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Overcoming Barriers to Native Species Restoration Using Gibberellic Acid and Fungicide Seed Coatings

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Master of Science

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ABSTRACT

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Master of Science

Many barriers can limit restoration success. In the first chapter of this thesis, the barrier of strong seed dormancy is addressed. While dormancy benefits the species' long-term survival, it can present a challenge within a restoration scenario where rapid establishment is required. Soaking seeds in gibberellic acid (GA_3) can overcome dormancy. An easier and potentially more effective method to apply this hormone is to coat seeds with a GA_3-impregnated polymer, which provides a slow release of the hormone. Seed dormancy can also be mitigated by creating a favorable microsite with increased soil moisture. We compared the emergence and establishment of penstemon seeds that were coated with GA_3 to uncoated seeds planted in shallow drill rows versus deep, U-shaped furrows. These treatments were evaluated in fall and spring plantings at three field sites in the Great Basin Region of the United States. Overall, coating with GA_3 improved the emergence and establishment of Palmer's penstemon (*Penstemon palmeri*; $p < 0.01$) and thickleaf penstemon (*P. pachyphyllus*; $p < 0.001$) but did not improve the emergence or establishment of firecracker penstemon (*P. eatonii*; $p = 1$). Between planting seasons, fewer seedlings emerged or established from spring than from fall planting ($p < 0.001$). Emergence and establishment were higher for all species in deep furrows than in shallow drill rows ($p < 0.001$). These results indicate that GA_3 seed coating and deep, U-shaped furrows may improve the restoration success of some native forbs. Land managers could use these techniques to restore native forbs in dry, disturbed areas.

The second chapter of this thesis addresses another barrier to successful restoration, specifically pathogenesis from soil and seed-borne fungus. Survival and growth of native seeds and seedlings can be limited by soil and seed-borne pathogens. Fungicides can combat fungal pathogens, but in some studies, fungicide treatments were ineffective at improving seedling emergence. These studies cite dry conditions leading to low fungal presence as the cause of the ineffectiveness of fungicide treatments for some years and sites. This study tested if fungicide treatment effectiveness is indeed related to the amount of fungus in the soil. We analyzed the emergence and biomass of uncoated, blank-coated, and fungicide-coated bluebunch wheatgrass (*Pseudoroegneria spicata*) across five soil fungal levels. For both percent emergence and total biomass, uncoated seed performed best in autoclaved soil and declined with increasing level of fungus, but the level of fungus did not impact fungicide-coated seed. When grown in autoclaved, untreated, or low fungal soils, percent emergence and total biomass from fungicide-coated seeds was not different from uncoated seeds. However, in medium and high fungal soils, the percent emergence and total biomass from fungicide-coated seeds were more than two times greater than uncoated seeds ($p < 0.05$). These results indicate fungicide seed coatings can be effective at increasing restoration success for bluebunch wheatgrass, but the effectiveness of this treatment depends on the microbial environment of the planting site.

Keywords: dormancy, forbs, furrows, fungal pathogens, habitat

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CHAPTER 1

Breaking Dormancy and Increasing Restoration Success of Native Forbs Using Gibberellic Acid Seed Coatings and U-shaped Furrows

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ABSTRACT

Many plant species exhibit strong dormancy. This attribute benefits the species' long-term survival but can present a challenge within a restoration scenario where rapid establishment is required. Soaking seeds in gibberellic acid (GA_3) can overcome dormancy and increase germination but this treatment may not be effective outside the laboratory. An easier and potentially more effective method to apply this hormone is to coat seeds with a GA_3-impregnated polymer, which provides a slow release of GA_3. Seed dormancy can also be mitigated by creating a favorable microsite with increased soil moisture. We compared the emergence and establishment of penstemon seeds that were coated with GA_3 to uncoated seeds planted in shallow drill rows versus deep, U-shaped furrows. These treatments were evaluated in fall and spring plantings at three field sites in the Great Basin Region of the United States. Overall, the GA_3 coating improved the emergence and establishment of Palmer's penstemon (*Penstemon palmeri*; $p < 0.01$) and thickleaf penstemon (*P. pachyphyllus*; $p < 0.001$) but did not affect the emergence or establishment of firecracker penstemon (*P. eatonii*; $p = 1$). Between our planting seasons, fewer seedlings emerged or established from spring than fall planting ($p < 0.001$). Emergence and establishment were higher for all species in deep furrows than in shallow drill rows ($p < 0.001$). These results indicate that GA_3 seed coating and deep, U-shaped furrows may improve the restoration success of some native forbs by breaking dormancy and providing a favorable microsite. Land managers could use these techniques to restore native forbs in dry, disturbed areas.

