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Use of Plant Growth Regulators to Expand the Period of Sagebrush Seed Germination and Reduce the Risk of Restoration Failure: Laboratory Trials

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ABSTRACT

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and Reduce the Risk of Restoration Failure: Laboratory Trials

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Seed germination during unhospitable environmental conditions can be a major barrier to direct seeding efforts in dryland systems. In the sagebrush steppe, *Artemisia tridentata* Nutt. ssp. *wyomingensis* and *Artemisia arbuscula* are important shrub species that are being used in restoration, but seeding success is highly sporadic due to inter-annual and intra-seasonal weather variability. Altering and expanding the period of germination, as a form of bet-hedging, may improve plant establishment. Our objective was to determine if we could expand the period of germination using plant growth regulators (PGRs) applied in a conglomerated seed coating treatment. In a laboratory study, the seed was either left untreated, conglomerated separately with two concentrations of a germination inhibitor, abscisic acid (ABA), or with two different germination promoters, gibberellic acid (GA_3) and 1-Aminocyclopropane carboxylic acid (ACC), a precursor to ethylene. Seeds were incubated in a loam soil at five constant temperatures (5-25 °C) for approximately three months. Results indicate that seed treatments with PGRs can delay or speed germination. The greatest response to the seed treatments was observed at 5 °C. For example, at this temperature PGRs delayed the time for 25% of the seeds to germinate by a maximum of 35 and 21 d and decreased this time by 5 and 25 d for *A. t.* ssp. *Wyomingensis* and *A. arbuscula*, respectively. Field studies are needed to determine if the bet-hedging strategy developed in this study will increase the likelihood that some seeds will germinate during periods that are more favorable for plant establishment.

ACKNOWLEDGEMENTS

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INTRODUCTION

Over the last century, the sagebrush (*Artemisia* L. spp.) steppe ecosystem in the western

United States has been modified or fragmented (Schroeder et al., 2004) due to historic

overgrazing, altered fire cycles, plant invasions, urban development, etc. (West, 2000; Davies,

2011; Svejcar et al., 2017). The loss of native plant species in this region has had far-reaching

effects on forage production, biodiversity, wildlife habitat, and ecosystem services (Dobkin &

Sauder, 2004; Gilbert & Chalfoun, 2011; Beck et al., 2012). Maintaining species richness and

native cover can make an area more resistant to annual plant invasions (Davies & Johnson,

2017; Urza et al., 2019). Additionally, the presence of a native plant community makes an area

more likely to recover after a disturbance such as fire, rather than converting to an invaded state

(Bates et al., 2014; Chambers et al., 2014). Effective restoration strategies focusing on ecosystem

resilience are critical to maintaining what native plant diversity is left and re-establishing what

has been lost (Scheffer et al., 2001). Unfortunately, native plant establishment from seed in

sagebrush steppe ecosystems is notoriously difficult and has had inconsistent success depending

on site conditions, elevation, species sown, and seeding techniques (Knutson et al., 2014).

Sagebrush is the keystone plant species within the sagebrush steppe (Davies et al., 2011).

Artemisia tridentata (big sagebrush) is widespread with the subspecies *A.t.* Nutt. ssp.

wyomingensis Beetle and Young (Wyoming big sagebrush) covering the largest area of all the

big sagebrush types. In more arid regions, it is generally found on moderately deep, well-drained

soils (Shultz, 2012). *Artemisia arbuscula* Nutt. (low sagebrush) is also an import species for

restoration that often grows in smaller populations, intermixed in stands of big sagebrush, though

it typically occupies different and distinct soil microsites (shallow clay or rocky soils) (Connelly

et al., 2004). These species are found in 13 western states and are important habitat and food

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sources for multiple obligate and near obligate species, including *Centrocercus urophasianus* (greater sage-grouse) and *Brachylagus idahoensis* (pygmy rabbit) (Shipley et al., 2006; Miller et al., 2011; Green et al., 2017).

Sagebrush sub-species have restrictive requirements for establishment (Call & Roundy, 1991; Hardegree et al., 2018a), such as high winter moisture regimes, dark chill periods under snow, and habitat and climate-related germination patterns (Meyer et al., 1990). Due to these specific requirements, high inter-annual and intra-seasonal weather variability in the sagebrush range result in episodic recruitment events (Perryman et al., 2001; Schlaepfer et al., 2014). Seeding efforts that are successful commonly occur at higher elevations, which tend to have cooler temperatures and higher precipitation (Davies et al., 2014), or at lower elevations under average or above-average precipitation conditions (Hardegree et al., 2016). At lower elevations, during years where snowpack in December and January is above the annual average, sagebrush emergence can be higher (Maier et al., 2001; Ziegenhagen & Miller, 2009). Typically in this region, however, sagebrush restoration from seed fails to increase plant density and cover (Lysne & Pellant, 2004; Arkle et al., 2014; Svejcar et al., 2017; Rottler, 2018).

