence by day seven was similar to non-primed pellets (Table 5). If seeds are germinating in the fall but not emerging (Svejcar et al. 2017), then the earlier and higher emergence exhibited by primed seeds in the first seven days of growth may overcome that barrier. Primed seeds sown in the fall may be more likely to germinate and emerge than bare seed prior to the onset of winter due to a reduction in the number of wet-thermal days needed for germination. The development of biomass before the arise of frozen winter conditions, however, may be a critical aspect as to whether emerged seedlings survive through to the spring.

No one priming water potential or priming duration for any species appeared to outperform any other regarding total emergence, T_{10}, T_{25}, or T_{50} (Tables 3 & 4). Madsen et al. (2018) showed that germination velocity would increase with priming duration. Madsen et al. (2018) noted that drier priming conditions (-1.5, -2.0, -2.5 MPa) primed for longer durations tended to produce the fastest germinating seeds. This study used water potentials of -0.7 and -1.0 MPa for priming. In the future,

dryer priming conditions and longer incubation periods might allow for a greater increase in emergence velocity over a shorter period of time. Water was also applied every four days in this study, so soil moisture was relatively available for seed germination on a consistent basis for both primed and non-primed treatments. Future studies should consider watering regimens designed to limit the availability of moisture in the top 2 cm of soil. This might produce differences in water potential and priming duration among primed treatments in order to determine the most competitive combination for each species.

Non-primed Pelleted Treatments vs. Bare Seed

The increases in total emergence over 21 days observed from FEID and KOMA shows that the pellet materials provided some positive benefit (Table 6) that may help transition seeds from germination to emergence. This transition has been identified as the primary bottleneck to establishment in many studies (James et al. 2011; Larson et al. 2015; Svejcar et a. 2017). For example, James et al. (2011) stated that pathogen attack prior to emergence, when the radicle is most vulnerable, likely account for a great deal of mortality. Chambers (2000) stated that moisture availability in the top 2 cm of soil was the most likely cause of mortality in germinated seeds. The inclusion of ingredients in our extruded seed pellets, designed to limit possible negative affects stemming from these sources of mortality, may have helped to limit mortality and improve emergence in non-primed pelleted seeds. The inclusion of a powder fungicide and peat/sphagnum moss, along with vermiculite, may protect the radicle from pathogen attacks, as well as retain moisture within the microsite for longer, thus protecting the radicle from drying out prior to emergence. It should be noted that in this study, we were unable to observe any mortality associated with the transition from germination to emergence.

Pellet Materials

Soils typical of rangelands often form hard crusts near the surface. We observed that as the pellets swelled with moisture, they elevated themselves to the surface breaking through the soils physical crust. The soil surfactant used, aside from improving the pellets ability to absorb moisture and dissolve in the environment, may also help to increase water infiltration surrounding the pellet. The materials used to make extruded pellets along with the properties of the pellets themselves may have help to overcome some barriers to emergence.

Looking forward, other possible benefits of the pellet materials themselves may be related to an increased cation exchange capacity surrounding the seed. Organic matter, like peat/sphagnum moss is known for its high cation exchange capacity, and vermiculite has been said to have the one of

the highest cation exchange capacities of any clay (Yong & Warkentin 1975). Further research would be needed to assess the different possible effects coming from the pellet itself.

The Curious Success of Non-primed KOMA

We questioned if KOMA hadn't responded to the priming process positively. Since all treatments were planted at the same depth, we did not consider planting depth a source of possible error affecting these results.

We determined from the results of this experiment that priming does have a positive effect on germination within the first seven days of growth for KOMA. Results of the pellet effect analysis showed that KOMA non-primed pellets had a significant increase in total emergence over 21 days (Table 6). We cannot ascertain at this point where the potential error resulting in the significant success of the non-primed pelleted treatment over all other treatments, in regard to emergence, may have come from.

Conclusion

The findings of this study support evidence from previous studies which utilized SMP and seed extrusion technology to improve germination rate, and microsite characteristics, along with improving total emergence within the first seven days of growth. SMP and seed extrusion technologies represent another tool that land managers can utilize to prevent the conversion of degraded landscapes to cheatgrass dominated monocultures, stabilize soils, and provide ecosystem function. Current research is looking at the effect of priming on seeded native emergence and biomass accumulation within a greenhouse setting. Field studies using these technologies are needed prior to their adoption in re-vegetation efforts. Further research and incorporation of seed technologies like time-delayed seed coatings and herbicide protected pellets alongside SMP may further provide a buffer to complete re-vegetation failures. Though seed technologies, such as the ones presented here, represent an extra up-front cost to land managers looking to re-vegetate degraded landscapes, the immediate costs may be alleviated by increases in re-vegetation success on disturbed rangeland sites.