INTRODUCTION

While habitat restoration efforts have historically focused on perennial grasses, native forbs are important to reestablish in ecosystems after disturbance (Walker and Shaw 2005, Svejcar et al. 2017, Kildisheva et al. 2019a). Forbs have value for wildlife and livestock forage, pollinator use, and ecosystem resilience (Shaw 2005, Rawlins et al. 2009, Fund et al. 2019). Use of these species, however, has historically been limited due to high costs and low success in getting plants to germinate and emerge (Walker and Shaw 2005, Kildisheva et al. 2019a, Tilley et al. 2021). One reason for the low emergence of` these forbs is that the majority of these species exhibit strong physiological dormancy (Finch-Savage and Leubner-Metzger 2006, Baskin and Baskin 2014, Kildisheva et al. 2020). Seed dormancy is especially prevalent in arid environments, where this characteristic minimizes risk by postponing germination to avoid conditions where the seedling is unlikely to survive, such as freezing temperatures and drought (Kitchen and Meyer 1991, Lewandrowski et al. 2017, Kildisheva et al. 2020). For restoration efforts, however, this adaptation can be a limitation. After a disturbance such as fire, it is often critical to establish plants quickly to prevent erosion and invasion of exotic weeds (Kildisheva et al. 2019a, Larson et al. 2021). To establish dormant species quickly, techniques must be used to break dormancy.

Previous studies on overcoming dormancy have shown that gibberellic acid (GA_3) increased germination and emergence of various native forb species that are physiologically dormant (Rogis et al. 2004, Payal et al. 2014, Bujak and Dougher 2017). These studies applied the hormone by soaking seeds in a solution of GA_3, ethanol, and water (Rogis et al. 2004, Payal et al. 2014, Bujak and Dougher 2017). Soaking seeds in GA_3 can overcome dormancy and

increase germination, but this treatment may not be effective outside of the laboratory (Kildisheva et al. 2019a). An easier and potentially more effective method to apply this hormone is to coat seeds with a GA_3- impregnated polymer (Larson et al. 2023). This coating technique slowly releases the active ingredient to the seed as the coating breaks down in the soil. This slow release may extend the effects of the gibberellic acid to the seeds as they germinate and as the seedlings grow.

With the use of GA_3 to decrease time to germination, planting dormant species in spring may be possible. Generally, cold, wet conditions over winter are needed to break the physiological dormancy of many forbs (Kitchen and Meyer 1991, Monsen and Steven 2004, Kildisheva et al. 2019b). By using GA_3 to decrease time to germination, spring planting may be an alternative to traditional fall restoration plantings, which may improve survival of seedlings (Madsen et al. 2018). When seeds germinate in the fall or winter, they are exposed to pathogens and freezing conditions for months before emerging in the spring (Kuhnert et al. 2012, Boyd and Lemos 2015, Gornish et al. 2015). This can result in a severe bottleneck between germination and emergence (James et al. 2011, Gornish et al. 2015, Hoose et al. 2022). Planting GA_3 coated seeds in the spring may circumvent the worst of that bottleneck by allowing seeds to germinate quickly when conditions are prime for emergence.

