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Emerging Seed Enhancements to Reduce the Risk of Sagebrush Post-Fire Seeding Failure

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A thesis submitted to the faculty of
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Master of Science

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ABSTRACT

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The sagebrush (*Artemisia* spp.) steppe is undergoing rapid ecological change. The degradation of sagebrush steppe rangelands has resulted in the listing of more than 350 animals and plants as species of conservation concern. In addition, there has been a decrease in recreational values, reduced forage production, degraded water resources, and an increase in fire frequency. In the sagebrush steppe, success rates for seeding sagebrush after wildfire are notoriously low. Not only are sagebrush seeds hard to sow due to their small size and associated flower parts, but seedlings are exposed to numerous stresses that lowers their survivability. To improve sowing efficiency and reduce the associated stresses to seedling development we use seed enhancement technologies. In Chapter 1, we explain how a rotary seed coater can be used to agglomerate and apply enhancements to Wyoming big sagebrush (*Artemisia tridentata* spp. *wyomingensis*) seed. Using a mix of compost and clay we used a rotary seed coater to create small uniform agglomerates that allowed for enhancements to be applied to the seed. Our study demonstrated that agglomerates have no negative effects on seed germination and increased the overall flow of seed. In Chapter 2, we analyzed the addition of the plant hormone abscisic acid (ABA) in seed coatings. This hormone may delay seed germination and allow seedlings to avoid mortality due to over-winter freezing. We determined effects of different concentrations of ABA on total germination and timing of germination. Using a wet-thermal accumulation model, we estimated germination timing of seeds using soil temperature and water potential data for six different sites in the Great Basin. These models illustrate the variation in germination timing across the Great Basin. From our results we proposed the idea of using ABA to create a bet-hedging strategy in seed mixes to increase the probability that some seeds would germinate when conditions are favorable for seedling success.

Keywords: sagebrush, seed enhancement, agglomerate, germination, abscisic acid, bet-hedge

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CHAPTER 1

A Novel Seed Coating Approach for Improving Sagebrush Seed Delivery

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ABSTRACT

Seed coating provides a novel approach for improving restoration efforts on rangelands by increasing seed flowability through seeding equipment and providing a medium to deliver various enhancements, which aid in germination and plant growth. Applying a seed coating to sagebrush (*Artemisia* spp.) seed is challenging due its small size and low purity. We developed a technique within a rotary seed coater that allows for the agglomeration of sagebrush seed using a combination of clay, compost, water, and a liquid binder. Trials were performed on Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis* [Beetle & A. Young] S. L. Welsh). We demonstrated improved flowability of sagebrush agglomerates over untreated seeds through measurements of the Hausner ratio (8 % decrease) and the angle of repose (21% decrease). Laboratory trials evaluated germination over five different temperatures ranging from 5 – 25 °C and showed that on average sagebrush agglomerates increased seed germination by 15%. In field trials at two different sites, seedling emergence was similar for untreated and agglomerated seed. With no deleterious effects observed from the agglomeration treatment, additional research is now merited for using agglomerates as a platform to apply various seed enhancements such as fungicides, pesticides, plant growth hormones, fertilizers, biologicals, soil surfactants and other

products. The potential outcomes of these strategies may have a significant impact on future seeding attempts by improving seed delivery and increasing overall seeding success rates.

INTRODUCTION

Drylands, which encompass arid and semi-arid ecosystems, span over one-third of the Earth's landscape (Anderson & Inouye, 2001; James et al., 2013). Millions of hectares of drylands are being degraded resulting in the loss of over US $40 billion dollars in productivity (Brauch & Oswald, 2009; Kildisheva et al., 2016). Current restoration practices do not fully address the technology and management needs necessary to restore these drylands in a way that is both efficient and successful (James et al., 2011; James et al., 2013; Kildisheva et al., 2016).

One specific case of degraded drylands is the sagebrush (*Artemisia* spp.)-steppe ecosystem. This ecosystem is one of the most widespread semi-arid ecosystems in North America, spanning across much of the Western United States (Pyke et al., 2015) and is considered critically endangered due to impacts from grazing, altered fire regimes, invasive species, and various human disturbances (Knick et al., 2011; Davies et al., 2014). Sagebrush steppe communities are estimated to remain on only 56% of their historic range, which has resulted in over 350 different plants and animals being identified as species of concern (Davies et al., 2011; James et al., 2013). Loss of the sagebrush ecosystem has also led to a decrease in recreational activities, reduced forage production, and degraded water resources (Davies et al., 2011; Pyke et al., 2015; James & Carrick, 2016). Despite large expenditures, the success of sagebrush restoration projects is low and often sporadic, particularly within the hotter, drier,

lower elevation sites (Gebert et al., 2008; Davies et al., 2011; Madsen et al., 2016b; Svejcar et al., 2017).

To preserve the sagebrush steppe ecosystem, new restoration techniques are needed to establish sagebrush from seed (Arkle et al., 2014; Madsen et al., 2016b). One of the difficulties associated with sagebrush seeding is the complications that arise with delivering seed through broadcast and drill seeders (Shaw et al., 2005). Sagebrush seed lots are typically low in purity, containing approximately 70-90% non-seed parts (i.e. seed bracts, leaves, and fine stems) (Young & Young, 1992; Jacobs et al., 2011). These non-seed parts can cause bridging, rat holing, and arching within the seed box, which reduces the flow of seed from the planter or broadcast seeder (Shannon, 1979). The small seed size of sagebrush (~1 mm or less) may result in the seed separating from other species in the mix during the seeding operation, which results in variable sagebrush seeding rates across the restoration area. Sagebrush seed is generally broadcast due to its' inability to emerge from below the soil surface (Lysne & Pellant, 2004; McAdoo et al., 2013). When small seeds, such as sagebrush, are broadcast aerially or by ground broadcast spreaders they have the potential to drift from the targeted seeding area (Chambers, 2000; Groen & Woods, 2008).

Technological enhancements to improve dryland seeding have begun to emerge in the form of seed coatings (Madsen et al., 2012; Madsen et al., 2016a; Pedrini et al., 2016). Emerging seed coating technologies have been tested for various species and designed to address specific problems associated with seed germination and plant establishment (Madsen et al., 2013). Seeds are coated using centrifugal forces to mix the seeds, while adhesives (or stickers) are pumped to the center of the coating chamber onto an atomizing disk, which redirects the liquid outward in small droplets onto the seed. With a binder providing a tacky base, coating powder is delivered

through an auger feeder onto the moist seeds. This process is repeated until the coating has reached the desired thickness. Due to the low purity and small size of sagebrush seed, standard coating procedures in a rotary seed coater are difficult to apply.

To address these seed coating problems, we developed a new seed enhancement technology that has the potential to improve sagebrush seed delivery and provide a means to treat the seeds to enhance their establishment. The technology works by clustering sagebrush seed and associated non-seed parts into relatively uniform spherical shaped agglomerates ≈ 2 mm in size. The objectives of this study were to: 1) determine if flow properties of Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis* [Beetle & A. Young] S. L. Welsh) seed could be improved through agglomeration, and 2) evaluate the influence of agglomeration on seed germination and seedling emergence.

MATERIALS AND METHODS

Seed Coating

Trials were performed on Wyoming big sagebrush seed, obtained from the Utah Division of Wildlife Resources Great Basin Research Center in Ephraim, UT, U.S.A. Seed was previously cleaned to 30% purity and had a germination of 80%. Seed coating was performed at the Brigham Young University Seed Enhancement Laboratory. Seeds were agglomerated in a 31 cm diameter rotary drum seed coater (Universal Coating Systems, Independence, OR, U.S.A.). Agglomerates were created using a mixture of Azomite®, compost, seed, and water (Table 1). Azomite® is a highly mineralized complex silica ore mined near Levan, UT, U.S.A. We used Azomite® based on previously-successful performance trials on agglomerates in which various

clays were used. Compost was made with organic yard waste from the grounds of Brigham Young University campus. Compost was dried in a plant drier at 60 °C for 3 d and then ground in a Wiley Mill (Model 4, Arthur H. Thomas Co., Philadelphia, PA, U.S.A.) using a 0.5 mm screen. Compost was added to improve the physical, biological, and chemical conditions of the agglomerates. Preliminary trials indicated that compost appeared to improve aeration and breakdown of the agglomerates after planting to allow for seedling emergence. Agglomerates were formed by combining compost, Azomite®, and seed in the rotary seed coater and while spinning the material (20% of maximum rotor speed) adding water onto a spinning disk in the center of the seed coater at a rate of approximately 6 ml s^{-1}. Once all the water was added, a second treatment of Azomite® was added into the rotary coater while the seeds remained spinning. During this stage, water was added as previously described and then in the final step a liquid binder was added onto the seed. Binder was added to help maintain the coatings integrity and to reduce the dusting off of the agglomerates during transportation and seeding. After all the binder was applied, agglomerates remained spinning in the coater for 30 s. Agglomerates were then dried for 13 min on a forced air dryer at 42 °C. Seeds were then sieved through a 5.0 mm sieve to eliminate the few large masses that occurred in coating.