To improve direct seeding efforts, it may be advantageous to expand the period of sagebrush germination over time, thus increasing the probability that some seeds will germinate within an environmental period that is more favorable for plant survival. Termed "bet-hedging," this approach has been seen in nature as a strategy for plants to deal with unpredictable environmental conditions over time (Simons, 2011). This strategy has also been suggested for use in ecological restoration by creating specialized biodiverse seed mixes with the goal that some seeds will be adapted to the environmental conditions faced during establishment (Rinella & James, 2017). Many of the invasive plants that are successful at establishing in degraded

sagebrush systems utilize bet-hedging with respect to seed germination. For example, *Bromus tectorum* L. (cheatgrass) seeds can germinate with a non-continuous moisture supply, so if environmental conditions are favorable, they germinate rapidly, but if moisture is no longer available, germination can be suspended (Meyer & Allen, 2009). Thus, some germination may occur in the fall, but some seeds may delay until winter or early spring, resulting in multiple germination events (Mack & Pyke 1983; Hardegree et al., 2010). *Halogeton glomeratus* (M. Bieb.) C.A. Mey (Halogeton) is another example of a successful invasive plant. It produces polymorphic seeds, some of which have no physiological dormancy and others with deep physiological dormancy (Khan et al., 2001a). *H. glomeratus* polymorphic seed is believed to be an important trait associated with its ability to establish in harsh environments; i.e., because this strategy assures that some seeds will carry over across years (Khan et al., 2001b). Additionally, in the monsoonal systems of southern Arizona, a non-native grass, *Eragrostis lehmanniana* Nees (Lehmann lovegrass) has more successful establishment compared to natives because it can delay germination of some seeds until precipitation increases later in the monsoon season (Roundy et al., 1996). It may be possible to improve restoration success of sagebrush by mimicking similar germination strategies to those that are commonly employed by non-native and invasive plants.

Seed coating technologies allow for the application of materials to the seed surface that can influence seed germination timing and plant establishment (Perring et al., 2015; Madsen et al., 2016). The period of seed germination could be manipulated by using a seed coating treatment containing plant growth regulators (PGRs), which are artificially synthesized plant hormones, that can either delay or accelerate seed germination. However, applying seed treatments using commercially available procedures that are designed to coat individual seeds evenly is not

practical for sagebrush due to its small seed size (~1.5 mm in diameter) and typical low purity (10 – 30%) (Jorgensen and Stevens 2004). Hoose et al., (2019) developed a new coating technique that uses a combination of clay, aeration medium (such as compost), water, and a polymer binder to conglomerate small, low-purity seeds, into small pellets. This conglomeration technology provides a platform for incorporating seed enhancement treatments such as PGRs.

Abscisic acid (ABA), gibberellic acid (GA), and ethylene are naturally occurring plant hormones that play important roles in regulating seed dormancy and germination (Zhao et al., 2011; Matilla, 2000; Corbineau et al., 2014; Meng et al., 2017). These could be applied within a seed conglomerate to influence germination timing. Dormancy is defined as a seed resisting germination even under appropriate temperature and moisture conditions, and ABA plays a crucial role in inducing and maintaining dormancy (Finch-Savage & Leubner-Metzger, 2006). Once a seed has met its after-ripening requirements and begins imbibing water, the ability to synthesize additional ABA is suppressed, and germination is stimulated, in part, through the production of GA and the catabolism of ABA (Ali-Rachedi et al., 2004). Two key processes lead to the completion of germination in endospermic species: embryo elongation and endosperm weakening. Embryo elongation requires cell expansion in specific regions of the embryo, which is promoted by GA and inhibited by ABA. ABA can also delay endosperm weakening and radicle growth, but its effects can be overcome by increased levels of GA and 1-Aminocyclopropane carboxylic acid (ACC), the direct precursor of ethylene, which does not affect ABA levels in the cell but rather interrupts ABA hormone signaling (Linkies & Leubner-Metzger, 2012; Villedieu-Percheron et al., 2014). Other phytohormones and specific signaling compounds, including brassinosteroids, auxin, cytokinins, salicylic acid, jasmonic acid,

strigolactones, and karrikins, also play crucial roles in inhibiting or stimulating germination (Corbineau et al., 2014).

Plant growth regulators that are commercially available can be applied to seeds exogenously (Camara et al., 2018). Recent laboratory studies using sagebrush steppe species have shown that ABA is effective at delaying germination of *Pseudoroegneria spicata* (Pursh) Á. Löve (bluebunch wheatgrass) (Badrakh, 2016; Richardson et al., 2019). In these studies, the rate of germination delay was correlated with the amount of ABA applied to the seeds. Synthetic applications of GA_3 PGRs can be applied to overcome physiological dormancy or accelerate seed germination (Tuna et al., 2008). In previous studies, seeds have typically been imbibed with a solution of GA_3 to break dormancy (Finch-Savage & Leubner-Metzger, 2006; Kildisheva et al., 2018). Because ethylene is a gas, ACC, which is the immediate precursor to ethylene in the seed and is rapidly metabolized to ethylene in metabolically active plant tissue, can be used for seed and plant treatments to stimulate germination (Matilla, 2000; Corbineau et al., 2014). The application of ABA and GA_3 have had limited use in exogenous applications within seed coatings on native dryland species in large-scale restoration projects (Pedrini et al., 2017) and ACC has not been used.