Another technique to improve emergence and establishment of dormant native species is to use furrows to create a favorable microsite (Chambers 2000, Anderson et al. 2023, Camp et al. In Review,). Within deep (~15 cm) furrows, soil moisture is higher and temperatures are more moderate than the surrounding areas (Anderson et al. 2023). This could be especially useful in arid landscapes like the Great Basin region of the United States where seedings commonly fail due to drought conditions (Boyd and Lemos 2015, Svejcar et al. 2017). Traditional furrows are

created with V-shaped furrowers (Anderson et al. 2023, Camp et al. In Review). Small native forbs planted in V-shaped furrows, however, are at risk of being buried too deep as the walls of the furrow slough off with precipitation and gravity (Ott et al. 2016, Camp et al. In Review). The general rule for the ideal planting depth is about two to three times the width of the seed (Monsen and Stevens 2004, Tilley et al. 2021). Since many forbs in the Great Basin have very small seeds, they should be planted shallowly (Ott et al. 2016, Hardegree et al. 2016, Jensen et al. 2022). U-shaped furrows with a wide bottom may decrease the amount of soil that collapses onto the seeds after planting (Camp et al. In Review). U-shaped furrows may provide the same microsite benefits of traditional V-shaped furrows without risking small forb seeds being buried too deeply (Anderson et al. 2023, Camp et al. In Review).

A large genus of small-seeded forbs common in the Great Basin are the penstemons (Kitchen and Meyer 1991, Kramer et al. 2011). Several species of penstemon are commonly used in restoration efforts in western North America as they are valuable for wildlife, livestock, and pollinators (Kitchen and Meyer 1991, St. John et al. 2011, Ogle et al. 2013a, 2013b). Additionally, these species can assist in erosion control and protect soils (Ogle et al. 2013a, 2013b). Many penstemon species show strong physiological dormancy (Kitchen and Meyer 1991, Baskin and Baskin 2014) and respond positively to applications of GA_3 (Palzkill et al. 1988, Kitchen and Meyer 1991). We selected three penstemon species to test our technologies: Palmer's penstemon (*Penstemon palmeri* A. Gray), thickleaf penstemon (*P. pachyphyllus* A. Gray ex Rydb.), and firecracker penstemon (*P. eatonii* A. Gray). Preliminary work has shown that GA_3 coatings may decrease dormancy and increase emergence of these species (Larson et al. 2023).

In this study, we tested GA_3 seed coating, fall and spring planting, and U-shaped furrows to improve restoration success of penstemon species at degraded rangeland sites across Utah, USA. We compared the emergence and establishment of native penstemon seeds that were coated with GA_3 to uncoated seeds planted in late fall and early spring. Additionally, we compared GA_3-coated and uncoated seed planted in shallow, drill rows and deep, U-shaped furrows. We predicted that seeds coated with gibberellic acid would emerge and establish at a higher rate than uncoated seed. We also predicted that GA_3-coated seed would emerge and establish at a higher rate when planted in the spring than when planted in the fall. Further, we predicted there would be higher emergence and establishment in deep, U-shaped furrows than in shallow, drill rows.

METHODS

Study sites

This study took place at three sites across Utah that had been altered by farming, grazing, or fire. From November through December 2021, studies were planted near Santaquin (39.9073, -111.8163), Sage Valley (39.5462, -112.0683), and Enterprise (37.5889, -113.7157), Utah, USA (Fig. 1-1). These sites were planted again in March 2022. The Santaquin site is located at an elevation of 1620 m and is classified as an upland stony loam (Soil Survey Staff 2022). The Sage Valley site is located at an elevation of 1500 m and is classified as an upland loam (Soil Survey Staff 2022). The Enterprise site is located at an elevation of 1620 m and is classified as a semidesert shallow loam (Soil Survey Staff 2022). Additional site information is available in table 1. From June to October 2021, we used glyphosate and 2,4-D to control existing vegetation prior to planting.