Tests of Flowability

Using the Hausner ratio and the angle of repose we can make comparative conclusions on the flow properties of untreated and agglomerated seed. The Hausner ratio compares bulk and tap densities where a lower ratio between the two densities indicates an increase in flowability (Abdullah & Geldart, 1999). Tests to determine bulk density and tapped density were similar to those used by Guzzomi et al. 2016. Bulk density was determined by dividing the mass by a 300

ml sample volume. Tapped density was measured by tapping the same sample volume in a cylinder 40 times, from a height of 15 cm, and then using the new volume to calculate density. These procedures were repeated five times where the order of the treatments was randomized, with a new batch of untreated and agglomerates for each repetition.

The angle of repose was determined by producing a cone-shaped pile by pouring a 1000 ml sample of material through a 3 cm pipe that was placed in a fixed position with the bottom of the opening 20 cm above a flat surface. The angle of repose was calculated by taking the inverse tangent of the height of the cone divided by the radius of the base of the cone. These procedures were repeated five times where the order of the treatments was randomized, with a new batch of untreated and agglomerates for each repetition.

Lab Germination Trial

Germination of untreated seed (control) and agglomerated seed was assessed over five constant temperatures (5, 10, 15, 20, 25 °C), in an environmental growth chambers, under (12h/12h) light/dark intervals (Precision Plant Growth Chambers, Thermo Fischer Scientific, Waltham, MA, U.S.A) Prior to starting germination trials, thirty ~0.1 g samples of the agglomerates were weighed to a thousandth of a gram, washed, and the number of seeds in the sample were counted. The same procedure was also performed for untreated seed, only the samples were not washed prior to counting. Average number of seeds g^{-1} of untreated seed was equal to 1233.16 ± 54.5 (mean ± SE) and agglomerates g^{-1} were 170.74 ± 8.06. Using the seeds g^{-1} estimation, ~35 seeds were weighed out and placed on soil inside petri dishes. Soil was collected from a degraded Wyoming big sagebrush site approximately 10 miles south of Santaquin, UT (lat 39°54’35”N log 111°48’45”W). Soil at the site was composed of ~42% sand,

38% silt, and 20% clay and is classified as a Donnardo stony loam with a pH of 7.4-7.8 and 1-3% organic matter (Soil Survey Staff 2018). After collection, soil was dried at room temperature, sieved through a 1.7 mm sieve to remove excess debris, then run through a soil grinder. Water was mixed into the soil bringing it to field capacity (0.247 g of water 1g $soil^{-1}$) and 25 g of the wet soil was uniformly placed across the bottom of a petri dish. Seeds were sown on surface of the soil within the petri dishes. Treatments were organized in a randomized complete block split-plot design. Temperature comprised the split-plot factor. Treatments were replicated within 10 blocks at each temperature. Each block was contained in a stack of petri dishes, with one petri dish for each treatment. The location of the blocks in the incubation chambers and the order of the petri dishes in the blocks was re-randomized at least once a week.

Germination was counted every 1-3 days. Seeds that had germinated were counted, recorded, and removed from the petri dishes. From daily germination counts, we calculated the following germination indices: 1) Final germination percentage (FGP), time to reach 10, 20, 50, and 90% germination (T_{10}, T_{20}, T_{50}, and T_{90}), and germination synchrony (T_{90}-T_{10}). Final germination percentage was corrected at the end of the study by counting the remaining amount of seeds in the petri dish at the end of the trial and calculating a percentage based off of how many seeds had germinated.

Time to reach T_{10}, T_{20}, T_{50}, and T_{90} was calculated as follows:

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

where: T = time (days) 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 10, 20, 50, or 90% of the total population.

Field Germination Trials

Seedling emergence of untreated seed and agglomerate seed was assessed at two different locations. Field studies were planted on 4 November 2016 at the same site where soil was collected. Vegetation at this site is predominantly weedy species, bulbous bluegrass *(Poa bulbosa* L.), and curveseed butterwort (*Ceratocephala testiculata [Crantz] Roth).* The second study site was planted on 5 November 2016 near Lookout Pass in Tooele County, UT, U.S.A. (lat 40°09'N, long 112°28'W). The Lookout Pass site was dominated by crested wheatgrass (*Agropyron cristatum* [L.] Gaertn). Prior to planting, existing vegetation was removed by spraying with 280 g ai · ha^{-1} of glyphosate (Accord Concentrate, Dow AgroSciences, Indianapolis, IN, U.S.A.) using a Chapin 61800 4-gallon ProSeries Backpack sprayer with a tank pressure of ~400 kPa, in April and again in October. The study was arranged in a randomized complete block design, where untreated (control) and agglomerated seed were randomly assigned a row within each of 10 blocks. Seeds were planted in 2 m rows with 30 cm between each row. Rows were seeded with ~250 pure live seeds m^{-1} using a push cone seeder (Kincaid Equipment, Haven, KS, U.S.A.). Seeds were weighed out using the same seeds g^{-1} weight calculation in the laboratory trials. The cone seeder was modified so the seeds were placed onto the soil surface and the back wheel of the seeder pressed the seeds into the soil 1-3 mm deep. The total number of emerged seedlings was determined by counting individual seedlings across the length of each row in May of 2017.

Statistical Analysis

The laboratory germination trial was analyzed using mixed model analysis (JMP®, Version *13,* SAS Institute Inc., Cary, NC, U.S.A., 2017) to determine the effects of agglomerating sagebrush seed. Models were used to analyze the effect that agglomerates have in relation to FGP, germination timing ($T_{10,}$ $T_{20,}$ $T_{50,}$), and synchrony. In the model, block was considered a random factor while incubation temperature, seed treatment, and the interaction of temperature x treatment were analyzed as fixed factors. We tested for differences in response of agglomerated seed compared to control seed at the incubation temperatures of 5, 10, 15, 20, and 25 °C using a single-tailed t test, where probability of control < agglomerated seed. Because residual plots and linearity tests indicated that $T_{10,}$ $T_{20,}$ $T_{50,}$ and synchrony values violated statistical assumptions for equal standard deviation and linearity, they were log transformed. Sagebrush seedling density in the field was analyzed using a mixed model analysis with blocks considered random and treatment, study site, and the interaction of treatment x study site being fixed factors. For all statistical comparisons a significance level of $P < 0.10$ was used; values were reported as mean ± *SE*.

RESULTS

Flowability

Successful agglomerates of sagebrush seed were created through our treatment technique (Fig. 1). Agglomerating sagebrush seed increased bulk density from 0.24 g $m1^{-1}$ to 0.58 g $m1^{-1}$, a 142% increase. The agglomeration process increased sagebrush seed tap density from 0.29 g ml^{-1} to 0.65 g ml^{-1}, a 124% increase. A Hausner ratio closer to 1.0 is indicative of better flow, we recorded an average ratio of 1.23 for untreated seed and 1.13 for agglomerated seed ($P < 0.01$),

indicating that agglomerated seeds have improved flow over untreated seed. Improved flowability through agglomeration was also indicated through the angle of repose measurement; the general principal is that the smaller the angle the better the flow properties. Angle of repose of untreated and agglomerated seed was 43° and 34°, respectively, giving a 21% decrease in the angle of repose between the seed types.