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Tables and Figures

Figure 1: Forms (15 cmx15cm) used to create extruded seed pellets, alongside extruded pellets.

Figure 2: Extruded pellets measuring approximately 1.25 x 1.25 cm on average (diameter x height).

Table 1: SMP medium ingredients and amounts used to create one batch of extruded seed pellets (≈ 100) under optimal priming conditions.

Ingredients		
Product	Supplier	Weight
Peat Moss	Premier Tech LTD (Riviere-du-Loup, Quebec)	15 (g)
Vermiculite	Therm-O-Rock West Inc. (Chandler, AZ)	15 (g)
Bentonite Clay	Western Bentonite (Guthrie, OK)	45 (g)
Fine Sand	Sakrete (Charlotte, NC)	45 (g)
Fungicide	Bonide Products Inc. (Oriskany, NY)	1 (g)
Surfactant	KALO inc. (Overland Park, KS)	350 (µL)

Priming water potential	Water content	Water required for priming
Mpa	%	(g)
-0.7	33	40
-1	26	32

species	seed required (g)
PSSP6	7.5
FEID	1.4
KOMA	0.41

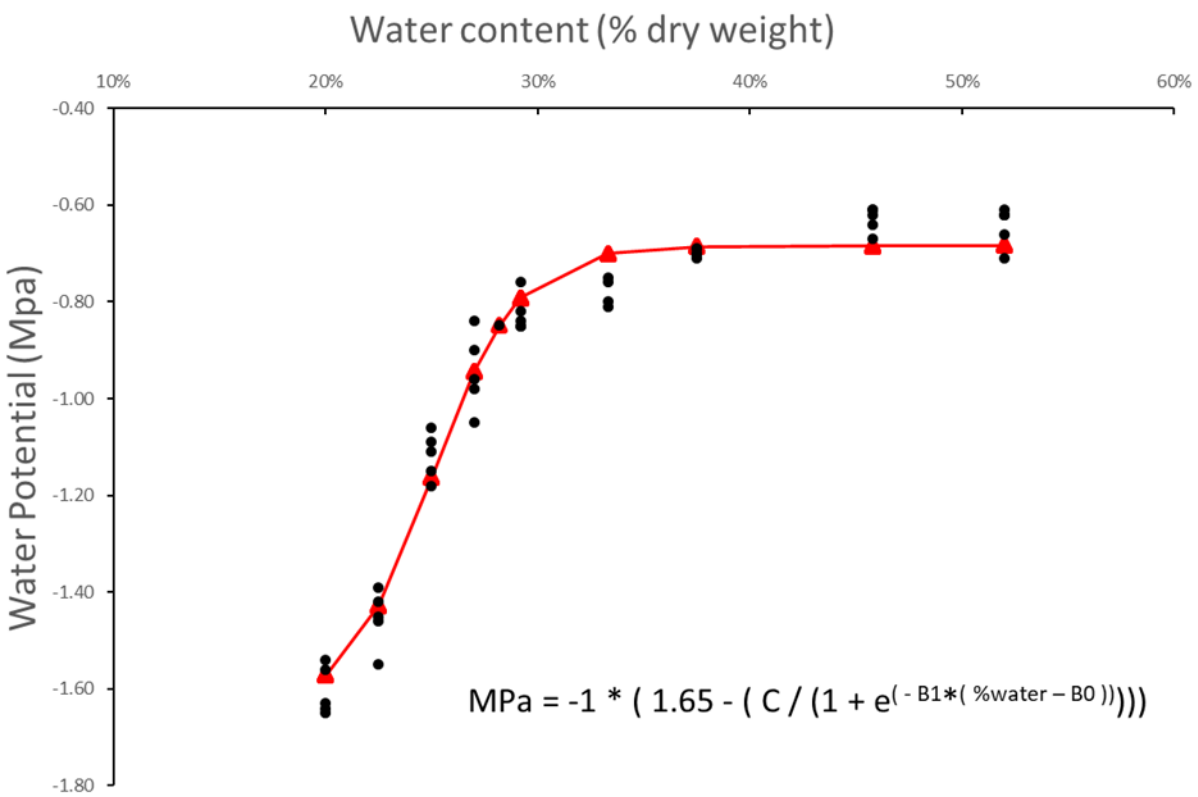

Figure 3: Moisture release curve developed for priming medium using a Decagon WP4-C Dewpoint Potentiometer. Water content (wet weight-dry weight)/dry weight)x100) is expressed along the x-axis. Water potential (MPa) values provided by the WP4-C expressed along the y-axis. The formula generated was used to