Developing Rangeland Restoration Techniques: A Look at Phosphorus Fertilizer as a Seed Coating to Improve Bluebunch Wheatgrass Growth

Morgan Elaine Parkinson
Brigham Young University

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ABSTRACT

Developing Rangeland Restoration Techniques: A Look at Phosphorus Fertilizer as a Seed Coating to Improve Bluebunch Wheatgrass Growth

Morgan Elaine Parkinson
Department of Plant and Wildlife Sciences, BYU
Master of Science

Planting native species after a major disturbance is a critical tool land managers use to stabilize soils, restore ecosystem processes, and prevent weed invasion. However, within the sagebrush steppe and other arid and semi-arid environments the percentage of sown seeds that produce an adult plant is remarkably low. Applying fertilizers at the time of planting may improve native plant establishment by increasing the ability of the seedlings to cope with environmental stresses. However, traditional fertilizer applications are often economically infeasible and may be counterproductive by encouraging weed invasion. Seed coating technology allows for the efficient application of fertilizers within the microsite of the seeded species. The objective of our research was to determine the optimal rate of fertilizer to apply to the seed to improve seedling emergence and plant growth. We applied a phosphorus (P) rich fertilizer (0.13 g P g\(^{-1}\)) to bluebunch wheatgrass (\textit{Pseudoroegneria spicata} (Pursh) Á. Löve) seeds in a rotary coater at rates ranging from 0 to 50 g of fertilizer 100 g\(^{-1}\) seed. Three separate studies were conducted to test germination, biomass, relative growth rate, and tissue nutrient uptake. Study one showed decreasing root and shoot biomass and increasing time to 50% germination as fertilizer rates increased. Study two showed no difference in relative growth rate between the controls and fertilizer treatments. Study three showed no difference in root and shoot biomass or nutrient concentration between treatments except in the lowest fertilizer treatment (10 g fertilizer 100 g\(^{-1}\) seed), which was significantly lower in root and shoot biomass than all other treatments but had higher P tissue concentrations than all other treatments. Collectively these results showed no evidence that a P fertilizer coating could aid in bluebunch wheatgrass seedling establishment. Because bluebunch wheatgrass and similar late-seral plants have evolved with low nutrient requirements they may not be physiologically capable of handling increased nutrient supply, which may explain the results of our studies. Continued studies and fieldwork need to be performed to evaluate the potential of fertilizer seed coatings in restoration efforts.

Keywords: bluebunch wheatgrass, phosphorus (P), fertilizer, sagebrush steppe, seed coating, seed enhancement
ACKNOWLEDGEMENTS

I would like to thank my committee members Matthew Madsen, Bryan Hopkins, and Neil Hansen for their constant assistance and guidance throughout this process. I am especially grateful to Matthew Madsen for his support in the research and writing of my thesis. He has been an excellent mentor and example during the time we have worked together. I am also extremely grateful to my good friend Rachel Buck who mentored me as I worked in BYU’s Environmental Analytical Lab and was the original supporter of me pursuing a Master’s degree. I would not have finished without her friendship and support. I am grateful for Jana Featherstone, the graduate program manager, who has been extremely helpful in meeting deadlines and providing guidance. I am also grateful for my wonderful family. My parents for providing unfailing support and strength. My wonderful husband, Shaun Parkinson, who has constantly been supportive and motivating in completing this thesis, and to our two beautiful daughters, Lucy and Avery, who were born while working on this thesis. Finally, I would like to express my gratitude to my Heavenly Father who guides and strengthens me each day.
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INTRODUCTION

Some of the biggest concerns for arid and semi-arid rangelands worldwide is the loss of plant productivity and diversity through degradation (Milton et al. 1994; Reynolds et al. 2007). Often this degradation is caused by major disturbances that compromise ecological resilience and impair recovery of native species (Bradley and Mustard 2006; Chambers et al. 2014). North America’s sagebrush steppe biome, in particular, has been an area prone to deterioration from disturbance (Brummer et al. 2016; Chambers et al. 2007). Historically this area has been threatened by disturbances from overgrazing, increased population pressures, and altered fire regimes to a point where it now only exists on 56% of its historical range (Hardegree et al. 2016; Harrison et al. 2003; Miller et al. 1994b; Suring et al. 2005). Such disturbances to the landscape leave these sites vulnerable to weed invasion, particularly by annual grasses, such as cheatgrass (*Bromus tectorum* L.), medusahead (*Taeniatherum caput-medusae* [L.] Nevski), and North Africa grass (*Ventenata dubia* [Leers] Coss.) The resultant shift in vegetation from native species to invasive annual grasses increases fire frequency, which in turn reduces rangeland productivity and causes a shift in vegetation, wildlife, and ecosystem processes (Baker 2006; Balch et al. 2013; Dantonio and Vitousek 1992; Evans et al. 1970).