Lab Seed germination

Final germination percentage was influenced by seed treatment ($P = 0.003$), and incubation temperature ($P = 0.002$) but not by their interactions ($P = 0.86$). Across all temperatures, mean final germination percentage was 15% higher for the agglomeration seed treatment, final germination percentage = 75.1 ± 2.8 % for control and 86.8 ± 2.7 % for agglomerated seed (Fig. 2). At 10, 15, and 20 °C final germination percentage of the agglomerates was 17, 17, and 15% higher, than the control, respectively; while at 5 and 25 °C a treatment effect was not detected (Fig. 2).

Measurements of T_{10}, and T_{20} were influenced by seed treatment ($P = 0.03$ - 0.06) and temperature ($P < 0.01$) but not by their interaction ($P = 0.43$ - 0.64). At 5 °C, sagebrush agglomerates decreased germination timing by 3.15 and 3.83 d for T_{10}, and T_{20} respectively (Fig. 2A-B). Above 5 °C, germination timing was similar between the treatments. Analysis showed T_{50} was not influenced by seed treatment ($P = 0.96$) or the interaction with treatment and temperature ($P = 0.32$) but T_{50} was influenced by temperature ($P < 0.01$). As temperature increased seed germination timing decreased, particularly between 5 and 10°C where T_{50} decreased on average between the treatments from 42.8 to 9.3 d, respectively (Fig. 3C).

Germination synchrony was influenced by seed treatment ($P < 0.025$) and temperature ($P = 0.01$) but not by their interaction ($P = 0.31$). At 10 and 15°C, agglomerates increased synchrony by 8.44, and 8.97 d respectively. As with T_{50}, as temperature increased germination synchrony decreased, with sharp contrasts between 5 and 10 °C (Fig. 2D).

Field emergence

The amount of emerged seedlings was not influenced by seed treatment ($P = 0.948$), site (0.239), or the interaction between them (0.883). The number of emerged seedlings at the Santaquin study site was 5.26 ± 0.92 seedlings m^{-1} for untreated seed and 5.1 ± 1.01 seedlings m^{-1} for agglomerated seed. The average number of emerged seedlings at Lookout pass was 8.33 ± 5.2 seedlings m^{-1} for untreated and 7.63 ± 2.48 seedlings m^{-1} from agglomerated seed (Fig. 4).

DISCUSSION

There have been few improvements in the technologies used for rangeland restoration over the last several decades. Concepts of aerial seeding and rangeland drills have been around for years with little adjustments made to improve seeding success. Seed coating in itself is a relatively new technique in rangeland restoration, but is quickly gaining headway as more research demonstrates its potential benefits (Madsen et al., 2016a). Our results provide evidence that agglomerating seeds produced a smaller Hausner ratio, and minimized the angle of repose, which demonstrates improved flowability of sagebrush agglomerates compared to untreated seed. Enhanced seed flow characteristics may improve land managers ability to distribute seed across the landscape and reducing the labor associated with mixing, handling, and seeding. Sagebrush agglomerates may further improve seeding efforts by enhancing the ballistic

properties of the seed, which should advance the ability of seeders to spread seed at a greater distances. Studies using broadcast seeders need to be conducted to fully understand the cost and benefits associated with agglomerating seeds and to verify an increase in flowability using traditional seeding equipment.

Agglomerating sagebrush also gives managers the ability to think in terms of altering the seed instead of altering the machinery or various other logistical aspects associated with seeding. Our laboratory experiments demonstrated that in controlled conditions agglomerates provided a moderate increase in overall seed germination and produced quicker initial seed germination times at cold temperatures. Field trials performed at two different sites, did not show a difference in seedling emergence between untreated and agglomerated seed. With no deleterious effects observed from the agglomeration treatment, additional research is now merited for using agglomerates as a platform to apply various seed enhancements such as: fungicides, plant growth hormones, herbicide protectants (i.e. activated carbon), water absorbent polymers, fertilizers, biologicals, soil surfactants and other treatments that may address factors controlling sagebrush recruitment (Madsen et al., 2012; Guzzomi et al., 2016; Merritt et al., 2016; Pedrini et al., 2016).

The agglomeration approach proposed in this study could potentially serve as a seed treatment for other species that have similar characteristics to Wyoming big sagebrush. Examples of such species may include but not limited to; low sagebrush (*Artemisia arbuscular* Nutt.), mountain big sagebrush (*Artemisia tridentata* Nutt. *ssp. Vaseyana* [Rydb.] Beetle) and black sagebrush (*Artemisia nova* A. Nelson.), along with various native forbs such as western yarrow (*Achillea millefolium L.*).

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FIGURES

Figure 1-1**.** Photo of (A) untreated and (B) agglomerated Wyoming big sagebrush seed using the recipe outlined in Table 1.

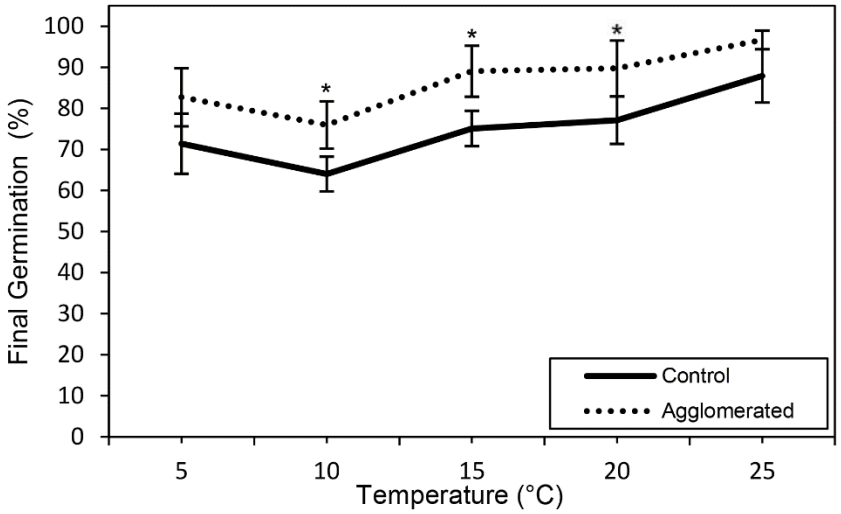

Figure 1-2**.** Influence of untreated (control) and agglomerated Wyoming big sagebrush seed on final germination percentage (mean ± SE) at temperatures ranging from 5-25 °C. Single asterisks indicate a difference in germination ($P < 0.10$) between the treatments at the specific temperature.