estimate -0.7 and -1.0 MPa (C = maximum asymptote; B1 = rate of increase; B0 = lag). Black circles represent values generated by the WP4-C. Red triangles represent mean values at each point estimated using the associated formula.

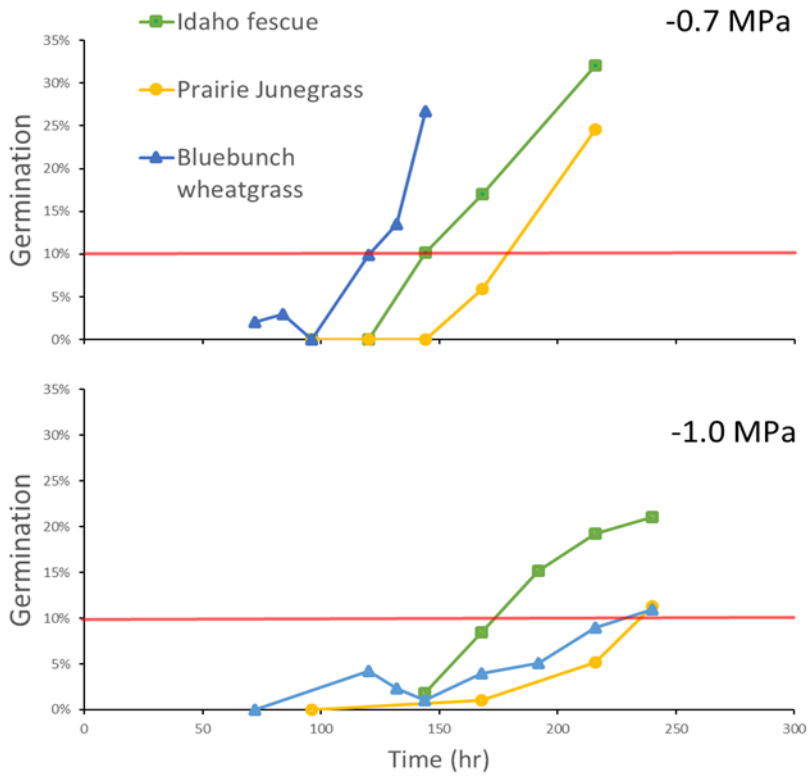

Figure 4: Germination curves for three species of bunchgrass at two water potentials (-0.7 & -1.0 MPa) with time (hrs) along the x-axis and germination (%) within the subsample (germinated seeds/total seeds)x100). Optimal priming duration is defined as the time necessary to reach 10% within the priming medium for this study. The red horizontal line indicates 10% germination.

Box Design (Placement of treatments)

1	2	3	4	5	6	7	8	9	10	11	12	13
14	15	16	17	18	19	20	21	22	23	24	blank (25)	blank (26)

Figure 5: Randomized block design. Rows (24) were systematically placed within the tray, treatments were randomly assigned to one row. Two blank rows were used to measure water added across the tray and were also randomly placed.

Table 2: Treatments for each block were randomized with 24 treatments and 2 blank rows. Below is one example. Treatments are read as follows: species, priming water potential (MPa), priming duration with p1 being the shortest and p3 being the longest.