Native and introduced perennial grasses are both major components of most seed mixes planted after a disturbance (Crawford et al. 2004; Pyke et al. 2015; Richards et al. 1998). Perennial grasses help to stabilize soils, restore ecosystem processes and functions, and prevent weed invasion (Brummer et al. 2016; Herron et al. 2001; Larson et al. 2018). Within the sagebrush steppe and other arid and semi-arid environments the percentage of sown seeds that produce an adult plant is remarkably low; often well below 10% (Chambers 2000; Hardegree et al. 2010; Merritt and Dixon 2011). This lack of seeding success can be attributed to high
mortality during early stages of plant development, i.e. seed germination, seedling emergence, 
plant establishment, and survival through the first year (Clark et al. 2007; James et al. 2019; 
James and Svejcar 2010; James et al. 2011). Consequently, the most successful seeding practices 
are aimed at helping seedlings through these limiting demographic stages.

Enhancing perennial grass nutrition may improve seedling vigor and subsequently 
increase the ability of the plant to cope with the many biotic and abiotic stressors that cause 
mortality during these early stages. The ability of individual plants to uptake nutrients for growth 
often determines plant species performance and drives successional processes (Krueger-Mangold 
et al. 2006; Radosevich et al. 2007). In nutrient-limited environments, appropriate applications of 
fertilizers may help improve plant nutrition, which in turn can favorably impact seedling vigor. 
However, the use of fertilizers is typically not recommended for rangeland restoration projects 
because traditional fertilizer applications are often economically infeasible and can inadvertently 
promote the colonization of weeds (Aerts 1999; Brooks 2003; Garnier et al. 1989; Herron et al. 
2001; Hillhouse et al. 2018; James 2008a; Walker et al. 2017; Yuan et al. 2007). Moreover, 
within the scope of rangeland restoration, “fertilizers” do not have a general treatment effect, but 
rather their impact is associated with the type and amount of individual nutrients in the fertilizer. 
Nitrogen (N) is the primary nutrient that increases the competitive ability of invasive annual 
weeds, because of the annual weeds ability to uptake and utilize N relatively faster than native 
perennial vegetation. Other issues with increased N usage in rangeland settings are related to 
excessive shoot growth at the expense of root growth (Geary et al. 2015; Rengel 2020), thus 
negatively impacting plant-water relations for native plants (Bown et al. 2010). As the soil dries 
in arid environments plants with more shoot growth are more likely to succumb to drought stress 
as there is more shoot biomass than the roots can support. Other nutrients, especially phosphorus
(P), are known to favor root over shoot growth (Hopkins 2015; Hopkins and Hansen 2019). Phosphorus also is an essential nutrient required by plants for use in photosynthesis, respiration, energy storage and transfer, cell division and several other processes. Fertilizers that have no or little N and high P may have positive effects on the root growth of native vegetation and subsequently, improve the plants drought tolerance during early seedling stages (Lambers and Poorter 1992).

Applying high P fertilizers directly to the seed through seed coating technology may offer an economical solution to improving rangeland seeding success. In this way, only low amounts of fertilizer may be required because the fertilizer seed coatings provide nutrients within the microsite of the seed—increasing the P use efficiency by minimizing soil reactions and modifying the rhizosphere (Hopkins et al. 2014). Additionally, this approach may enhance seedling establishment and plant survival, without making the increased fertilizer available to surrounding weeds. While fertilizer seed coatings have been commonly studied and used to successfully promote growth in agricultural settings (Mašauskas et al. 2008; Pedrini et al. 2017; Peltonen-Sainio et al. 2006; Ros et al. 2000), there is a lack of understanding on how this technology works in rangeland settings. Our studies aimed to understand the potential of a fertilizer seed coating to facilitate native grass establishment in the sagebrush steppe biome.

The fertilizer used for seed coating in our study was TerraFuze P® (9-30-1 as N-P₂O₅-K₂O percentages; Landview Inc., Rupert, ID, USA), which is an inorganic P-rich fertilizer. This fertilizer also has a small amount of N, which may further enhance P activity (Hopkins and Hansen 2019). The N in the fertilizer can also promote overall growth as it is used by plants for DNA and RNA synthesis, and is a major component of amino acids, chlorophyll, and ATP (Bilbrough and Caldwell 1997; James and Richards 2007). Although the concentration is very
low, the potassium (K) in TerraFuze P® may further enhance seedling vigor as it plays an indispensable role in plant water relations and physiological processes, including regulation of stomata, osmoregulation of water and other salts in plant tissues and cells, protein and starch synthesis, and activation of enzymes for the generation of ATP.