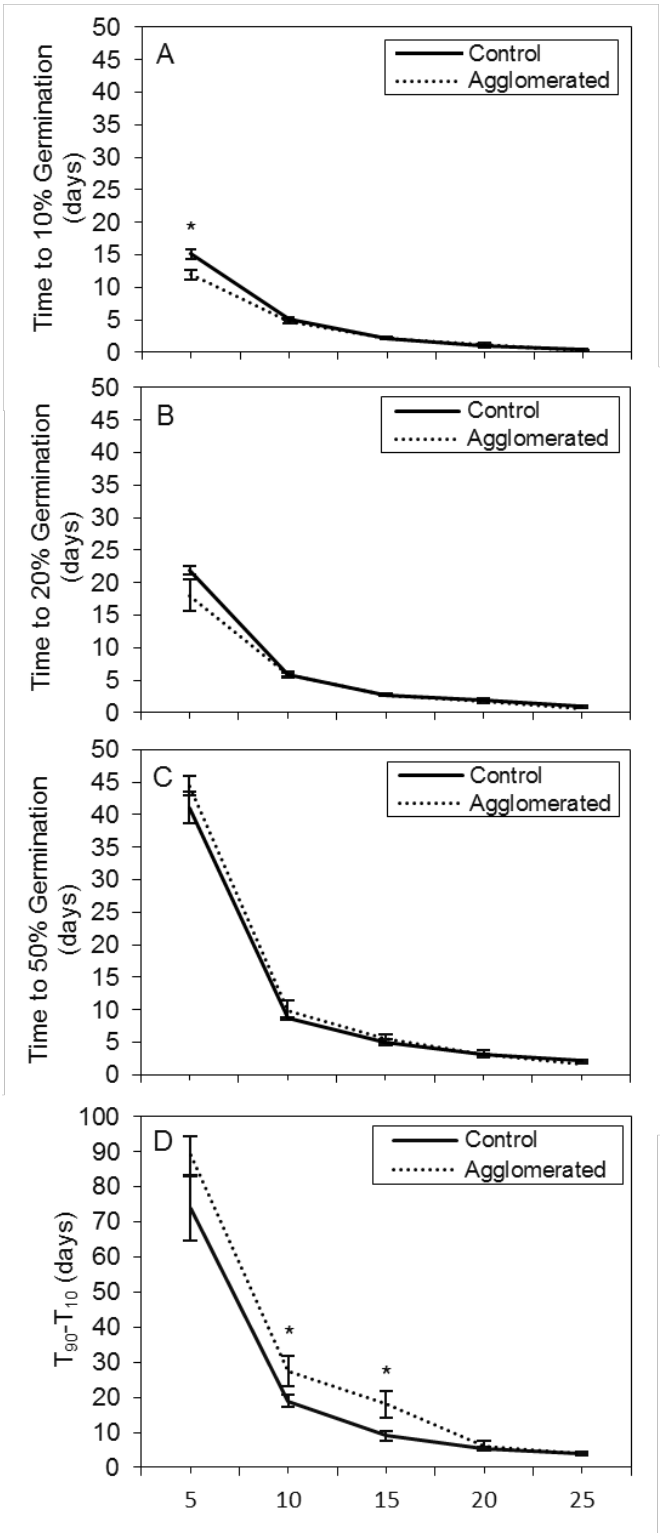

Figure 1-3. Influence of untreated (control) and agglomerated Wyoming big sagebrush seed for time to reach (A)10%, (B) 20% and (C) 50% germination, and (D) germination synchrony (mean ± SE) at temperatures ranging from 5-25 °C. An asterisk indicates a difference in germination ($P < 0.10$) between the treatments at that temperature.

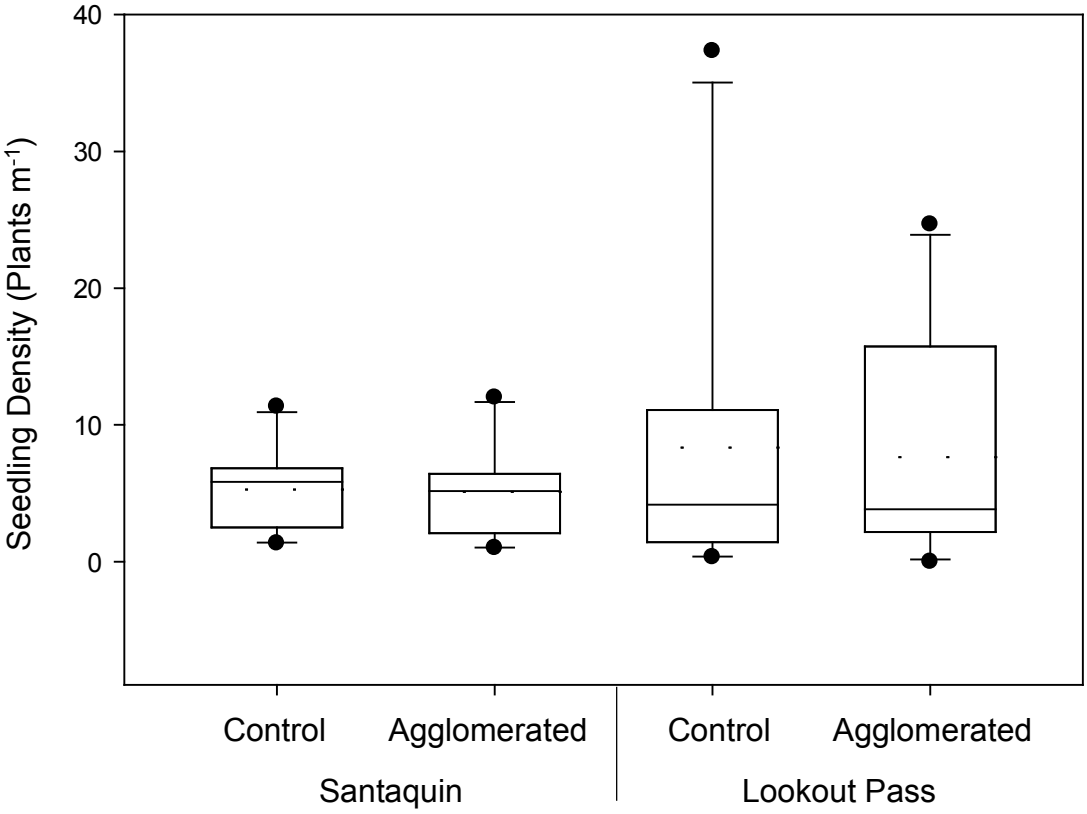

Figure 1-4**.** Box plots showing emergence for untreated (control) and agglomerated seed at two different study sites (Santaquin and Lookout Pass, UT) counted in May 2017. The boxes indicate the 25th to 75th percentiles where the solid line is the median and dashed line is the mean. The upper and lower bars represent the range from 0 to 100th percentile with individual dots representing outliers.

TABLES

Table 1-1**.** Recipe used to create a batch of sagebrush seed agglomerates. The table shows the different amounts of each ingredient used and at what step in the agglomerating process the ingredient is applied.

Agglomeration Step	Seed	Clay	Compost	Water	Binder
	----------g----------				
1	43	194	41	130.0	0.0
2	0.0	194	0.0	30.0	40.0
Total	43	388	41	160.0	40.0

CHAPTER 2

Modeled estimates of Wyoming big sagebrush seed germination timing and use of abscisic acid to delay seed germination

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ABSTRACT

Wyoming big sagebrush (*Artemisia tridentata* ssp. *wyomingensis*) is a dominant shrub on the more arid portions of the sagebrush range. Efforts to re-establish this species through direct seeding efforts have had limited success. Low seeding success may be due to seeds germinating during inopportune periods that are not suitable for plant establishment. Our objectives were to: 1) model when sagebrush seeds would germinate with different simulated planting dates, and 2) determine if the plant growth hormone abscisic acid (ABA) can delay germination and broaden the germination window. We evaluated sagebrush seeds that was either left untreated, coated, and coated with ABA at six different rates ranging from 1.0 - 10.0 g ABA formulation 100 g^{-1} of seed. Seed germination was assessed at five different temperatures ranging from 5 - 25 °C. Wet thermal accumulation models were created from laboratory data and applied to historic soil moisture and temperature data collected across six different sites in the western United States. Germination models predicted that with a 15 October planting date, 48 % of the seeds on average would germinate during late fall through winter, and the majority of remaining seeds would germinate in early spring. Seeds treated with high rates of ABA decreased the amount of seeds that germinated prior to and during winter, and spread the period seeds germinated in spring. Spreading out germination during the spring period with ABA treatments might lead to an

increased probability that some seeds would germinate under conditions that are more suitable for survival.

INTRODUCTION

Sagebrush (*Artemisia* spp.) ecosystems in the western United States are of critical concern due to altered fire regimes, woodland expansion, invasive species, and various anthropogenic disturbances including over-grazing, mining, oil and gas extraction, and urban development (Noss, 1995; Han et al., 2008; Knick et al., 2011; Davies et al., 2014; Davies & Bates, 2017). Degradation of sagebrush plant communities negatively impacts recreational activities, forage production, water resources, and hundreds of plant and animal species (Suring et al., 2005; Davies, 2011; Pyke et al., 2015; James & Carrick, 2016). Land managers spend millions of dollars annually on restoration projects to restore degraded sagebrush sites (Knutson et al., 2009; Davies et al., 2011; Knutson et al., 2014; Kildisheva et al., 2016).