Row	Treatment
1	KOMA, -.70, p1
2	KOMA, -1.0, p3
3	FEID, -.70, p2
4	Blank row (nothing planted)
5	Blank row (nothing planted)
6	PSSP, -1.0, 93
7	PSSP, -1.0, p1
8	PSSP, non-primed pellet
9	KOMA, -1.0, p2
10	PSSP, -.70, p3
11	FEID, -1.0, p1
12	FEID, -1.0, p2
13	FEID, bare seed
14	FEID, -1.0, p3
15	PSSP, bare seed
16	KOMA, bare seed
17	KOMA, -.70, p2
18	KOMA, non-primed pellet
19	FEID, -.70, p1
20	PSSP, -.70, p1
21	PSSP, -.70, p2
22	KOMA, -.70, p3
23	KOMA, -1.0, p1
24	FEID, non-primed pellet
25	PSSP, -1.0, p2
26	FEID, -.70, p3

Table 3: The effect of priming water potential (ψ) and incubation period (days) on time to 10, 25, and 50% emergence (T_{10}, T_{25}, T_{50}) for three species of bunchgrass. KOMA = *Koeleria macrantha*, FEID = *Festuca idahoensis*, PSSP6 = *Pseudoroegneria spicata*. Too few subpopulations of *Festuca idahoensis* and *Koeleria macrantha* emerged to 50% to analyze T_{50} for those two species.

			——— T_{10} ———				——— T_{25} ———				——— T_{50} ———			
Species	Ψ	Incubation Period (days)	Mean Days to 10%	Lower.CL	Upper.CL	Group	Mean Days to 25%	Lower.CL	Upper.CL	Group	Mean Days to 50%	Lower.CL	Upper.CL	Group
Pseudoroegneria spicata		non-primed pellet	5.3	4.4	6.1	B	6.8	6.0	7.5	B	19.7	13.9	25.5	B
	-0.7 Mpa	4.5	2.9	2.1	3.7	A	3.6	2.9	4.4	A	8.4	4.4	12.5	A
		5.0	3.1	2.3	3.9	A	4.2	3.5	5.0	A	10.0	5.0	15.0	AB
		5.5	3.3	2.5	4.1	A	4.6	3.9	5.4	A	12.5	8.5	16.6	AB
	-1.0 Mpa	8.5	2.8	2.0	3.6	A	3.7	2.9	4.4	A	6.9	2.6	11.2	A
		9.0	3.2	2.3	4.0	A	4.2	3.5	5.0	A	7.1	2.0	12.1	A
		9.5	3.1	2.3	4.0	A	4.3	3.5	5.0	A	11.3	6.2	16.3	AB
Festuca idahoensis		non-primed pellet	8.2	4.7	11.7	A	13.7	8.0	19.3	A	-	-	-	-
	-0.7 Mpa	5.5	7.5	4.0	11.0	A	12.2	5.3	19.2	A	-	-	-	-
		6.0	9.0	5.8	12.1	A	8.2	1.2	15.1	A	-	-	-	-
		6.5	6.6	3.1	10.1	A	9.7	4.7	14.6	A	-	-	-	-
	-1.0 Mpa	6.5	6.7	3.6	9.9	A	10.0	5.6	14.3	A	-	-	-	-
		7.0	4.6	1.5	7.8	A	10.1	6.1	14.1	A	-	-	-	-
		7.5	6.8	3.6	10.0	A	11.8	6.9	16.8	A	-	-	-	-
Koeleria macrantha		non-primed pellet	6.2	4.5	7.9	A	7.1	4.9	9.2	A	-	-	-	-
	-0.7 Mpa	7.0	4.3	2.2	6.4	A	6.6	3.9	9.3	A	-	-	-	-
		7.5	5.3	3.5	7.2	A	4.9	1.5	8.3	A	-	-	-	-
		8.0	7.9	5.4	10.3	A	-	-	-	-	-	-	-	-
	-1.0 Mpa	9.5	5.2	3.3	7.0	A	5.3	2.6	8.0	A	-	-	-	-
		10.0	3.6	1.7	5.4	A	4.9	2.5	7.4	A	-	-	-	-
		10.5	5.1	3.3	7.0	A	10.6	6.7	14.5	A	-	-	-	-

Lower.CL = lower edge of its confidence interval
Upper.CL= upper edge of its confidence interval
Group = letters represent significant differences within species ($P \leq 0.05$)

Table 4: The effect of water potential (Ψ) and Incubation period (days) on cumulative emergence for primed treatments vs. non-primed pellets over the first seven days of growth for three species of bunchgrass. KOMA = *Koeleria macrantha*, FEID = *Festuca idahoensis*, PSSP6 = *Pseudoroegneria spicata*.