The objective of this study was to evaluate the efficacy of a fertilizer seed coating to improve seedling germination and plant growth, and to determine the optimal rate of fertilizer to apply to the seed. We hypothesized that: i) there is an optimal rate of fertilizer that when applied to the seed would increase germination percentage and overall seedling biomass relative to lower and higher fertilizer rates; ii) fertilizer application at the optimum rate would result in higher relative growth rate (RGR) and increased seedling root biomass and tissue P concentration.

Bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) Á. Löve) was the model species used in these studies. This species was selected because it is an integral climax species in the sagebrush steppe, and is commonly used by land managers for restoration projects because of its extensive distribution, abundance, and palatability to wildlife and all classes of livestock (Miller et al. 1994a).

**MATERIALS AND METHODS**

**Study 1**

The trial was performed on ‘Columbia’ bluebunch wheatgrass seed obtained from the Utah Division of Wildlife Resources Great Basin Research Center (Ephraim, UT, USA). Seven different rates of TerraFuze P® fertilizer were evaluated in the trial (0.5, 1, 2, 4, 8, 16, and 32 g fertilizer 100 g⁻¹ seed), which were applied within a polymer seed coating. The trial also contained two experimental controls by leaving seed untreated (control) and treating seeds with a
polymer coating that did not contain fertilizer (blank). The polymer binder used was a 45% solution of Agrimer-15 (Ashland Inc., Covington, KY, USA) and water, which served to hold and stabilize the seed coating. A powder filler material of calcium carbonate powder (limestone) was also used to absorb the applied liquid and increase the seed coating thickness.

Seed coatings were applied in a 30-cm diameter rotary seed coater from Universal Coating Systems (Independence, OR, USA) in a two-step process. For rates 0.5 - 8 g fertilizer 100 g\(^{-1}\) seed, the fertilizer was mixed with polymer binder to reach 10 g of total liquid, which was then applied in the coater to the seed through a centralized atomizing disk. In the second step, 65 g of polymer binder and 175 g of limestone powder were slowly added using standard seed coating techniques. For the coating rates of 16 and 32 g fertilizer 100 g\(^{-1}\) seed, 10 g of fertilizer was applied directly to the seed in the first step, using the same method as above. In the second step, the fertilizer was mixed with polymer binder to reach 65 g of total liquid. This mixture of fertilizer and polymer binder was then coated onto the seed with 175 g of powdered limestone using the same technique described previously. Once coated, all seeds were dried using a forced-air dryer at 43°C (Universal Coating Systems, Independence, OR, USA).

Twenty-five seeds were placed, from each of the nine treatments described above, in 13 x 13 cm acrylic boxes filled with 140 g of fine sand. Before planting, the sand was watered to field capacity. Seeds were planted by lightly pressing them into the soil and boxes were sealed to maintain moisture. Boxes were placed in a walk-in growth chamber (Environmental Growth Chambers, Chagrin Falls, OH, USA) and held at a constant 5°C. This incubation temperature was chosen to mimic spring conditions when seedlings are starting to emerge from the soil in the sagebrush steppe. Lights in the chamber provided a 12 h photoperiod, with a maximum photosynthetically active radiation flux density of approximately 700 μmol m\(^{-2}\)s\(^{-1}\) at plant height.
Each treatment was replicated ten times, with the boxes arranged within the growth chamber in a randomized complete block design. Seedling emergence was counted every 1-3 d for 53 d from planting. After counting, the sand was watered to field capacity and the boxes, within a block, were re-randomized and placed on a new shelf within the growth chamber. At the conclusion of the study, 52 d from seeding, plants were harvested by washing the sand from the roots and drying the plants at 105°C for three days. After drying, above and below ground biomass was separately weighed.

From daily germination counts, we calculated time to reach 50% germination ($T_{50}$) and final germination percentage. $T_{50}$ was calculated as follows:

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

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.

**Study 2**

Preliminary results from study 1 indicated decreased germination rates and biomass with increasing rates of fertilizer, which we hypothesized could be resultant of a fertilizer toxicity caused by putting the fertilizer too close to the naked seed in the coating process. In consequence of this, we designed a study to test if applying fertilizer on the outside of the seed coating would improve the efficacy of the fertilizer. This was done as part of an addition to our predesigned study looking at seedling growth rate over time. The treatments tested in this trial included 1)
applying fertilizer at the start of the coating (fertilizer on seed), and 2) applying the fertilizer on the outside the seed coating (fertilizer on coating). This trial also contained the same two experimental controls used in the previous study (control and blank).