Efforts to re-establish dryland species through direct seeding efforts have had limited success (Davies et al., 2011; James et al., 2011; Erickson et al., 2016; Guzzomi et al., 2016; James & Carrick, 2016; Madsen et al., 2016). While the reasons for seeding failures are not fully understood, the factors impairing seeding success occur during early stages of plant development (Madsen *et al.* 2016a). Specifically the highest probabilty of mortality for sagebrush occurs during its' first year (Schlaepfer et al., 2014). Germination rates are usually high within sagebrush species, meaning that germination in itself is not usually considered a limiting factor (Harniss & Murray, 1973; Ziegenhagen & Miller, 2009). However, germination timing and the amount of precipitation received plays a key role in establishment, where success of Wyoming big sagebrush is dependent on high levels of precipitation (Young et al., 1990; Schuman et al.,

1998). When the amount of precipitation received between December and January was above the annual average Wyoming big sagebrush was shown to have higher long-term establishment (Maier et al., 2001; Ziegenhagen & Miller, 2009). It is also notable that temperature plays a key role in sagebrush establishment where the optimal range for sagebrush germination is above 10 °C (Hardegree, 2006; Schlaepfer et al., 2014). It is probable that sagebrush seeds germinate during unfavorable conditions when either temperature and or moisture is not suitable for seedling survival, which creates high mortality within the first year.

Abscisic acid (ABA) helps regulate dormancy in seeds (Ali-Rachedi et al., 2004a; Meng et al., 2017). Seed germination is not delayed strictly due to the concentration of the hormone but in relation to the ratio of ABA to gibberellic acid (GA) (LeonKloosterziel et al., 1996; Lefebvre et al., 2006). When the relative concentration of ABA is higher than GA, seeds are more likely to stay in their dormant state (Kermode, 2005; Duclos et al., 2014). ABA decline and dormancy is alleviated through cold stratification or through after-ripening that occurs during storage within the seed warehouse (Walkersimmons, 1987; Bewley, 1997; Ali-Rachedi et al., 2004b). Sagebrush seeds are harvested in the winter and then stored for up to a year or more prior to planting. Upon planting, these stored seeds have meet their after ripping requirements and will readily germinate when provided with adequate water, oxygen, and suitable temperatures. If seeds are germinable when sown in fall, they may germinate prior to winter and have an increased probability of exposure to unsuitable environmental conditions that could result in seedling mortality (James et al., 2011). It may be possible to improve seedling survival by delaying the germination of fall planted seeds until late winter or early spring using an exogenous application of ABA applied directly to the seed.

Seed germination modeling could provide an efficient approach prior to field planting to predict how ABA seed treatments impact seed germination timing. Wet thermal accumulation models are based on the premise that germination timing for non-dormant seeds can be predicted as a function of temperature accumulation when seeds are imbibed (Rawlins et al., 2012a). Rawlings et al. (2012b) showed that wet thermal accumulation models could accurately predict seed germination in the field between 50-95% of the time. It has also been suggested that due to strong limitations of temperature and moisture these models would give insights into sagebrush germination timing (Schlaepfer et al., 2014).

It is probable that wet thermal accumulation models can be built for individual ABA seed treatments and then applied to long term historical soil microclimate data to predict seed germination timing based off of simulated planting dates. If models were run using seedbed data from multiple sites and across several years it should provide a robust prediction of how ABA seed treatments may perform in the field. While this approach does not circumvent field research it may stream-line the development of ABA seed treatments.

The objective of this research was to determine for Wyoming big sagebrush: 1) how varying rates of ABA seed treatments influenced seed germination percentage, seed germination timing, and germination synchrony under different constant temperatures, and 2) estimate for each ABA seed treatment the timing of germination at different sites in the Great Basin region of the western United States using wet thermal accumulation models applied to soil moisture and temperature data sets.

MATERIALS AND METHODS

Seed Coating

Trials were performed on Wyoming big sagebrush seed obtained from the Utah Division of Wildlife Resources Great Basin Research Center in Ephraim, Utah. The seed was stored in cold storage for over one year and previously cleaned to a 30% purity with a germination of 80%. Seeds were treated at Brigham Young University's Seed Enhancement Laboratory. Seeds were coated using an agglomeration procedure and materials described previously (Chpt. 1, pg 4-5). Agglomerates were created using a mixture of clay, compost, seed, water, and an ABA solution. Clay used is sold under the trade name Azomite® and is a highly mineralized complex silica ore mined near Levan, UT, U.S.A. Compost was made with organic yard waste and grass clippings from the grounds of Brigham Young University campus (Provo, UT, U.S.A.). Compost was dried in a plant drier at 60 °C for 3 d and then ground in a Wiley Mill (Model 4, Arthur H. Thomas Co., Philadelphia, PA, U.S.A) using a 0.5 mm screen. The ABA coating solution was made using the plant growth regulator BioNik™, and is comprised of a 25% formulation of s-abscisic acid (s-ABA) (Valent BioSciences Corp., Libertyville, IL, U.S.A.). The ABA application rates used were 1, 2, 4, 6, 8, and 10 g BioNik 100 g^{-1} of seed. ABA was measured out and mixed with water to make 130 g of solution. ABA infused agglomerates were formed by combining 40.9 g of compost, 193.8 g of Azomite®, and 42.6 g of seed in the rotary seed coater and while spinning the material (20% of maximum rotor speed) adding 130 g of the previously mixed ABA solution onto the spinning disk in the center of the seed coater at a rate of approximately 6 ml s^{-1}. Once all the ABA solution was added, a second treatment of 193.8 g of Azomite® was added into the rotary coater while the seeds remained spinning. During this stage, tap water followed by 40 g of a liquid binder (Agrimer™ TF binder, Ashland Inc., Covington,

KY, U.S.A.) was added. Binder was added at the end of the coating to help maintain the coatings integrity and dusting off of the agglomerates during transportation and seeding. After all ingredients were applied, the agglomerates remained spinning in the coater for 30 s. Agglomerates were then dried for 13 min is an air dryer at 42 °C. After seeds were dried they were sieved through a 1.4 mm sieve to eliminate the large particles.

Germination Experiment

Seed germination of untreated seed (control), agglomerated seed, and nine rates of ABA were assessed over five constant temperatures (5, 10, 15, 20, 25 °C), in a randomized complete block split-plot design, with germination temperature as the split-plot factor. Seeds were incubated in environmental growth chambers, under (12h/12h) light/dark intervals (Precision Plant Growth Chambers, Thermal Fischer Scientific, Waltham, MA). Prior to starting germination trials, thirty ~0.1 g samples of the agglomerates were washed and the number of seeds in the sample were counted; thirty ~0.1 g samples of untreated seed were also weighed out and the number of seeds were counted. Average number of seeds g^{-1} of agglomerates was equal to 170.74 ± 8.06 (mean ± SE), untreated seed was 1233.16 ± 54.5 (mean ± SE). Using the seeds g^{-1} calculation, approximately 35 seeds were weighed out and placed on soil (to mimic field setting) inside petri dishes. Soil was collected from a degraded Wyoming big sagebrush site approximately 10 miles south of Santaquin, Utah (lat 39°54’35”N log 111°48’45”W). Soil at the site was composed of ~42% sand, 38% silt, and 20% clay and is classified as Donnardo stony loam with a pH of 7.4-7.8 and 1-3% organic matter (Soil Survey Staff 2017). After collection, soil was dried at room temperature, sieved through a 1.7 mm sieve to remove rocks and debris, then run through a soil grinder. Water was mixed into the soil bringing it to field capacity

(0.247g of water 1g soil^{-1}) and 25 g of the wet soil was uniformly placed across the bottom of a petri dish.

Germination was counted every 1-3 days. Seeds that had germinated were counted, recorded, and removed from the petri dishes. From daily germination counts, we calculated the following germination indices: Final germination percentage (FGP), time to reach 10, 50, and 90% germination ($T_{10,}$ $T_{50,}$ T_{90}), and germination synchrony (T_{90}-T_{10}). FGP was estimated by counting the remaining amount of seeds in the petri dish at the end of the trial and calculating a percentage on how many seeds had germinated.

Time to reach $T_{10,}$ $T_{50,}$ and T_{90} was calculated as follows:

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

where: T = time (days) 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 10, 50 or 90% of the total population. Germination synchrony measures the spread of germination, where a larger value represents a greater spread at which germination occurs and was estimated by subtracting T_{90} from T_{10}.