Our objective was to develop novel seed coatings for sagebrush seed, which incorporated PGRs to expand the period of seed germination. We conglomerated *A. arbuscula and A. t. ssp wyomingensis* seeds with or without PGRs and then assessed the influence of these on seed germination rate and final germination percentage under different constant temperatures. We hypothesized that ABA would delay germination while the germination promoters would increase the germination rate. By inhibiting germination in some seeds and promoting it in others, we hoped to expand the period of germination to several discrete events.

MATERIALS AND METHODS

Trials were conducted at Brigham Young University's Seed Enhancement Laboratory. *A. arbuscula* seed was purchased from Granite Seed and Erosion Control (Lehi, Utah, U.S.A.). *A. t. ssp wyomingensis* seed was obtained from the Utah Department of Wildlife Resources (Ephraim, Utah, U.S.A) (Table 1). The seed of each species was: left untreated (control), 2) conglomerated as described later with no addition of PGRs (blank), 3) conglomerated and treated with ABA at a low (ABA low) or high (ABA high) rate, and conglomerated and treated with GA_3 or with ACC.

Conglomeration Process

Ingredients to create conglomerates included sagebrush seed, Azomite® (Azomite Mineral Products, Inc., Levan, UT, U.S.A.), ground compost, water, and SCPII polymer binder (Ashland Inc., Covington, KY, U.S.A.). Azomite® is a highly mineralized complex silica ore (hydrated sodium calcium aluminosilicate). Compost was obtained from organic yard waste on Brigham Young University campus (Table 1). It was dried in a plant drier at 60 °C and then ground in a Wiley Mill (Model 4, Arthur H. Thomas Co., Philadelphia, PA, U.S.A.) using a 0.5 mm screen.

Conglomerates were formed in a 31 cm Universal Rotary Drum Seed Coater (Universal Coating Systems, Independence, OR, U.S.A). In the first step, sagebrush seed (42.6 g), Azomite® (193.75 g), and compost (40.9 g) (Table 2) were pre-mixed in a metal bowl then added to the seed coater. While spinning the coating pan at 20% of maximum speed, water (130 ml) was slowly added, followed by a second measure of Azomite® (193.75 g), and finished with 30 ml of water and 20 ml of SCP II binder. The liquids were applied onto the central atomizing disk, while powders were added directly onto the seeds. After the ingredients were added, the formed conglomerates were allowed to mix for an additional 60 s, and then they were removed

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from the coater and dried with a forced-air dryer (36 °C) for approximately ten minutes. Once dry, the samples were sieved to remove conglomerates outside of the target size of ~ 2 - 4 mm in diameter.

When adding a PGR treatment to the seed, the desired amount was combined with water during the first water addition in the coating process. The PGRs used in the study were obtained from Valent BioSciences LLC (Libertyville, IL, U.S.A.). The ABA formulation is sold commercially under the trade name BioNik^{TM} and is comprised of a formulation of 25% S-ABA. The conglomerated mixture was treated with either 5 g or 10 g of BioNik 100 g^{-1} of seed, to make the ABA low and ABA high treatments, respectively. Valent BioSciences LLC supplied GA$_3$ and the ACC in the form of experimental formulations. We applied GA$_3$ and ACC within the conglomerated mixtures at 4.41 g and 2.20 g 100 g^{-1} of seed, respectively. The PGR rates applied to the seed were based on preliminary laboratory trials conducted before the start of the study.

Seed Germination Experiment

We determined an average number of seeds per gram for each conglomeration treatment by weighing out approximately 0.8 g of conglomerated seed, washing the conglomerated mixture over a 0.074 mm sieve, and then counting the number of seeds in the sample. For control seed, approximately 0.2 g of seed was weighed out and then counted. We counted an average of 105 seeds per sample of *A. arbuscula* and an average of 305 seeds per sample of *A. t. ssp wyomingensis*. This seed estimation process was repeated six times to get an average number of seeds per gram for each treatment. Based on the seeds per gram value, we weighed out ~ 35 viable seeds of each treatment and placed them on top of 25 g of moistened field soil that was approximately 0.7 cm deep within 15-cm diameter Petri dishes. The field soil was collected from

a *A. t. ssp wyomingensis* site near Santaquin, Utah (lat 39°54'35" N log 111°48'45" W). The soil

at the site was composed of approximately 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. Before the experiment,

the soil was dried and sieved through a 0.25 mm sieve, and then brought to field capacity by

applying 0.25 g of water for every 1.0 g of soil.