Lack of treatment response in study 1 to fertilizer may have also been due to the Agrimer polymer binder or limestone powder decreasing the efficacy of the fertilizer. In this trial, a new polymer binder, Selvol-205 (Sekisui Specialty Chemicals America, Dallas, TX, USA), was used, which was prepared with a 10% solid content, according to Sekisui Specialty Chemicals solution preparation guidelines. We also applied a new powder filler, diatomaceous earth (Perma-Guard, Inc., Albuquerque, NM, USA), in place of limestone. The species and fertilizer were the same as described in the above methods. Fertilizer was applied to the seed at 5 g 100 g\(^{-1}\) seed.

The fertilizer on seed treatment was produced by applying a mixture of 5 g of fertilizer and an equal amount of polymer binder directly to the seed in the first step. In the second step, 90 g of polymer binder and 175 g of diatomaceous earth were added using standard seed coating techniques. The fertilizer on coating treatment was applied by coating the seeds with 90 g of polymer binder and 175 g of diatomaceous earth and then in the second step using 5 g of fertilizer and an equal amount of polymer binder. After seeds were coated, they were dried following procedures in Study 1.

The study was conducted in the same walk-in growth chamber as used in study 1. The experiment was arranged within a completely randomized design consisting of the four treatments described above and four harvests with eight replicates per treatment per harvest. Twenty-five seeds were planted in each of the four treatments in 100 cm\(^3\) plastic pots with 456 g of soil (sieved to 1.18 mm) and 22 g of vermiculite. Soil was brought to field capacity when planted and watered twice a week. Pots were placed in the walk-in growth chamber. Based on
preliminary results from study one showing increasingly delayed periods of germination from low temperatures we increased the temperature to 10°C. After a month, pots were thinned randomly to 10 seedlings per pot. The four harvests were included so we could look at relative growth rates between the treatments to see if the fertilizer impact varied with time. The first harvest was conducted one week after thinning. The second, third and fourth harvests were conducted at one-month intervals after the first harvest. At the time of harvest, soil was washed from the plants, and roots were separated from shoots. Harvested plants were dried at 105°C for three days and above and belowground biomass was separately weighed. The RGR was calculated for the periods of initial to early harvest, early to mid harvest, and mid to late harvest as follows:

\[ \text{RGR} = \frac{\ln(\text{biomass}_{\text{final}}) - \ln(\text{biomass}_{\text{initial}})}{t} \]

where \( \text{biomass}_{\text{final}} \) = biomass of plants at early harvest, mid harvest, and late harvest respectively, \( \text{biomass}_{\text{initial}} \) = biomass of plants at initial harvest, early harvest, and mid harvest respectively, and \( t \) = time (d).

Study 3

Results from study 2 indicated a minimal treatment effect from the fertilizer. We postulated that perhaps we were not obtaining a strong treatment response because our fertilizer application rate was not high enough. Subsequently, we initiated this study to determine if relatively higher rates of fertilizer would produce a treatment response. We included four rates of fertilizer in the trial (10, 20, 30, and 50 g fertilizer 100 g\(^{-1}\) seed) as well as the same two experimental controls used in previous studies (control and blank). The species, fertilizer, polymer binder (Selvol-205), and powder filler (diatomaceous earth) were all the same as
described in study 2. However, to apply this level of fertilizer onto the seed a different coating method was required to maintain the durability of the seed coating. In this study, 30 g of dry Selvol powder was mixed with 200 g of liquid fertilizer and then heated and mixed according to Sekisui Specialty Chemicals solution preparation guidelines. Through this approach the fertilizer was modified so that it could also function as a binder within the seed coating.

Seeds were coated in a two-step process. The first step consisted of mixing 110 g of polymer binder with 87.5 g of diatomaceous earth. This was done slowly to ensure a solid coating on the seed before the fertilizer was added. Once a solid base had formed on the seed, the fertilizer/Selvol mixture was added at rates of 10, 20, 30, or 50 g. Diatomaceous earth was also added during this time at rates of 8.7, 13.2, 16.2, and 25 g respectively. Once coated, seeds were dried using the same method as above. A blank was also included and produced using only step one of the coating process and excluding the fertilizer.

Twenty-five seeds were planted in 0.004 cubic meter plastic pots filled with a mix of 1,046 g of sieved soil (< 1.18 mm) and 138 g of perlite. Seeds were planted on top of the soil/perlite mixture, covered with ~1 cm of soil, and watered to field capacity from the bottom up. Each treatment was replicated eight times in a randomized complete block design and all pots were placed in a glasshouse. The temperature of the glasshouse was set at ~23°C with a 12-h photoperiod.