These sites receive limited precipitation every year. Historic annual precipitation for Santaquin, Sage Valley, and Enterprise was 438 mm, 321 mm, and 384 mm, respectively, based on 30-year averages (PRISM Climate Group 2022). Precipitation for 2021 to 2022 was below average for all sites (Fig. 1-2). Santaquin, Sage Valley, and Enterprise received 430 mm, 278 mm, and 315 mm of precipitation, respectively, from October 2021 through 2022. While precipitation was lower than average for most months, precipitation was higher than average in October 2021, December 2021, and August 2022 (Fig. 1-2). Mean temperatures during this study were similar to 30-year averages for all sites (Fig. 1-2).

Seed treatments

We obtained Palmer's penstemon, thickleaf penstemon, and firecracker penstemon seed from the Utah Division of Wildlife Resources Great Basin Research Center and Seed Warehouse in Ephraim, Utah, USA. To evaluate the effectiveness of GA_3 seed coatings, we compared seeds coated with a polymer imbibed with GA_3 to uncoated seeds. The GA_3 polymer was prepared by impregnating ethylcellulose (Ethocel™, Dow Chemical, Midland, MI, USA) with GA_3 (Gold Biotechnology, St. Louis, MO, USA). To prepare this polymer, we dissolved 4.62 g of ethylcellulose in 50 mL of acetone on a stir plate. At the same time, we dissolved 0.382 g of GA_3 in 10 mL of ethanol on a stir plate. We then mixed the ethylcellulose and GA_3 solutions and stored the polymer in a sealed Erlenmeyer flask in a 4ºC cooler until we coated the seed.

We coated seed in 200-g batches using a 31-cm rotary seed coater (Universal Coating Systems, Independence, OR, USA) following standard seed coating protocols (Halmer 2008, Pedrini et al. 2017). We used a 45% polyvinylpyrrolidone (PVP) solution (Agrimer-15, Ashland Inc., Covington, KY, USA) as a binder, prepared by mixing 45 g of PVP powder for every 100 g

of water. First, we added 50 mL of GA_3 solution directly to the atomizer disk by syringe and allowed the polymer to dry onto the seed. Then we added calcium carbonate powder (Clayton Calcium, Parma, ID, USA) over the seed while pumping binder onto the atomizer disk. Amounts of lime and binder applied to each species varied by size and surface area of the seed (Table 2). Coated seed was dried at 20 to 25°C on a forced-air dryer (Universal Coating Systems, Independence, OR, USA).

Field planting

We planted our study in the fall (between 20 November and 8 December 2021) and spring (from 3 March to 22 March 2022). The study was a randomized split-split plot design. Each block was randomly divided between planting in deep, U-shaped furrows (hereafter "deep furrows") and shallow drill rows (hereafter "shallow rows"). These split plots were further divided into fall- and spring-planted plots. Species and treatments were randomized within split-split plots.

We planted seed using a JD Four-Row Planter (Kincaid Equipment Manufacturing Inc., Haven, KS, USA), which was adjusted to plant 2-m long rows 0.5 m apart. We fit modified furrowers in front of the planting disks to create furrowed rows that were 30 cm wide and 10 cm deep. Furrowers were modified by cutting off their bottom edge, to make furrows with a flattened bottom. Furrowers were removed for shallow rows. Before planting, the seeder was calibrated to plant seed ~2 to 5 mm below the soil surface, and the depth was periodically checked during planting to ensure uniform planting across blocks and furrow treatments. All species were planted at a rate of 246 pure live seed m^{-1}. Furrows for spring planting were made in the fall. Then we returned to each site in March and planted the seeds by hand to avoid complications associated with using heavy equipment in the spring mud. We took care to plant at

the same depth (~2 to 5 mm) as the seeder had been calibrated to plant in the fall. Emerged seedlings at each site were counted from 20 May to 3 June 2022. Established seedlings were counted on 10 August 2022.