The germination study was arranged as a randomized complete block split-plot design. Petri

dishes with lids were placed in Precision Plant Growth Chambers (Thermal Fischer Scientific,

Waltham, MA, U.S.A.) at five constant temperatures: 5, 10, 15, 20, and 25 °C, under light/dark

intervals (12 h/12 h). Each growth chamber/constant temperature contained six replicates of each

treatment (control, blank, 2 rates of ABA, 1 rate of GA_3, and 1 rate of ACC). Each of the

replicate treatments within a growth chamber was grouped into blocks and placed in plastic bags,

to prevent drying out. This study design resulted in a total of 300 Petri dishes for each species in

the study (5 temperatures X 6 seed treatments X 10 blocks = 300 Petri dishes).

Seed germination was counted daily for the first 14 d, and then every 2-3 d until germination

ceased. Germinated seeds, defined as those with radicals protruding at least 1 mm from the seed,

were removed after they were counted. At the time of counting, Petri dishes were re-randomized

within the block, and the location the block was placed within the growth chamber was also re-

randomized to reduce any temperature or light variation bias. This process was repeated using

both *A. arbuscula* and *A. t. ssp wyomingensis* seed.

From daily germination counts, we calculated the time to initial germination, time to reach 25

% germination (T_{25}) and final germination percentage (FGP). Time to initial germination was

calculated as the time between planting and initial germination. T_{25} was calculated as follows:

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

where: T = time (d) to subpopulation germination, t_a = incubation day when subpopulation germination was reached, t_b = incubation day before subpopulation germination was reached, n_a = number of germinated seeds on day that subpopulation germination was reached, n_b = number of germinated seeds on day before subpopulation germination was reached, N = number of germinated seeds equal to 25% of the total population. For Petri dishes that had zero germination (1 ABA high and 1 ABA low treatment for *A. arbuscula* and 1 ABA high treatment for *A. t. ssp wyomingensis*), T_{25} was not calculated, but rather replaced with 94 d (*A. arbuscula*) and 97 d (*A. t. ssp wyomingensis*), which are the upper limits of the study lengths (Kildisheva et al., 2018). To determine FGP, we counted the remaining number of seeds in the Petri dish at the end of the trial and calculated a percentage based on how many seeds had germinated. This procedure was performed because seeding rates in each Petri dish were based on weight estimates as described and not on the exact number of seeds.

Statistical Analysis

We analyzed time to initial germination, T_{25} and FGP using a standard least-squares analysis in JMP® version 13 (SAS Institute Inc., Cary, NC, U.S.A.). In the model, block was considered a random factor, and species, temperature, and seed treatments were analyzed as fixed factors. Based on residual plots and linearity tests, time to initial germination, $T_{25,}$ and FGP violated the statistical assumptions of normality and equal variance. The data for the time to initial germination and T_{25} were log transformed to better meet these assumptions, while an arcsin transformation better suited FGP data. We tested for differences between seed treatments across each incubation temperature using a Tukey pairwise comparison test ($P < 0.05$). The data

averages in the figures are labeled with letters to indicate significance between treatments. Cumulative germination curves were created using the software R (R Core Team 2015). Non-linear regression models were fit with the function 'drm' of the 'DRC' package following the method used by Ritz et al., (2013), which uses a three-parameter log-logistic model.

RESULTS

Comparison of Germination Between the Species at Different Constant Temperatures

Incubation temperature had a strong influence on the rate of germination and the degree of response was different between species (Table 3; Fig. 1). For both species, germination was delayed at cold temperatures but *A. t. ssp wyomingensis* seed germinated faster than *A. arbuscula*. For example, at 5 °C, seed germination was first detected for *A. arbuscula* at 40 d, while for *A. t. ssp wyomingensis,* germination was first detected at 11 d (29 d difference). At this same temperature, T_{25} for *A. arbuscula* and *A. t. ssp wyomingensis* was 55 d and 17 d, respectably (38 d difference).

The difference in seed germination timing was dramatic between the lowest and highest temperatures, with T_{25} values decreasing with increasing temperatures (Fig. 1). *A. arbuscula* and *A. t. ssp wyomingensis* had a 52 and 15 d difference in T_{25} between 5 and 25 °C, respectively (Fig. 1). The initial slope of the cumulative germination curves also indicates evidence of faster germination as temperature increased (Fig. 2). Additionally, cumulative germination curves indicate that *A. t. ssp wyomingensis* has a more uniform germination rate in comparison to *A. arbuscula* (Fig. 2).

Analysis of cumulative germination curves and FGP indicated that there was a strong thermal threshold inhibiting germination of *A. arbuscula* but not *A. t. ssp wyomingensis* (Table 3; Fig. 1

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and 2). For *A. arbuscula,* FGP at 5 and 10 °C was approximately less than half that at warmer temperatures. However, this degree of separation may have been less if the study would have continued for a longer period, as it appears from cumulative germination curves that germination had not yet leveled off at 5 and 10 °C (Fig. 2). Optimal temperatures for *A. arbuscula* FGP appear to be at 20 - 25 °C, while for *A. t. ssp wyomingensis* 15 °C produced the highest FGP.