Pots were watered every 3-4 d and rotated to ensure even light. Seedlings were thinned to 9 plants, 19 days after planting, and harvested 59 days after planting. Plants were harvested using the same method described above and root and shoot biomass was recorded. Additionally, we analyzed for P, K, sulfur (S), calcium (Ca), magnesium (Mg), boron (B), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), and sodium (Na) nutrient concentrations, in the shoots using a
hydrogen peroxide microwave digestion (Ethos EZ, Milestone, Shelton, CT, USA) followed by ICP-OES Analysis (iCAP 7400, Thermo Electron, Madison, WI, USA). Total N was analyzed by combustion using the Dumas method (LECO TruSpec CN Determinator, LECO Instruments, St. Joseph, MI, USA).

Statistical Analysis

Time to 50% germination, final germination, shoot biomass, root biomass, RGR, and nutrient concentrations were analyzed using JMP® version 14 (SAS Institute Inc., Cary, NC, USA). The data was analyzed using analysis of variance (ANOVA) to determine overall model significance with the means between treatments separated for significance using a Dunnett’s multiple comparison test ($P < 0.05$) to compare each treatment to the control and the blank treatment. Significance was noted when a treatment was different from both the control and blank. Assumptions of normality and equal variance were checked against a normal distribution.

RESULTS

Study 1. Evaluation of Fertilizer Rate on Seed Germination and Early Seedling Growth

Fertilizer delayed seed germination timing when applied at the highest rate (32 g fertilizer 100 g⁻¹ seed; Fig. 1A). At this rate, the time to 50% germination was on average 5 d slower than the uncoated and unfertilized control ($P = 0.03$) and 7 d slower than the unfertilized, but coated blank ($P < 0.001$; Fig. 1A). There was no significant impact at the lower rates, although there was an overall trend for slower germination with rate of fertilizer added to the coating.

Coating the seed (blank) increased final germination over the control by 14% ($P < 0.001$; Fig. 1B). Fertilizer coatings had similar final germination percentages as the blank coating (Fig.
Fertilizer coatings generally had a similar increase in final germination over the control with the exception of the application rates of 2 and 32 g fertilizer 100 g\(^{-1}\) seed, which showed no difference from the control (\(P = 0.05\) and \(P = 0.63\); Fig. 1B). This suggests that the very highest rate of fertilizer may have had a negative impact on germination.

The polymer coating improved seedling growth as indicated by the blank treatment having 39% and 32% more shoot and root biomass than the control (\(P = 0.002\) and 0.01), respectively (Fig. 1C; Fig. 1D). As fertilizer rates increased in the coating the positive impact of the coating was negated for shoot and root biomass (Fig. 1C; Fig.1D). There was, however, no rate that had statistically different shoot biomass from both the control and the blank. The lower rates (0.5 and 1 g 100 g\(^{-1}\)) had more shoot biomass than the control (but not the blank) and higher rates (8, 16, and 32 g fertilizer 100 g seed\(^{-1}\)) had less shoot biomass than the blank (but not the control; Fig. 1C). The only treatment to show a difference in root biomass from both the control and blank was the highest rate of fertilizer (32 g 100 g seed\(^{-1}\); Fig. 1D). At this rate, root biomass was on average 3 g lower than the control (\(P = 0.03\)) and 6 g lower than the blank (\(P < 0.001\); Fig. 1D). Lower application rates (0.5 and 1 g 100 g\(^{-1}\)) had more root biomass than the control but showed no difference from the blank (Fig. 1D). Higher rates (8 and 16 g fertilizer 100 g seed\(^{-1}\)) had less root biomass from the blank but showed no difference from the control (Fig. 1D).

**Study 2. Evaluation of Fertilizer Coating Techniques on Seedling Growth Rates**

There was no difference in shoot and root biomass at any of the fertilizer application rates for each of the four harvest periods (\(P > 0.05\); Fig. 2A; Fig. 2B). Differences between treatments began to increase, particularly for shoot biomass at the third and fourth harvest, but no treatment
was statistically different from the other (Fig. 2A). There was also no difference between
 treatments for RGR at the early, mid, and late harvest periods ($P > 0.05$; Table 1).

**Study 3. Evaluation of Fertilizer Application Rate on Plant Growth and Nutrient Uptake**

The difference in shoot and root biomass was drastically lower ($P < 0.001$) for the lowest
fertilizer rate (10 g fertilizer 100 g$^{-1}$ seed) in comparison to the control, blank, and other fertilizer
rates (Fig. 3A; Fig. 3B). Shoot biomass was an average of 444 g lower than all other treatments
(Fig. 3A) and root biomass was an average of 217 g lower than all other treatments for the 10 g
fertilizer 100 g$^{-1}$ seed treatment (Fig. 3B). All other treatments had similar shoot and root
biomass to each other.