Statistical analysis

For the field study, we analyzed the proportion of seeds that emerged and the proportion of seeds that were established using generalized linear mixed-effects models with a Poisson distribution. We constructed models for each species using the 'glmer' function of the 'lme4' package (Bates et al. 2022) in R (R Core Team 2022). Block was defined as a random effect. Treatment, furrows, and season of planting were defined as fixed effects. We tested for two-way interactions for each model between treatment, season, and furrows. Pairwise comparisons were then conducted using the Tukey method with the 'emmeans' function of the 'emmeans' package (Lenth et al. 2022).

RESULTS

Study sites

We excluded data from Sage Valley and Enterprise, Utah, USA from analyses because emergence was severely limited or absent for most treatments at those sites. There was no emergence at Sage Valley, and only 12 of 144 rows (8%) contained seedlings at Enterprise. For these reasons, all results presented below come from the Santaquin site.

Palmer's penstemon

Fall planting, seed coating, and deep furrows all improved seedling emergence, with the combination of these treatments resulting in the highest number of Palmer's penstemon seedlings (Fig. 1-3). Seed treatment had the strongest influence on seedling emergence ($F = 13.8$, $p < 0.001$), followed by furrows ($F = 7.5$, $p = 0.006$), and planting season ($F = 6$, $p = 0.01$). There

were no significant interactions, so the model was analyzed without interactions. When planted in the fall, no Palmer's penstemon emerged in shallow rows unless the seed was coated with GA_3 ($p < 0.001$; Fig. 1-3). Fall planting in deep furrows had five times higher emergence from seed coated with GA_3 ($\bar{x} = 1.25$ plants m^{-1}) than uncoated seed ($\bar{x} = 0.25$ plants m^{-1}; $p = 0.005$). Overall, two times more seedlings emerged when planted in the fall ($\bar{x} = 0.52$ plants m^{-1}) than when planted in the spring ($\bar{x} = 0.21$ plants m^{-1}; $p = 0.01$). All treatments had relatively minimal emergence for spring planting, except for GA_3-coated seed planted in deep furrows. Spring planting in deep furrows had seven times higher emergence from seed coated with GA_3 ($\bar{x} = 0.58$ plants m^{-1}) than uncoated seed ($\bar{x} = 0.08$ plants m^{-1}; $p < 0.005$; Fig. 1-3). Across both planting seasons and seed treatments, three times more Palmer's penstemon emerged when planted in deep furrows ($\bar{x} = 0.54$ plants m^{-1}) than in shallow rows ($\bar{x} = 0.19$ plants m^{-1}; $p = 0.006$).

As with seedling emergence, the density of established Palmer's penstemon at the end of the growing season was highest for GA_3-coated seed planted in the fall in deep furrows (Fig. 1-4). There was a significant interaction between seed treatment and planting season ($p = 0.007$), so we included that interaction in the model. When planted in the fall in shallow rows, there was eight times higher establishment from GA_3-coated seed ($\bar{x} = 0.67$ plants m^{-1}) than uncoated seed ($\bar{x} = 0.08$ plants m^{-1}; $p = 0.005$). This increase in establishment was more pronounced when GA_3-coated seeds were sown in deep furrows. For fall planting, establishment of GA_3-coated seed planted in deep furrows ($\bar{x} = 1.67$ plants m^{-1}) was 20 and 10 times higher than uncoated seeds planted in shallow rows ($\bar{x} = 0.08$ plants m^{-1}; $p < 0.001$) and deep furrows ($\bar{x} = 0.17$ plants m^{-1}; $p = 0.006$), respectively (Fig. 1-4). For spring planting, plant establishment did not vary by seed treatment or furrow type ($p = 1$; Fig. 1-4).