Influence of Plant Growth Regulator Treatments on Germination

Significant two-and three-way interactions were found as the responses to the seed treatments varied by species and temperature (Table 3; Fig. 2). The response of the PGR treatments tended to be greater for *A. arbuscula.* For both species, the effect of the PGR treatments was higher at lower temperatures. The blank and the control were not statistically different in their germination responses (Fig. 1). Tukey's test showed that the PGR treatments spread the time for initial germination and T_{25} into 2-4 different time periods depending on species and incubation temperature (Table 3; Fig. 1). The greatest difference in seed treatment effects was found at 5 °C. For *A. arbuscula* at this temperature, in comparison to the control, ABA high increased T_{25} by 21 d, while GA$_3$ and ACC decreased T_{25} by 25 and 17 d, respectively. For *A. t. ssp wyomingensis*, in comparison to the control, ABA low and ABA high increased T_{25} by 24 and 34 d, respectively, while GA$_3$ and ACC decreased T_{25} by 4 and 5 d, respectively. *A. arbuscula* and *A. t. ssp wyomingensis* seed treatments continued to have differences in germination timing at other temperatures, but the degree of difference between the treatments and the control generally declined with increasing temperatures. Seeds treated with ABA showed the greatest difference in germination timing from the control, with slower germination rates across all temperatures for both species.

Cumulative germination curves (Fig. 2) showed that GA_3 and ACC increased the number of seedlings that had germinated early in the study. FGP was generally not influenced by GA_3 and ACC seed treatments. The ABA seed treatments generally had notably lower final germination percentages compared to the other treatments at every temperature.

DISCUSSION

The results of this study support our hypothesis that the three PGRs applied through a seed treatment can expand the germination timing of *A. arbuscula* and *A. t. ssp wyomingensis* seeds (Fig. 1). The findings of this research represent a proof of concept that these seed treatments can cause some seeds to germinate rapidly and others more slowly, thus allowing for the use of seed mixtures that could expand the period of germination to several different germination events.

It could be advantageous to speed up sagebrush germination with GA_3 or ACC PGRs (Fig. 1) to improve plant establishment. In general, the rate that seeds germinate can be explained primarily by soil temperature when moisture is above a base water potential (Hardegree et al., 2018b). Sagebrush is a small-seeded species that is planted on the soil surface, and progress towards germination can be inhibited because the soil surface is highly susceptible to drying, particularly on years with low snow accumulation (Meyer & Monsen, 1992). Additionally, the rate that sagebrush progresses towards germination is relatively slow under cold temperatures, which are typical of the soil seed bed conditions of the Great Basin (Richardson et al. 2018). We observed that GA_3 and ACC treatments both decreased T_{25} of sagebrush at cold temperatures (e.g. at 5 °C, T_{25} was decreased using GA_3 and ACC by approximately one week for *A. arbuscula* and three weeks for *A. t. ssp wyomingensis*). On years and sites when soil moisture and temperature are marginal or inadequate for sagebrush germination, accelerated germination

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provided by GA$_3$ or ACC may allow a greater proportion of the seeds to meet their germination requirements.

Additional evidence that increasing the germination rate of sagebrush may improve restoration success may be found in the comparison of seeding success with *Achillea millefolium* L. var. *occidentalis* DC. (Western yarrow). *A. millefolium* is a small-seeded species that is commonly used in restoration seed mixes, which has a relatively higher establishment success rate than sagebrush (Laughlin et al., 2008). Richardson et al. (2018) developed wet thermal accumulation models for several common native rangeland plant species, and *A. millefolium* was shown to have considerably faster germination rates compared to sagebrush at cold temperatures, which may explain, at least in part, *A. millefolium's* success in direct seeding projects (Barak et al., 2018). The ability of GA$_3$ and ACC treatments to speed up sagebrush germination at cold temperatures may help sagebrush seeds have higher seeding success rates, such as those found for *A. millefolium.*

Alternatively, it could be beneficial in some years to delay germination with ABA (Fig. 1). At 5 °C, the ABA high treatment delayed germination approximately one month longer than the control of both species. Sagebrush is typically seeded in late fall or early winter (Shaw et al., 2005), but winter precipitation is highly variable from year to year. When winter snowpack levels are above average, sagebrush emergence is higher (Maier et al, 2001), however, when snowpack is low or absent, seedlings can experience high mortality from winter drought, damaging freeze-thaw cycles, and increased pathogen attack (Boyd & Lemos, 2013; Gornish et al. 2015; Roundy & Madsen, 2016). Roundy & Madsen (2016) showed that across 14 sagebrush steppe sites, there was an average of 58 freeze-thaws cycles in the upper 1-3 cm of soil between October and March during four years of monitoring. Because yearly weather conditions are

unpredictable, in some years, it could be advantageous for seeds to delay germination until late winter or early spring, avoiding these potentially hazardous conditions.