At the lowest fertilizer rate, N, Ca, Mg, P, and S tissue concentrations were significantly
higher from all other treatments ($P < 0.001$). N for the lowest fertilizer rate had an average
increase of 26% above the average of all treatments, likewise, Ca had 48%, Mg had 41%, P had
17%, and S had 22% (Fig 3C). It should be noted that because of laboratory errors only one
replicate for the rate of 10 g fertilizer 100 g$^{-1}$ seed was tested therefore these results should be
viewed with caution.

**DISCUSSION**

Contrary to our hypotheses, we found that a P-rich fertilizer coating did not improve seeding
success, regardless of the application rates used in these studies. Specifically, no improvements
were found in germination timing, final germination, plant biomass, or RGR (Fig. 1-3). In study
1, the highest rate of fertilizer slowed germination, decreased final germination, and reduced
plant growth (Fig. 1). This finding is generally unsurprising, considering that other studies have
shown fertilizer toxicity occurring when high rates are placed in close proximity to a seed (Duncan and Ohlrogge 1958; Munns 2002). However, study 2 did not confirm our assumption that by providing a barrier between the seed and the fertilizer, we would improve the efficacy of the seed coating. In this study, we found no evidence that a fertilizer coating, regardless of proximity to the seed, could increase plant shoot and root biomass or RGR (Fig. 2). Additionally, study 2 provided evidence that a lack of a treatment effect from the fertilizer coating was not due to the relatively short period of time the plants were allowed to grow in study 1 (53 d). It has been noted in other studies with native perennial species that juvenile plants lack the ability to quickly acquire and utilize nutrients and that these traits are established as the plants enter later developmental phases (Bateman et al. 2018; Lambers et al. 2008). Each of our three monthly harvest dates, over a 97 d period, provided no evidence the fertilizer was affecting plant growth (Fig. 2).

Our alternative hypothesis as to why we did not see a treatment effect from the fertilizer seed coating is there was not enough soluble fertilizer available to promote plant growth. This could be particularly true for study 1, which used a limestone powder in the seed coating. In the presence of lime, P fertilizer can undergo a series of reactions that convert it to less soluble compounds, such as dicalcium phosphate, dehydrate, octacalcium phosphate, or hydroxyapatite (Hopkins 2015; Sharpley et al. 1989). However, studies 2 and 3, used an alternative powder (diatomaceous earth), in place of limestone, to minimize the formation of calcium compounds, and we still did not see a treatment effect with this coating. Additionally, in study 3, we applied a relatively high fertilizer seed coating rate (ranging from 10-50 g fertilizer 100 g⁻¹), and we saw no improvement in plant growth. In study 3, we also examined the impact of the fertilizer treatment on the nutrient concentrations in the plant tissue. The only rate that showed an increase
in nutrient concentration was 10 g fertilizer 100 g seed$^{-1}$ (Fig. 3C). This fertilizer rate was also associated with a decrease in root and shoot biomass (Fig. 3A-B). This concurrent decrease in biomass and increase in nutrient tissue concentration may be explained by the necessitation for bluebunch wheatgrass to allocate a relatively high amount of resources to future production and growth. One of the greatest advantages native perennials have over annual plants is the ability to store nutrients for future use (Jeuffroy et al. 2002). However, allocating significant energy into nutrient storage can prove detrimental when these nutrient gains are offset by a large decrease in overall biomass production (Lambers et al. 2008; Lambers and Poorter 1992; Rodgers and Barneix 1988), as seen in our study. While this phenomenon of a synchronous low biomass and high nutrient tissue concentration may be explained by the necessitation of the perennial plant to store nutrients, the same phenomenon should logically have occurred in all our fertilizer treatments and not just the lowest rate.