Thickleaf penstemon

As with Palmer's penstemon, fall planting, seed coating, and deep furrows all improved seedling emergence of thickleaf penstemon, with the combination of these treatments resulting in the highest number of plants (Fig. 1-3). Seedling emergence was most influenced by seed treatment ($F = 17.8$, $p < 0.001$) and planting season ($F = 17.8$, $p < 0.001$) but was also influenced by furrows ($F = 4.8$, $p = 0.03$). Thickleaf penstemon had relatively minimal emergence across all planting season and depth combinations unless coated with GA_3 ($p < 0.001$; Fig. 1-3). When planted in the fall in shallow rows, thickleaf penstemon had 12 times higher emergence when coated with GA_3 ($\bar{x} = 1$ plants m^{-1}) than uncoated seed ($\bar{x} = 0.08$ plants m^{-1}; $p < 0.001$). This increase in emergence with GA_3 seed coating was more pronounced in deep furrows. Fall planting in deep furrows had 29 times higher emergence from seed coated with GA_3 ($\bar{x} = 2.42$ plants m^{-1}) than uncoated seed ($\bar{x} = 0.08$ plants m^{-1}; $p < 0.001$). Overall, 21 times more thickleaf penstemon emerged when planted in the fall ($\bar{x} = 0.90$ plants m^{-1}) than when planted in the spring ($\bar{x} = 0.04$ plants m^{-1}; $p < 0.001$). When planted in the spring, thickleaf penstemon only emerged when coated with GA_3 and planted in shallow rows. However, overall, two times more thickleaf penstemon emerged when planted in deep furrows ($\bar{x} = 0.63$ plants m^{-1}) than in shallow rows ($\bar{x} = 0.31$ plants m^{-1}; $p = 0.025$; Fig. 1-3).

The density of established thickleaf penstemon at the end of summer was also highest for GA_3-coated seed planted in the fall in deep furrows (Fig. 1-4). Thickleaf penstemon had little to no establishment across all planting season and depth combinations unless coated with GA_3 ($p < 0.001$; Fig. 1-4). When planted in the fall in shallow rows, thickleaf penstemon had 12 times higher establishment when coated with GA_3 ($\bar{x} = 1$ plants m^{-1}) than uncoated seed ($\bar{x} = 0.08$ plants m^{-1}; $p < 0.001$). Additionally, no seedlings from deep furrows established unless the seed

was coated with GA_3 ($p < 0.001$). For fall planting, there was 27 times higher establishment from seed coated with GA_3 and planted in deep furrows ($\bar{x} = 2.25$ plants m^{-1}) than uncoated seed planted in shallow rows ($\bar{x} = 0.08$ plants m^{-1}; $p < 0.001$). Additionally, two times more thickleaf penstemon established overall when planted in deep furrows ($\bar{x} = 0.63$ plants m^{-1}) than in shallow rows ($\bar{x} = 0.31$ plants m^{-1}; $p = 0.026$; Fig. 1-4).

Firecracker penstemon

For firecracker penstemon, fall planting ($F = 45.5$, $p < 0.001$) and deep furrows ($F = 22.1$, $p < 0.001$) improved seedling emergence, but GA_3 seed coating did not influence emergence ($p = 0.92$; Fig. 1-3). The greatest seedling emergence for this species resulted from the combination of fall planting in deep furrows. There was a significant interaction between planting season and furrows ($F = 3.8$, $p = 0.01$). For spring planting, emergence was not different between shallow rows and deep furrows ($p = 1$), but for fall planting, deep furrows had greater emergence than shallow rows. For the fall planting, four times more seedlings emerged from deep furrows ($\bar{x} = 3$ plants m^{-1}) than shallow rows ($\bar{x} = 0.83$ plants m^{-1}; $p < 0.001$; Fig. 1-3).

Similar to seedling emergence, the establishment of firecracker penstemon at the end of the growing season was highest for seeds planted in the fall in deep furrows. There was a significant interaction between planting season and furrows ($p < 0.001$). For spring planting, establishment was not different between shallow rows and deep furrows ($p = 1$), but for fall planting, deep furrows had greater establishment than shallow rows. For fall planting, three times more plants established from deep furrows ($\bar{x} = 2.46$ plants m^{-1}) than shallow rows ($\bar{x} = 0.71$ plants m^{-1}; $p < 0.001$; Fig. 1-4). GA_3 seed coating did not improve establishment over uncoated seeds for any planting season and furrow combination ($p = 1$; Fig. 1-4).