			Day 1				Day 2				Day 3				Day 4				Day 5				Day 6				Day 7			
species	Ψ	Incubation Period (days)	Mean Emergence	Lower.CL	Upper.CL	Group	Mean Emergence	Lower.CL	Upper.CL	Group	Mean Emergence	Lower.CL	Upper.CL	Group	Mean Emergence	Lower.CL	Upper.CL	Group	Mean Emergence	Lower.CL	Upper.CL	Group	Mean Emergence	Lower.CL	Upper.CL	Group	Mean Emergence	Lower.CL	Upper.CL	Group
Pseudoroegneria spicata		non-primed pellet	0.0	-	-	-	0.0	-	-	-	0.0	-	-	-	1.0	-4.9	6.9	A	6.8	1.0	12.7	A	17.8	12.0	23.7	A	25.2	19.3	31.0	A
	-0.7 Mpa	4.5	0.0	-	-	-	0.5	-5.4	6.4	A	9.7	3.8	15.5	AB	31.7	25.8	37.5	C	44.8	39.0	50.7	C	51.2	45.3	57.0	D	52.7	46.8	58.5	C
		5	0.0	-	-	-	0.5	-5.4	6.4	A	6.8	1.0	12.7	AB	21.2	15.3	27.0	BC	32.7	26.8	38.5	B	37.2	31.3	43.0	BC	40.7	34.8	46.5	B
		5.5	0.0	-	-	-	0.7	-5.2	6.5	A	5.0	-0.9	10.9	AB	19.0	13.1	24.9	B	27.7	21.8	33.5	B	34.7	28.8	40.5	B	37.5	31.6	43.4	B
	-1.0 Mpa	8.5	0.0	-	-	-	1.5	-4.4	7.4	A	12.8	7.0	18.7	B	30.5	24.6	36.4	C	37.7	31.8	43.5	BC	46.0	40.1	51.9	CD	46.7	40.8	52.5	BC
		9	0.0	-	-	-	1.0	-4.9	6.9	A	5.8	0.0	11.7	AB	23.8	18.0	29.7	BC	36.3	30.5	42.2	BC	40.3	34.5	46.2	BC	42.8	37.0	48.7	BC
		9.5	0.0	-	-	-	0.0	-	-	-	7.0	1.1	12.9	AB	21.5	15.6	27.4	BC	28.0	22.1	33.9	B	36.3	30.5	42.2	BC	38.8	33.0	44.7	B
Festuca idahoensis		non-primed pellet	0.0	-	-	-	0.0	-	-	-	0.0	-	-	-	0.0	-	-	-	0.2	-3.1	3.5	A	1.3	-2.0	4.6	A	3.5	0.2	6.8	A
	-0.7 Mpa	5.5	0.2	-3.4	3.8	A	0.2	-3.4	3.8	A	0.8	-2.8	4.4	A	3.0	-0.6	6.6	A	3.4	-0.2	7.0	AB	7.8	4.2	11.4	AB	8.6	5.0	12.2	AB
		6	0.0	-	-	-	0.2	-3.1	3.5	A	1.0	-2.3	4.3	A	3.2	-0.1	6.5	A	6.5	3.2	9.8	ABC	9.5	6.2	12.8	BC	9.8	6.5	13.1	AB
		6.5	0.0	-	-	-	0.5	-2.8	3.8	A	1.3	-2.0	4.6	A	4.0	0.7	7.3	A	8.0	4.7	11.3	BC	10.2	6.9	13.5	BC	12.5	9.2	15.8	BC
	-1.0 Mpa	6.5	0.0	-	-	-	0.0	-	-	-	0.7	-2.6	4.0	A	3.5	0.2	6.8	A	7.8	4.5	11.1	BC	9.7	6.4	13.0	BC	11.8	8.5	15.1	B
		7	0.0	-	-	-	0.7	-2.6	4.0	A	2.5	-0.8	5.8	A	6.0	2.7	9.3	A	11.7	8.4	15.0	C	15.8	12.5	19.1	C	18.7	15.4	22.0	C
		7.5	0.0	-	-	-	0.3	-3.0	3.6	A	1.7	-1.6	5.0	A	4.7	1.4	8.0	A	8.7	5.4	12.0	BC	10.2	6.9	13.5	BC	11.7	8.4	15.0	B
Koeleria macrantha		non-primed pellet	0.0	-	-	-	0.0	-	-	-	0.0	-	-	-	0.3	-3.5	4.2	A	3.0	-0.9	6.9	AB	9.0	5.1	12.9	AB	16.0	12.1	19.9	C
	-0.7 Mpa	7	0.0	-	-	-	0.7	-3.2	4.5	A	2.2	-1.7	6.0	A	4.3	0.5	8.2	AB	8.3	4.5	12.2	AB	10.2	6.3	14.0	AB	11.3	7.5	15.2	ABC
		7.5	0.0	-	-	-	1.0	-2.9	4.9	A	1.7	-2.2	5.5	A	4.5	0.6	8.4	AB	8.0	4.1	11.9	AB	8.5	4.6	12.4	AB	8.8	5.0	12.7	ABC
		8	0.0	-	-	-	0.0	-	-	-	0.3	-3.5	4.2	A	1.5	-2.4	5.4	AB	2.7	-1.2	6.5	A	3.5	-0.4	7.4	A	3.7	-0.2	7.5	A
	-1.0 Mpa	9.5	0.0	-	-	-	0.7	-3.2	4.5	A	2.2	-1.7	6.0	A	4.7	0.8	8.5	AB	8.7	4.8	12.5	AB	9.2	5.3	13.0	AB	9.8	6.0	13.7	ABC
		10	0.0	-	-	-	1.8	-2.0	5.7	A	3.3	-0.5	7.2	A	8.5	4.6	12.4	B	10.7	6.8	14.5	B	12.7	8.8	16.5	B	13.0	9.1	16.9	BC
		10.5	0.0	-	-	-	0.7	-3.2	4.5	A	1.2	-2.7	5.0	A	3.2	-0.7	7.0	AB	5.7	1.8	9.5	AB	6.8	3.0	10.7	AB	7.5	3.6	11.4	BC