Our results are not consistent with the literature linking P fertilizer coatings with increased germination times, earlier growth, and increased final shoot and root biomass (Mašauskas et al. 2008; Pedrini et al. 2017; Peltonen-Sainio et al. 2006; Ros et al. 2000). However, this discrepancy may be accounted for by taking into account the difference in species used in these studies. The majority of work done with fertilizer seed coatings has been performed on annual crops that have been cultivated over many millennia to have rapid growth rates, such as barley ($Hordeum vulgare$ L.), oat ($Avena sativa$ L.), and rice ($Oryza sativa$ L.). Studies examining nutrient addition among native perennial species, although applied through traditional methods and not through a seed coating, tell a much different story. Such studies show that an increase in nutrient concentration, especially N does little to improve plant growth of native perennial species, especially when grown alongside invasive annual weeds (Aerts 1999; Brooks 2003;
Garnier et al. 1989; Herron et al. 2001; Hillhouse et al. 2018; James 2008b; Walker et al. 2017; Yuan et al. 2007). These studies found that native perennial species were unable to compete with invasive annual weeds with added N and P because fast growing invasive species were able to react rapidly to these increased nutrient levels by increasing their uptake kinetics, thus utilizing available nutrients faster than native perennials (Caldwell et al. 1996; Jackson and Caldwell 1996). Native perennial species have also been shown to be incapable of incorporating large amounts of nutrients into organic matter (Lewandrowski et al. 2017), which may explain the limited growth we observed in our trials, even when the plants were given optimum nutrition from the addition of the fertilizer coating. Additionally, because bluebunch wheatgrass has adapted to low nutrient availability, it is likely that some favorable traits are suppressed when exposed to periods of increased nutrient supply. For example, slow growing, late seral, species such as bluebunch wheatgrass have been shown to respond to low nutrient supplies by initiating second order laterals and root hairs (Boot and Mensink 1990; Clarkson 1985; Jungk and Claassen 1989). These root hairs are important for the acquisition of ions that slowly diffuse in the soil like phosphate (Clarkson 1985). Plants can respond to soils with a low nutrient supply by increasing both the density and length of their root hairs, which likely contributes to their successful performance in nutrient-poor environments. Although not measured, there was a visible difference in root hair formation of plants grown with and without the fertilizer coating (Fig. 4). Those plants grown without the fertilizer coating exhibited longer, and more fibrous root structures, with more root hairs than those grown with the fertilizer coating (Fig. 4). There is also a possibility that the physical proximity of the P to the seed hindered the plants potential for deep root growth. Because P is immobile in the soil, roots must extend outward to reach a sufficient P source (Bucher 2007). It is therefore feasible that the plants in the study exhibited
decreased root extension as a result of the high P supply placed in close proximity to the roots through the fertilizer seed coating.

Although we did not see a major difference between treatments in response to the fertilizer coating, we did see a consistent difference in study 1 between the blank and the control (Fig. 1). The blank outperformed the control in regards to final germination (Fig. 1B), shoot biomass (Fig. 1C), and root biomass; Fig. 1D), suggesting that the coating by itself was advantageous to the seeds germination and growth. This was likely the result of the limestone powder in the coating retaining moisture close to the seed during the critical processes of germination and early seedling growth. This phenomenon has been recorded in the literature by McWilliam and Dowling (1970) who found that a coating of limestone powder and methocel adhesive applied to ryegrass increased seed moisture content and resulted in faster germination and increased total germination compared to uncoated seeds. Research continues to progress on the work of hydrophilic seed coatings and their potential to enhance germination and early seedling growth (Adak et al. 2016; Gorim and Asch 2012).

CONCLUSION

As the degradation of rangelands advances at an ever-increasing rate it becomes vitally important to identify key components of effective efforts in native vegetation restoration. In nutrient limited environments appropriate fertilizer application through a seed coating seems a novel and compelling solution to supplying nutrients to select native perennials without allowing access to invasive annual neighbors. In our studies, however, there was no evidence to suggest that fertilizer as applied through a seed coating could increase germination percentage, germination timing, or shoot or root biomass of bluebunch wheatgrass. There was some evidence
suggesting small amounts of fertilizer could increase nutrient tissue concentration but at a major cost to overall biomass production. While the limited scope of our studies only included one species and one fertilizer, the patterns of response observed across multiple studies suggest a fertilizer coating consisting of a similar makeup of macro- and micronutrients will do little to help in the establishment of bluebunch wheatgrass and other similar late-seral, slow growing native plants. Future research should be done on early to mid-seral native species that have higher nutrient demands and greater nutrient uptake rates, as they may benefit from a fertilizer seed coating. Additionally, the research on fertilizer seed coatings as employed in rangeland settings is extremely limited and it may be that other coatings formulations could be developed that would produce a treatment effect. Because of the isolated and controlled environments our plants were grown in, further trials incorporating field studies are also needed to explore the effects of a fertilizer coating in a realistic environment.
Figure 1. Influence of fertilizer (9-30-1) as applied through seed coatings on (A) time to reach 50% germination (B) final germination percentage (C) shoot biomass and (D) root biomass at rates ranging from 0-32 g of fertilizer 100 g\(^{-1}\) seed. Statistical difference from control at \(P < 0.05\) are indicated by crosses (+). Statistical difference from blank at \(P < 0.05\) are indicated by diamonds (\(\ast\)).
Figure 2. Seedling biomass for shoot (A) and root (B) over time from seeds that were untreated (Control), coated without fertilizer (Blank), coated by applying 5 g fertilizer 100 g seed\(^{-1}\) at the start of the coating (Fertilizer on Seed), or coated by applying the same amount of fertilizer after, on the outside of the coating (Fertilizer on Coating). Fertilizer was a 9-30-1. There were no statistically significant differences between any treatments ($P < 0.05$).
Figure 3. Influence of fertilizer (9-30-1) as applied through seed coatings at rates ranging from 0-50 g fertilizer 100 g⁻¹ seed on (A) shoot biomass, (B) root biomass, and (C) nutrient tissue concentrations of nitrogen (N), calcium (Ca), magnesium (Mg), phosphorus (P), and sulfur (S). At a rate of 10 g fertilizer 100 g⁻¹ seed, tissue nutrient concentrations were statistically higher than all other treatments. At this rate root and shoot biomass was statistically lower than all other treatments. There was no difference between treatments for boron, copper, iron, potassium, manganese, sodium, and zinc (Table 2). It should be noted that because of lab errors only one replicate for the rate of 10 g fertilizer 100 g⁻¹ seed was tested therefore the results for total N should be viewed with some caution.
Figure 4. Belowground biomass produced from a seed that was either (left) coated but did not contain any fertilizer and (right) coated with a fertilizer coating treatment 50 g of product 100 g$^{-1}$ seed.
Table 1. Relative growth rate of plants grown from seed that were left untreated (Control), coated but without a fertilizer (Blank), coated by applying 5 g fertilizer 100 g seed\(^{-1}\) at the start of the coating (Fertilizer on Seed), or coated by applying the same amount of fertilizer after, on the outside of the coating (Fertilizer on Coating). Fertilizer was a 9-30-1. There were no statistically significant differences between any treatments (\(P < 0.05\)).