Germination modeling

Wet-thermal accumulation models were created, based on the modeling done previously by Rawlins *et al.* 2012b and Richardson et al [In Preparation]. Linear and curvilinear regression equations were created from the ABA lab data. These equations estimated the time it takes for sagebrush to germinate (T_x) in relation to incubation temperature. Models were created across

each of the listed ABA application rates, for every sub-population between 10% and 90% at 5 percent increments (T_{10}, T_{15}, T_{20}, etc.). To increase the models accuracy we used the inverse of the time it takes for sagebrush to germinate ($1/T_x$) (Rawlins et al., 2012a). Models were than applied to historic soil and moisture data to estimate when germination would occur for each ABA treatment. Soil microclimate data was obtained at a depth of 1-3 cm and was collected hourly from the Sagebrush Step Treatment and Evaluation Project (SageSTEP) (Cline et al., 2017). Through the SageSTEP program soil temperature and water potential data is measured at hourly intervals using thermocouples and gypsum blocks (Delmhorst, Inc., Towaco, NJ), respectively (Cline, 2014; Cline et al., 2017). The SageSTEP network had 19 different study sites across six different states. For our models we chose four different sites within Wyoming big sagebrush communities, Saddle Mountain, WA; Moses Coulee, WA; Hart Mountain, OR; and Onaqui, UT. Two more sites were chosen in Mountain and Basin big sagebrush communities encroached by juniper, Blue Mountain, CA; and Bridge Creek, OR. We picked these sites to give a good distribution across the Great Basin Region of the western United States.

Seed germination was estimated using the soil temperature and moisture data at each of the six sites over a five year period (2011-2015). We made two different models: the first model determined the month that > 50% of the population would germinate, based on a 15 October planting date. The second model ran simulations with daily planting dates between 1 September and 31 December, which are common periods for fall seedings. For each simulated planting date we analyzed when the population of seed would reach 50% germination. For both models the progress towards germination was estimated using the wet-thermal models (Roundy et al., 2007). These models estimate progress towards germination by accumulating thermal time when temperature > 0 °C and water potential > -1.5 MPa. Water potential of -1.5 MPa was used based

off of previous models where -1.5 MPa was shown to have the highest accuracy across several species (Rawlins et al., 2012b) and due to mortality occurring in sagebrush for prolonged durations at or below -1.5 MPa (Daubenmire, 1975; Schlaepfer et al., 2014). Progress towards germination was calculated by dividing hourly soil temperature by the time to reach T_x at the temperature of that data point (determined using the regression models described above). Progress towards germination, was then converted to a percentage and accumulated until 100% was reached. At that point, we determined that the germination interval of the regression model used was also reached (10%-90%, at 10% intervals). This process was repeated for each individual wet thermal model. For our results we looked at germination predictions using the standard of a 1 March germination date.

Statistical Analysis

Results were subjected to mixed model analysis (JMP®, Version 13. SAS Institute Inc., Cary, NC, 2017) to first look at the effects of ABA concentration, incubation temperature, and their interactions. In the model, block was considered a random factor, and incubation temperature and seed treatments were analyzed as fixed factors. Any interaction that was not significant ($P > 0.05$) was left out of the model. This was done for final germination percentage (FGP), time to reach 50% germination (T_{50}), and synchrony (T_{90}-T_{10}). Based off of residual plots and linearity tests the T_{50}, and synchrony violated the statistical assumptions of linearity and equal standard deviation. The data for T_{50} and synchrony was log transformed to better meet these assumptions. Comparisons between each ABA concentration rate and the untreated seed were analyzed using a Tukey pairwise comparison test ($P < 0.05$) across each incubation

temperature. Significant values from the mixed model analysis on FGP were analyzed in comparison to the untreated seed using a Dunnett's multiple comparison test.

RESULTS

Laboratory Seed Germination

Incubation temperature did not influence final germination percentage ($F_{4, 234} = 4.35$, $P = 0.002$). Final germination percentage varied depending on ABA treatment ($F_{7, 234} = 15.36$, $P < 0.01$) and the interaction between temperature and ABA treatment ($F_{28, 234} = 3.73$, $P < 0.01$). Germination of agglomerated seed with no ABA, and seed with 1 and 2 g BioNik 100 g^{-1} was 13, 21, and 21 % higher germination than the control, respectively. The higher ABA seed treatments were similar to the control.

Germination timing was influenced by incubation temperature ($F_{4, 234} = 638.39$, $P < 0.01$), ABA concentration ($F_{7, 234} = 145.93$, $P < 0.01$) and the interaction between incubation temperature and ABA concentration ($F_{28, 234} = 9.44$, $P < 0.01$). As temperature increased T_{50} decreased (Fig. 1) with a mean of 47.98 d required for T_{50} at 5° C and 16.1, 10.1, 7.2, and 7.2 d for each subsequent temperature. Generally speaking, at each incubation temperature, T_{50} increased as ABA concentration increased (Fig. 1). As an example, at 10 °C, mean T_{50} values increased in comparison to the control by 1.95, 4.97, 9.84, 12.97, 18.56 and 21.59 d for seeds treated with 1, 2, 4, 6, 8, and 10 g BioNik 100 g^{-1} of seed.

Synchrony was influenced by incubation temperature ($F_{4, 234} = 203.4$, $P < 0.01$), ABA concentration ($F_{7, 234} = 46.10$, $P < 0.01$), and the interaction between them ($F_{28, 234} = 8.17$, $P < 0.01$). Synchrony generally decreased as temperature increased with values at 72 d for 5 °C and

38, 21, 20, and 15 d for each subsequent temperature. Typically as the concentration of ABA increased the synchrony decreased, with the highest concentration of ABA having the highest value of synchrony. The exception to this was at 5 °C where no general pattern was observed in synchrony with values being at 68.9, 88.9, 78.3, 77.4, 68.0, 62.1, 61.2, and 68 d for concentrations of 0, 1, 2, 4, 6, 8, and 10g. From 10 °C to 25 °C the general trend was that higher concentrations of ABA had higher values of synchrony. For example the range of synchrony for untreated seed was between 18.9-3.85 d compared to the highest concentration (10 g BioNik 100 g^{-1}) that had a range from 52.5-33.38 d.

Cumulative germination predictions in the field

Model fitness for our curvilinear regression equations were in a range to accurately predict germination time (adjusted $R^2 = 0.90 - 0.56$). Models estimated that with a 15 October simulated planting date 48, 40, and 12% of the time the majority of germination would occur during October – February, March, and April, respectively (Fig. 2). As ABA concentration increased, less seeds germinated in the fall, and more seeds were estimated to germinate in spring or even into early summer (Fig. 2).

Individual Site Germination Predictions

The predicted planting date to have >50% germination to occur after 1 March was highly variable between each site. At Hart Mountain, our models predicted that to achieve >50% germination by 1 March you would need to plant untreated seed around 11 October. Each subsequent ABA concentration increased the date at which >50% germination would occur (Fig. 3). Meaning that to achieve the same 1 March germination date you would have to plant earlier

in the year. With concentrations of 1 and 2 g BioNik 100 g^{-1} of seed you would have to plant in mid-September to gain a 1 March germination date (Fig. 3). The higher concentrations would still not achieve a 1 March germination date with a mid-September planting date.

Moses Coulee showed a similar pattern in germination to Hart Mountain where >50% germination occurring for untreated seed by 1 March would be achieved using a 15 October planting date. Again similar to Hart Mountain using ABA you would have to plant earlier in the year to achieve a 1 March germination date (Fig. 3).

To achieve a 1 March germination date on Bridge Creek and Saddle Mountain you would have to plant after 31 December. At these sites you could achieve a 1 March germination date using different ABA concentrations, where depending on the concentration you could plant from late-October to early-November (Fig. 3).

Blue Mountain's model had the longest natural occurring delay in germination, where to achieve a 1 March germination date for untreated seed you would have to plant as early as 4 September. Using 2 and 4 g of BioNik 100 $g^{-1}$of seed and the 4 September planting date, you would achieve a mid-March germination. In contrast to this, there is no change in when germination would occur between September and October, in essence you could plant seeds in September and achieve the same germination as you would for seeds planted in October. The different concentrations of ABA were similar in that you would still achieve the mid-March to mid-April germination date if you planted anytime between September and October.

To have >50% germination occur after 1 March at Onaqui Sage you would need to plant around the 28 November for untreated seed. Although you would need to plant on 28 November to achieve a 1 March planting date for untreated seed, you could plant earlier in September-

October using different concentrations of ABA and still achieve >50% germination after 1 March (Fig. 3).