Lower.CL = lower edge of its confidence interval
Upper.CL= upper edge of its confidence interval
Group = letters represent significant differences for each species within days ($P \leq 0.05$)

Table 5: The effect of priming water potential (ψ) and incubation period (days) on time to 10, 25, and 50% emergence (T_{10}, T_{25}, T_{50}) for non-primed pellets vs. bare seed for three species of bunchgrass. KOMA = *Koeleria macrantha*, FEID = *Festuca idahoensis*, PSSP6 = *Pseudoroegneria spicata*. Too few subpopulations of *Festuca idahoensis* and *Koeleria macrantha* emerged to 50% to analyze T_{50} for those two species.

		------ T_{10} ------				------ T_{25} ------				------ T_{50} ------			
Species	Incubation Period (days)	Mean Days to 10%	Lower.CL	Upper.CL	Group	Mean Days to 25%	Lower.CL	Upper.CL	Group	Mean Days to 50%	Lower.CL	Upper.CL	Group
Pseudoroegneria spicata	bare seed	7.8	7.0	8.6	B	8.3	6.7	9.8	A	11.6	-	-	-
	non-primed pellet	5.3	4.4	6.1	A	6.8	5.4	8.1	A	19.7	13.9	25.5	A
Festuca idahoensis	bare seed	7.5	5.1	9.9	A	12.9	-	-	-	-	-	-	-
	non-primed pellet	8.2	7.1	9.2	A	12.6	4.1	21.1	A	-	-	-	-
Koeleria macrantha	bare seed	7.5	5.8	9.1	A	-	-	-	-	-	-	-	-
	non-primed pellet	6.2	4.5	7.9	A	7.1	4.9	9.2	A	-	-	-	-

Lower.CL = lower edge of its confidence interval
Upper.CL= upper edge of its confidence interval
Group = letters represent significant differences for each species within time to emergence category ($P \leq 0.05$)

Table 6: A comparison of non-primed pelleted seed vs. bare seed control emergence over 21 days for three species of bunchgrass. KOMA = *Koeleria macrantha*, FEID = *Festuca idahoensis*, PSSP6 = *Pseudoroegneria spicata*.