<table>
<thead>
<tr>
<th></th>
<th>Early Harvest</th>
<th>Mid Harvest</th>
<th>Late Harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.06 ± 0.010</td>
<td>0.03 ± 0.010</td>
<td>0.010 ± 0.002</td>
</tr>
<tr>
<td>Blank</td>
<td>0.06 ± 0.004</td>
<td>0.03 ± 0.004</td>
<td>0.009 ± 0.010</td>
</tr>
<tr>
<td>Fertilizer on Seed</td>
<td>0.07 ± 0.010</td>
<td>0.01 ± 0.010</td>
<td>0.010 ± 0.005</td>
</tr>
<tr>
<td>Fertilizer on Coating</td>
<td>0.06 ± 0.010</td>
<td>0.02 ± 0.004</td>
<td>0.006 ± 0.010</td>
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</tbody>
</table>

Table 2. Nutrient tissue concentration of plants grown from seed that was left untreated (Control), coated but without a fertilizer (Blank), coated by applying fertilizer at a rate of 10, 20, 30, or 50 g fertilizer 100 g seed\(^{-1}\). Fertilizer was a 9-30-1. There were no statistically significant differences between any treatments (\(P < 0.05\)).

<table>
<thead>
<tr>
<th>Nutrient Tissue Concentration</th>
<th>B (ppm)</th>
<th>Cu (ppm)</th>
<th>Fe (ppm)</th>
<th>K (%)</th>
<th>Mn (ppm)</th>
<th>Na (ppm)</th>
<th>Zn (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12.0 ± 1.3</td>
<td>13.3 ± 0.6</td>
<td>304 ± 68</td>
<td>2.8 ± 0.2</td>
<td>70 ± 4.0</td>
<td>914 ± 243</td>
<td>25.6 ± 1.0</td>
</tr>
<tr>
<td>Blank</td>
<td>11.7 ± 1.4</td>
<td>13.6 ± 1.4</td>
<td>255 ± 49</td>
<td>2.8 ± 0.2</td>
<td>69 ± 1.8</td>
<td>862 ± 303</td>
<td>16.5 ± 1.2</td>
</tr>
<tr>
<td>10</td>
<td>15.2 ± 1.8</td>
<td>14.3 ± 0.9</td>
<td>392 ± 113</td>
<td>3.3 ± 0.2</td>
<td>73 ± 5.8</td>
<td>1650 ± 89</td>
<td>27.2 ± 1.1</td>
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<tr>
<td>20</td>
<td>8.70 ± 0.7</td>
<td>12.0 ± 1.2</td>
<td>229 ± 19</td>
<td>3.0 ± 0.1</td>
<td>62 ± 2.5</td>
<td>814 ± 266</td>
<td>23.6 ± 1.2</td>
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<tr>
<td>30</td>
<td>9.10 ± 1.3</td>
<td>11.7 ± 1.2</td>
<td>261 ± 46</td>
<td>2.7 ± 0.1</td>
<td>62 ± 2.6</td>
<td>709 ± 185</td>
<td>23.3 ± 1.6</td>
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<tr>
<td>50</td>
<td>9.80 ± 1.3</td>
<td>11.7 ± 0.7</td>
<td>209 ± 37</td>
<td>2.8 ± 0.1</td>
<td>59 ± 2.9</td>
<td>988 ± 314</td>
<td>22.8 ± 1.0</td>
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