DISCUSSION

Seed coating has emerged as a technology to overcome limitations to seeding success in rangeland systems (Guzzomi et al., 2016; Madsen et al., 2016). Seed coating was previously limited to seeds that were conducive to being coated (i.e. relatively large and high purity seeds), however, Call et al [Chapter 1] seed agglomeration coating provided a platform for coating and applying seed enhancements. This is the first study that shows the application of an enhancement (ABA) to Call et. al [Chapter 1] agglomeration platform, and its influence on germination timing.

Understanding germination relationships to temperature and moisture play a crucial part in determining how to approach sagebrush seeding failures. Our predictive models help us to understand germination timing in relationship to these two important thresholds influencing sagebrush success. The predictive models in this study also demonstrated that Wyoming big sagebrush germination timing differs from commonly sown restoration species such as bluebunch wheatgrass (*Pseudoroegneria spicata* [Pursh] A. Love) (Hardegree et al., 2003; Hardegree et al., 2010; James et al., 2011; James et al., 2013). Where bluebunch wheatgrass planted in fall would yield upwards of 80% germination prior to the onset of winter (Boyd & James, 2013), our sagebrush modeling predicts that a large portion of sagebrush seeds would not germinate until after 1 March.

Our results indicate that sagebrush's relatively long delay in seed germination timing is due to its relatively slower germination rate at low temperatures. For example, Richardson et al.

(In preparation) demonstrated that T_{50} for bluebunch wheatgrass was only ~7 d slower at 5 °C than 10 °C (T_{50} = 6.2 and 13.4 d at 5 °C than 10 °C, respectively). Hardegree et al. (2003) showed for bluebunch wheatgrass that T_{50} was ~4 d slower at 6 °C than 9 °C (T_{50} =12.0 and 8.1 d at 5 °C than 10 °C, respectively). Cheatgrass (*Bromus tectorum* L.) and big squirreltail (*Elymus multisetus* [J.G. Smith] M.E. Jones) only had a ~4 d difference in T_{50} between 6 and 9 °C (T_{50} = 13.8 and 9.6 d at 5 °C than 10 °C, respectively), and a ~10 day difference for big squirreltail between 3 and 9 °C (T_{50} = 9.0 and 5.4 d at 5 °C than 10 °C, respectively). In contrast, our findings for big sagebrush showed that T_{50} was ~33 d slower at 5 °C than 10 °C (T_{50} = 41.1 and 8.6 d at 5 °C than 10 °C, respectively). This demonstrates a strong temperature threshold at which sagebrush begins to progress towards germination that is unique in comparison to previously modeled species in the sagebrush steppe. For the sites used in this study to predict seed germination timing in the field, soil temperatures were frequently below 10 ° C between November and March, which as previously stated is not optimal for sagebrush germination. Sites such as Moses Coulee and Onaqui Sage stayed below 5 ° C between November and December, consequently our models predicted that germination would be delayed the longest at these sites.

The Great Basin's annual and seasonal fluctuations in temperature and precipitation create a highly variable and unpredictable environment (Bates et al., 2006; Boyd & James, 2013). Our models showed that similar planting dates would potentially result in different germination times due to site temporal and spatial variability (Fig. 3). These unpredictable factors can have large impacts on sagebrush (Bates et al., 2006) making it crucial to limit these factors in order to have optimal conditions for success (Loik & Redar, 2003). To address the issues of environmental unpredictability and site variation a bet-hedging strategy could be implemented for sagebrush. Bet-hedging creates an aversion to risk, creating higher long-term

success, and is often associated in areas with variable climate (Philippi, 1993; Venable, 2007; Simons, 2011). The idea of bet-hedging is not a new concept and has been suggested several times as a means to increase restoration success by helping to reduce the risks associated with seasonal uncertainty (Philippi, 1993; Adondakis & Venable, 2004; Simons, 2011; Boyd & James, 2013; Rinella & James, 2017). Since bet-hedging is not a native strategy to sagebrush you would have to regulate germination by altering the planting dates or germination timing. Our results showed that ABA could delay germination ranging from 1.6-30.2 d depending on temperature and applied ABA concentration. Different concentrations of ABA would allow for germination to occur at periodic times throughout the year, with the potential that seeds would germinate under optimal windows for seeding success.

Previous research has shown the specific potential benefits of bet-hedging. Davies et al., (2018) compared success rates from sagebrush seed with and without a seed enhancement treatment across an elevation gradient on two different planting years and found that the treatment that was most successful varied with site and planting year. When a single treatment was seeded there was a 36% establishment success rate (defined as ≥ 0.25 sagebrush $\cdot$ m^{-2}); however, if the two treatments were combined it was expected that if both methods were used together success would have risen to 86% (Davies et al., 2018). It is probable that differences in seeding success between the two seed treatments was caused by differences in germination timing.

There are some limitations to this study. Although field conditions were mimicked using different incubation temperatures and native soil, we will need field data to understand how close the correspondence is between laboratory ABA trials and its affect in the field. Before this correspondence is analyzed the results of this study need to be taken with discretion. Field

conditions have the potential to expose the ABA coating to adverse conditions. For example, ABA may break down in the field more rapidly due to UV light exposure, leaching, pathogens and other factors that we were not able to synthesize in the laboratory.

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FIGURES

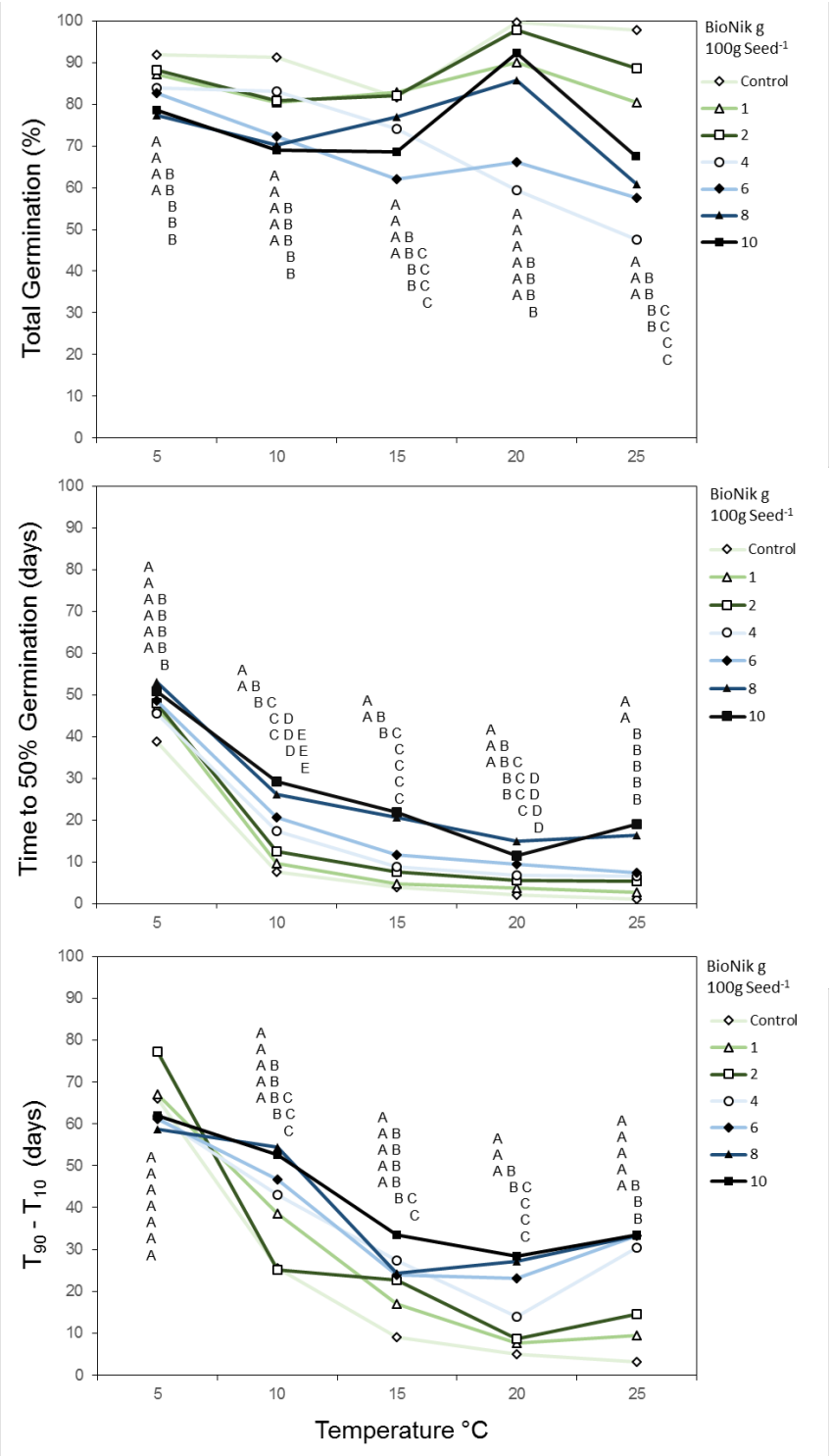

Figure 2-1. Influence of abscisic acid concentrations on total germination percentage, time to 50% germination, and synchrony at each incubation temperature. Values with the same incubation temperature with different letters are significantly different ($P < 0.05$) at that temperature. The letters correspond with the data points from top to bottom.