Festuca idahoensis

	Bare Seed				Non-primed Pellet			
Day	Mean Emergence	Lower CL.	Upper CL.	Group	Mean Emergence	Lower CL.	Upper CL.	Group
1	0.0	-	-	-	0.0	-	-	-
2	0.0	-	-	-	0.0	-	-	-
3	0.0	-	-	-	0.0	-	-	-
4	0.0	-	-	-	0.0	-	-	-
5	0.2	-7.2	7.5	A	0.2	-7.2	7.5	A
6	0.7	-6.7	8.0	A	1.3	-6.0	8.7	A
7	1.5	-5.9	8.9	A	3.5	-3.9	10.9	A
8	4.7	-2.7	12.0	A	7.5	0.1	14.9	A
9	6.8	-0.5	14.2	A	12.5	5.1	19.9	A
10	7.8	0.5	15.2	A	13.8	6.5	21.2	A
11	7.8	0.5	15.2	A	13.2	5.8	20.5	A
12	7.7	0.3	15.0	A	14.5	7.1	21.9	A
13	8.2	0.8	15.5	A	14.8	7.5	22.2	A
14	8.2	0.8	15.5	A	15.5	8.1	22.9	A
15	11.3	4.0	18.7	A	17.0	9.6	24.4	A
16	11.2	3.8	18.5	A	18.8	11.5	26.2	B
17	11.2	3.8	18.5	A	19.2	11.8	26.5	B
18	11.2	3.8	18.5	A	19.2	11.8	26.5	B
19	10.7	3.3	18.0	A	19.2	11.8	26.5	B
20	10.5	3.1	17.9	A	19.5	12.1	26.9	B
21	10.0	2.6	17.4	A	20.0	12.6	27.4	B

Koeleria macrantha

	Bare Seed				Non-primed Pellet			
Day	Mean Emergence	Lower CL.	Upper CL.	Group	Mean Emergence	Lower CL.	Upper CL.	Group
1	0.0	-	-	-	0.0	-	-	-
2	0.0	-	-	-	0.0	-	-	-
3	0.0	-	-	-	0.0	-	-	-
4	0.0	-	-	-	0.7	-13.5	14.9	A
5	0.0	-	-	-	2.3	-11.9	16.5	A
6	0.7	-13.5	14.9	A	11.3	-2.9	25.5	A
7	0.0	-14.2	14.2	A	13.3	-0.9	27.5	A
8	3.0	-11.2	17.2	A	24.7	10.5	38.9	B
9	1.3	-12.9	15.5	A	24.7	10.5	38.9	B
10	4.7	-9.5	18.9	A	35.0	20.8	49.2	B
11	1.7	-12.5	15.9	A	23.7	9.5	37.9	B
12	4.7	-9.5	18.9	A	35.7	21.5	49.9	B
13	1.3	-12.9	15.5	A	28.7	14.5	42.9	B
14	4.7	-9.5	18.9	A	34.3	20.1	48.5	B
15	2.0	-12.2	16.2	A	27.3	13.1	41.5	B
16	6.0	-8.2	20.2	A	36.7	22.5	50.9	B
17	2.7	-11.5	16.9	A	29.7	15.5	43.9	B
18	5.7	-8.5	19.9	A	36.0	21.8	50.2	B
19	2.3	-11.9	16.5	A	30.7	16.5	44.9	B
20	5.3	-8.9	19.5	A	36.7	22.5	50.9	B
21	2.0	-12.2	16.2	A	26.0	11.8	40.2	B

Pseudoroegneria spicata

	Bare Seed				Non-primed Pellet			
Day	Mean Emergence	Lower CL.	Upper CL.	Group	Mean Emergence	Lower CL.	Upper CL.	Group
1	0.0	-	-	-	0.0	-	-	-
2	0.0	-	-	-	0.0	-	-	-
3	0.0	-	-	-	0.0	-	-	-
4	0.0	-	-	-	1.0	-6.9	8.9	A
5	2.0	-5.9	9.9	A	5.0	-2.9	12.9	A
6	2.3	-5.6	10.2	A	22.0	14.1	29.9	B
7	19.7	11.8	27.6	A	20.7	12.8	28.6	A
8	10.0	2.1	17.9	A	35.3	27.4	43.2	B
9	42.3	34.4	50.2	B	30.3	22.4	38.2	A
10	18.3	10.4	26.2	A	42.0	34.1	49.9	B
11	42.3	34.4	50.2	A	31.7	23.8	39.6	A
12	19.7	11.8	27.6	A	42.3	34.4	50.2	B
13	48.3	40.4	56.2	B	34.7	26.8	42.6	A
14	21.0	13.1	28.9	A	43.0	35.1	50.9	B
15	48.3	40.4	56.2	B	36.0	28.1	43.9	A
16	24.7	16.8	32.6	A	43.0	35.1	50.9	B
17	49.0	41.1	56.9	A	39.0	31.1	46.9	A
18	26.0	18.1	33.9	A	42.7	34.8	50.6	B
19	47.7	39.8	55.6	A	41.3	33.4	49.2	A
20	24.0	16.1	31.9	A	43.3	35.4	51.2	B
21	45.7	37.8	53.6	A	40.3	32.4	48.2	A