Due to high inter-annual and intra-seasonal weather variability in the sagebrush steppe, seeding success may vary by the type and amount of PGRs applied to the seed. For this reason, it may be advantageous to plant a mixture of seeds that have different types and amounts of PGR treatments to expand the germination window. Planting coated seeds that allow for multiple germination events (Fig. 1) could function as a bet-hedging strategy to minimize seedling mortality during unfavorable environmental conditions by staggering the timing of seed germination throughout the population. For example, at 5 °C, the PGRs spread *A. arbuscula* germination by about three months with GA_3 germinating 24 d faster and ABA high 22 d slower compared to the control. Seed germination of *A. t. ssp wyomingensis* at 5 °C was spread over a two-month period with GA_3 germinating 5 d faster and ABA high 35 d slower compared to the control. This degree of separation in germination timing provided by PGR treatments may be sufficient to significantly manipulate germination timing in the field into several germination events.

An example of bet-hedging with sagebrush seed treatments was shown by Davies et al. (2018). They compared success rates from sagebrush seed with and without being incorporated into a seed pillow (designed to improve seed-soil contact) 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 m^{-2}); however, if the two treatments were combined the expected success would have risen to 86%. It is probable that differences in seeding success between the two seed treatments were caused by differences in germination timing and that

planting seeds that contain a mix of different types and rates of PGRs will also result in a higher establishment compared to untreated seed alone.

As climate change alters long-term temperature and precipitation patterns, (Pachauri et al., 2014) PGR treatments that manipulate germination timing may become increasingly important. It has already been shown that as the minimum atmospheric temperature increases, there is a net decrease in sagebrush cover even in undisturbed stands (Shi et al., 2018). Schlaepfer et al. (2014) hypothesized that the combination of warmer winters, spring droughts, and unpredictable frost events, predicted with climate change, could have negative effects on sagebrush regeneration. The PGRs evaluated in this study could likely be used to correct for some of the effects of climate change by expanding the window of, and possibly realigning germination timing.

Further research is needed to evaluate the FGP response from PGR treatments in this study. Although our study showed suppressed germination from the ABA treatment (Fig. 1), it is probable that most of the seeds maintained their viability and that germination was delayed beyond the period of the study. Low germination was most likely a result of the seeds remaining in Petri dishes that prevented ABA from leaching away from the seed. Additionally, we observed, particularly at higher temperatures, that the delay in seed germination produced by the ABA seed treatment subjected seeds to a longer period of potential pathogen attack. Seeds sown in the field may not necessarily experience the same level of pathogen attack as under laboratory conditions. Further trials are needed to explore how these seed treatments fare under field conditions to effectively evaluate ABA's influence on the seeds' FGP.

Although our study focused on sagebrush specifically, adding PGRs to conglomeration seed treatments have the potential to be applied to other native plant species. In highly variable environments, bet-hedging strategies using PGR treatments may spread the risk of seeding

failure for native species in other ecological systems as well. Continued studies and field work needs to be performed to evaluate further how the PGR treatments used in this study may improve ecological restoration efforts.

TABLES

Table 1. Seed lot details for the two sagebrush species used in the study. Viability and purity analyses were performed by the Seed Laboratory of the Utah Department of Agriculture and Food (Salt Lake City, UT, U.S.A.). In their analysis, seed viability was determined using a tetrazolium chloride (TZ) test.

Species	Seed Supplier	Viability (%)	Purity (%)	Collection Location	Collection Elevation (m)
A. arbuscula	Granite Seed and Erosion Control	90	18.0	Cassia Co., ID, U.S.A.	1,651
A. t. ssp wyomingensis	Utah Division of Wildlife Resources, Great Basin Research Center and Seed Warehouse	90	26.7	Elko Co., NV, U.S.A.	1,707

Table 2. Materials used to conglomerate a single batch of sagebrush seeds. The table shows the different amounts of each ingredient and at what step in the conglomerating (coating) process they were applied.

Conglomeration Step	Seed	Clay*	Compost†	Water	Binder$^\beta$
			------------g------------		
1	42.6	193.75	40.9	130.0	0.0
2	0.0	193.75	0.0	30.0	20.0
Total	42.6	387.5	40.9	160.0	20.0

*Clay comprised of the commercial product Azomite® (Azomine Mineral Products, Inc., Nephi, UT, U.S.A), which is a highly mineralized complex silica ore (hydrated sodium calcium aluminosilicate), with a pH of 8.0. Azomite nutrient breakdown; 0 % Nitrogen, 0 % Phosphoric Acid, 0.2 % Potash, 1.8 % Calcium, 0.5 % Magnesium, with a typical CEC range of 25-30 meq 100 g^{-1}.