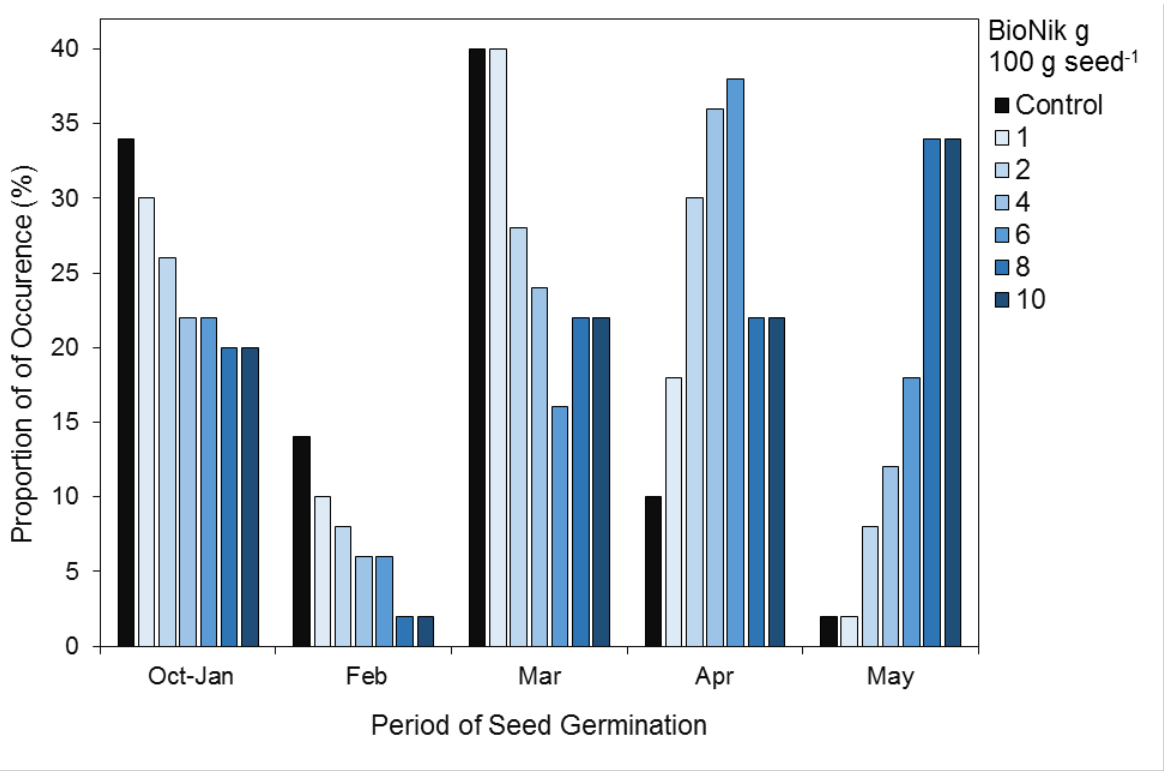

Figure 2-2. The period of the year when greater than 50% of the seed germinated based on a 15 October planting date. Values represent the percentage of occurrence across all sites (6 sites) and planting years (5 years) for untreated seed and seed treated with BioNik at rates ranging from 0-10 g Bionik 100 g^{-1} seed.

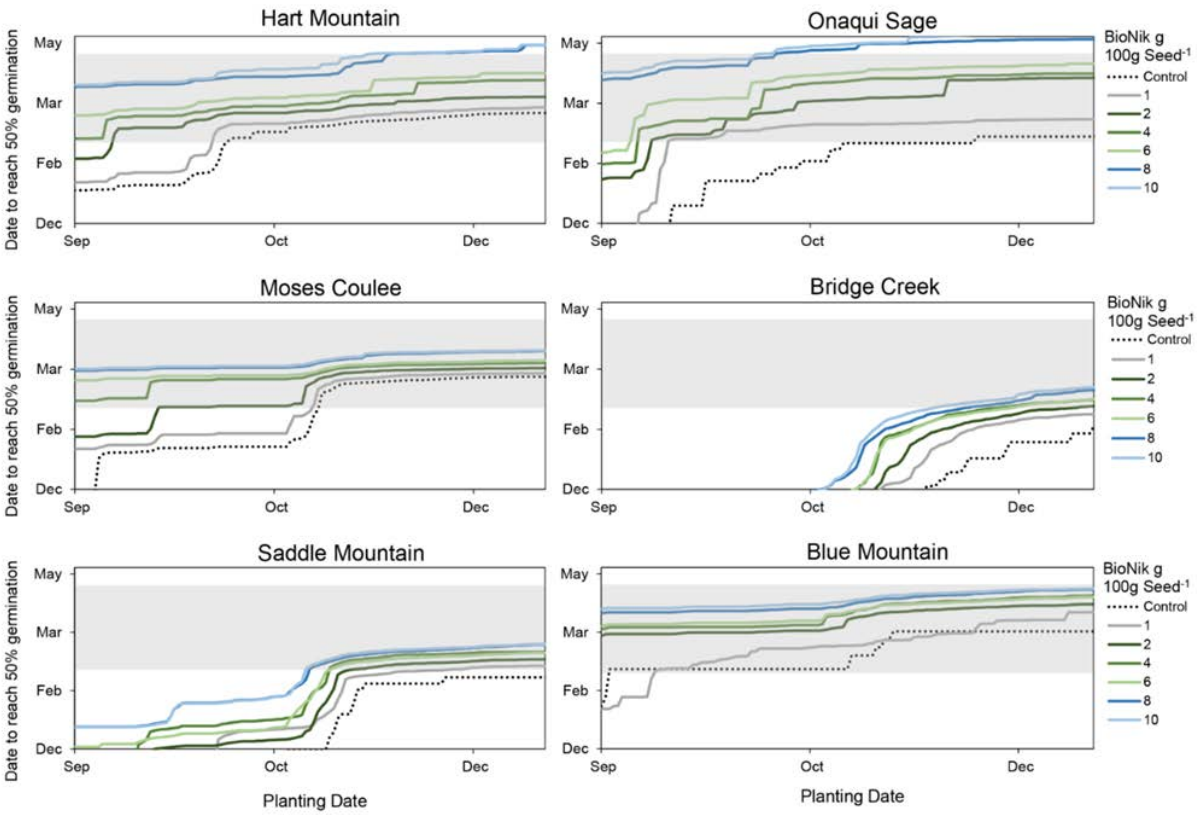

Figure 2-3**.** Modeled estimates of the date at which T_{50} would be reached based on simulated planting dates between 1 September and 31 December. Gray bars indicate the germination window between 1 March and 30 April

TABLES

Table 2-1. Displays the increments of ABA applied to each batch of agglomerates. Batch size was equal to 42.6 g of sagebrush seed. ABA was mixed with the appropriate amount to create the ABA solution that is applied in the first step of the agglomeration process.

BioNik 100 g^{-1} of Seed	**BioNik mg^{-1} Batch**	**Water (ml)**
1	426	128.57
2	852	128.09
4	1704	127.24
6	2556	126.38
8	3408	125.53
10	4260	124.68