Lower.CL = lower edge of its confidence interval

Upper.CL= upper edge of its confidence interval

Group = letters represent significant differences within species ($P \leq 0.05$)

Table 7*:* The effect of water potential (Ψ) and Incubation period (days) on cumulative germination for primed treatments vs. bare seed over the first seven days of growth for KOMA (*Koeleria macrantha*).

			--------- Day 1 ---------				--------- Day 2 ---------				--------- Day 3 ---------				--------- Day 4 ---------				--------- Day 5 ---------				--------- Day 6 ---------				--------- Day 7 ---------			
species	Ψ	Incubation Period (days)	Mean Emergence	Lower.CL	Upper.CL	Group	Mean Emergence	Lower.CL	Upper.CL	Group	Mean Emergence	Lower.CL	Upper.CL	Group	Mean Emergence	Lower.CL	Upper.CL	Group	Mean Emergence	Lower.CL	Upper.CL	Group	Mean Emergence	Lower.CL	Upper.CL	Group	Mean Emergence	Lower.CL	Upper.CL	Group
Koeleria macrantha	Bare seed		0.0	-	-	-	0.0	-	-	-	0.0	-	-	-	0.0	-	-	-	1.0	-0.3	2.3	A	2.8	1.5	4.1	A	4.4	3.1	5.7	A
	-0.7 Mpa	7.5	0.0	-	-	-	0.0	-	-	-	9.4	8.1	10.7	B	11.0	9.7	12.3	B	12.8	11.5	14.1	B	13.0	11.7	14.3	B	13.4	12.1	14.7	B
	-1.0 Mpa	10.0	0.0	-	-	-	0.0	-	-	-	11.2	9.9	12.5	B	13.0	11.7	14.3	B	14.8	13.5	16.1	B	14.8	13.5	16.1	B	15.0	13.7	16.3	B

Lower.CL = lower edge of its confidence interval
Upper.CL= upper edge of its confidence interval
Group = letters represent significant differences for each species within days ($P \leq 0.05$)

Table 8: The effect of priming water potential (ψ) and incubation period (days) on time to 10, 25, and 50% germination (T_{10}, T_{25}, T_{50}) for KOMA (*Koeleria macrantha*).

			------- T10 -------				------- T25 -------				------- T50 -------			
Species	Ψ	Incubation Period (days)	Mean Days to 10%	Lower.CL	Upper.CL	Group	Mean Days to 25%	Lower.CL	Upper.CL	Group	Mean Days to 50%	Lower.CL	Upper.CL	Group
Koeleria macrantha		bare see	5.1	4.9	5.3	B	6.4	6.1	6.6	B	7.9	7.7	8.2	B
	-0.7 Mpa	7.5	2.1	1.9	2.3	A	2.4	2.1	2.7	A	2.7	2.5	3.0	A
	-1.0 Mpa	10.0	2.1	1.9	2.3	A	2.3	2.0	2.6	A	2.6	2.3	2.8	A

Lower.CL = lower edge of its confidence interval
Upper.CL= upper edge of its confidence interval
Group = letters represent significant differences within time to emergence category ($P \leq 0.05$)

Appendix A - Commercial Operators Contacted

Craig Edminster, Pacific Northwest Natives, Albany, Oregon

Don Regan, Pitkin Forest Nursery, Moscow, Idaho

Kathy Hutton, Plants of the Wild, Tekoa, Washington

Kelly DeMasters and Clark Fleege, Lucky Peak Nursery, Boise, Idaho

Matt Benson, Benson Farms, Moses Lake, Washington

Randy Gilmore, Sun Mountain Natives, Moscow, Idaho

Todd Harris, Western Reclamation, Inc., Eltopia, Washington