† Compost nutrient breakdown; 0.3 % Total Nitrogen, 0.15% Phosphorous, 0.65% Potassium, 2.7 % Total Carbon, 3.42 % Calcium, pH 7.28

βAgrimer™ SCP2 polymer (Ashland Specialty Ingredients, Bridgewater, NJ, U.S.A.)

Table 3. Statistical summary for analysis of variance for the fixed main effects and interactions of species, temperature, and seed treatment on time to initial seed germination, time to 25% germination (T_{25}) and final germination percentage (FGP).

Effect	Df	Initial germination		T_{25}		FGP	
		F	P	F	P	F	P
Species (S)	1	335.9	< 0.001	387.7	< 0.001	59.6	< 0.001
Temperature (T)	1	4144.9	< 0.001	3248.5	< 0.001	427.6	< 0.001
S X T	1	90.5	< 0.001	63.2	< 0.001	335.5	< 0.001
Treatment (Tr)	5	588.3	< 0.001	437.7	< 0.001	114.7	< 0.001
S X Tr	5	10.1	< 0.001	9.1	< 0.001	7.6	< 0.001
T X Tr	5	7.0	< 0.001	19.7	< 0.001	3.3	0.007
S X T X Tr	5	6.6	< 0.001	6.5	< 0.001	1.5	0.202

FIGURES
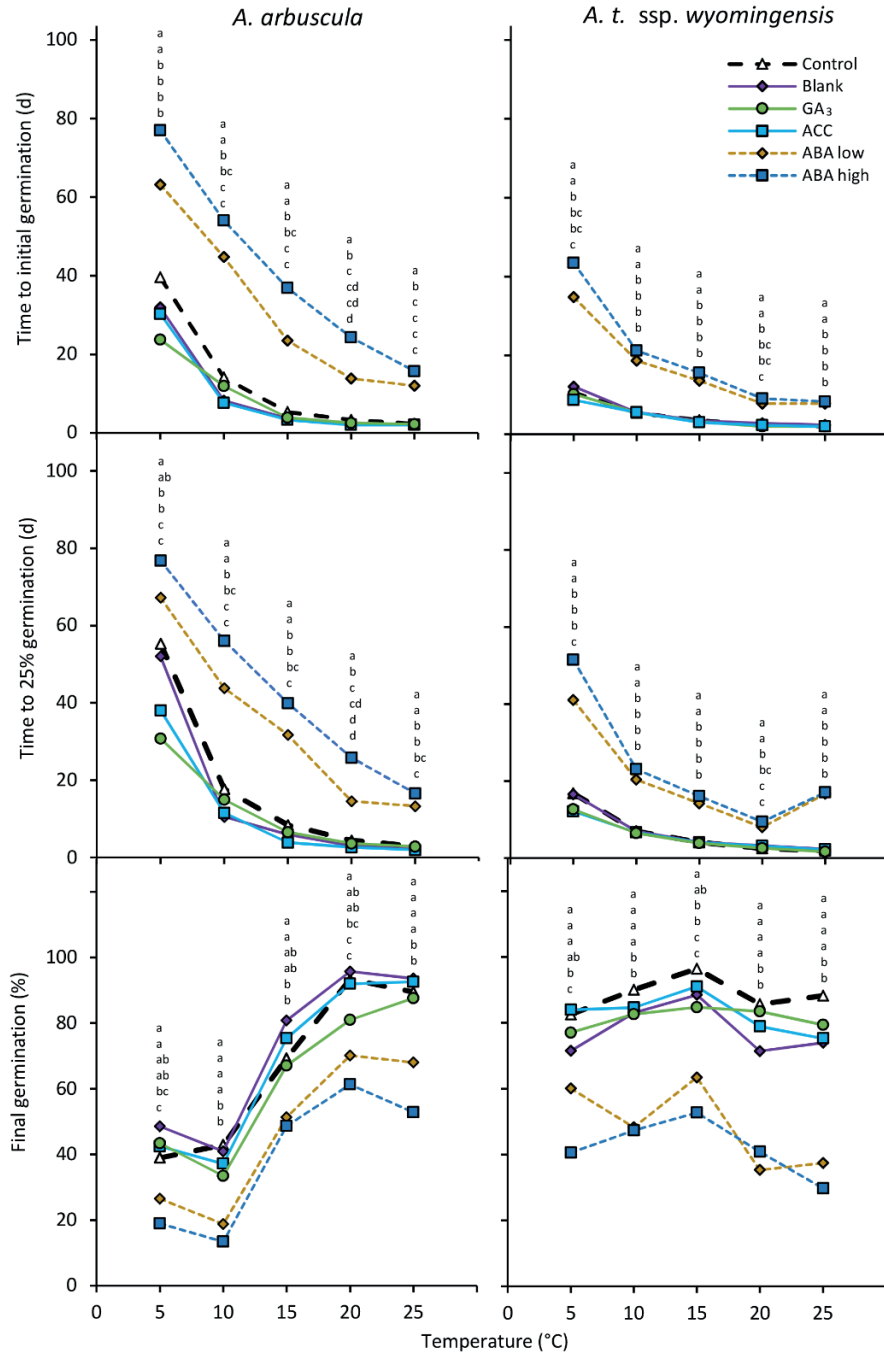
Figure 1. Seed germination responses for *Artemisia tridentata* Nutt. ssp. *wyomingensis* and *Artemisia arbuscula* that were untreated (control), conglomerated with no active ingredient (blank), or conglomerated with a formulation of 25% S-abscisic acid at a low (ABA low) and high (ABA high) rate, gibberellic acid (GA₃), and 1-Aminocyclopropane carboxylic acid (ACC). Values for a given temperature that are superseded by the same letter are not significantly different as determined with Tukey pairwise comparisons (*P* < 0.05). Letters correspond to the order of the data points in the figure.
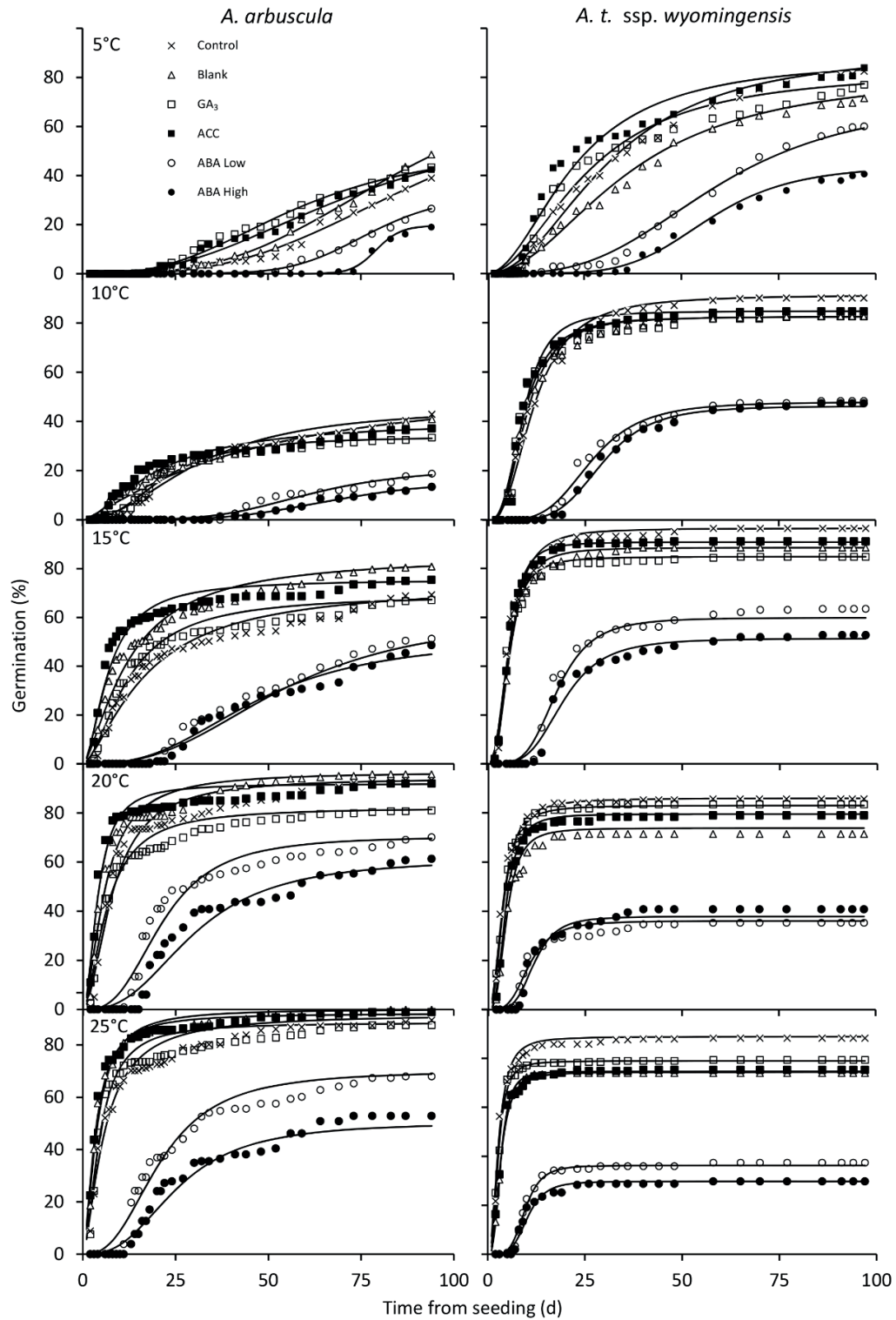
Figure 2. Cumulative germination curve of each seed treatment for *Artemisia tridentata* Nutt. ssp. *wyomingensis* and *Artemisia arbuscula.* The solid line represents cumulative germination over time estimated from a three-parameter log-logistic curve, and the symbols indicate germination recorded on a specific day.

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