DISCUSSION

GA_3 Seed Coating

Our prediction that GA_3 seed coating would increase the emergence and establishment of Palmer's, thickleaf, and firecracker penstemon was partially supported. In this study, GA_3 increased the emergence and establishment of Palmer's and thickleaf penstemon. These results build on other studies which show germination of these species increases when the seeds are treated with GA_3 (Palzkill et al. 1988, Kitchen and Meyer 1991, Larson et al. 2023). Although these studies also show that GA_3 may increase germination of firecracker penstemon (Palzkill et al. 1988, Kitchen and Meyer 1991); in our study, GA_3-coating did not improve emergence or establishment of that species.

Differences in seed dormancy among the plant materials in this study may have influenced the response of GA_3 seed coatings. Seed dormancy can be a complex, species-specific characteristic influenced by the environmental conditions where plant material was obtained (Kitchen and Meyer 1991, Meyer and Kitchen 1994, Meyer et al. 1995). Plant materials from higher elevations tend to have stronger seed dormancy (Baskin and Baskin 2014, Kucera et al. 2021, Brown and Allen In Review). Our firecracker seed was sourced from 1770 m, whereas our Palmer's and thickleaf penstemon seeds were from higher sites at 1890 m and 2130 m, respectively. As a heritable trait, seed dormancy may be influenced by the maternal environment where the seed was grown (Herman and Sultan 2011, Penfield and MacGregor 2017). Penstemon seeds produced by cultivated plants tend to germinate more readily without treatment than penstemon collected from wild populations (Meyer and Kitchen 1994, Kucera et al. 2021). In our study, the firecracker seed lot was three generations removed from the wild collection, whereas both the Palmer's and thickleaf penstemon seed lots were wild collections. The maternal

environment of the seed production plot likely differed from that of the wild seed collection sites. This difference in the maternal environments may have influenced the inherited dormancy of the penstemon species used in this study (Herman and Sultan 2011, Penfield and MacGregor 2017). Assuming the plant materials we tested in this study had differences in seed dormancy, we would expect GA_3 seed coatings to be most successful in the restoration of species with high levels of seed dormancy.

Although we can decrease the dormancy of some species with GA_3 coatings, it may not be in the best interest of restoration practitioners to break the dormancy of all seeds before planting. Evolutionarily, dormancy benefitted these species by allowing them to postpone germination to years when they would be more likely to establish (Kitchen and Meyer 1991, Lewandrowski et al. 2017, Kildisheva et al. 2019b, 2020). A bet-hedging strategy may increase long-term restoration success by planting a combination of GA_3-coated and noncoated seeds (Davies et al. 2018, Kildisheva et al. 2020). This would provide a mix of dormancy characteristics, so if the planting year is wet and favorable, the GA_3-coated seeds can emerge and establish. If the planting year is unfavorable, however, the uncoated, dormant seeds may remain viable in the seedbank and germinate when conditions may be better for plant survival (Meyer and Kitchen 1994).

Planting season

Our prediction of increased emergence from spring planting of GA_3-coated seed was not supported. Spring planting may have failed because of dry field conditions due to drought restrictions from January through June 2022 (PRISM Climate Group 2022). Spring planting of GA_3-coated seed may be more successful in years with higher precipitation. However, high precipitation levels can make spring planting difficult due to muddy or frozen soils that make the

site inaccessible to planting equipment (Hardegree et al. 2016, Svejcar et al. 2017). Future studies are merited to test GA_3-coated seed in fall and spring plantings across multiple years to account for annual variations in moisture availability. Based on the findings of this study and the understanding that spring is a challenging period to plant, fall appears to be the best time to plant GA_3-coated seed.

Deep, U-shaped furrows

As predicted, deep furrows increased seedling emergence and plant establishment of all species of penstemon tested in this study. Presumably, increased seeding success from the deep furrows resulted from the treatment improving the microsite of the seed. Anderson et al. (2023) measured increased soil moisture and moderated temperatures within deep, U-shaped furrows similar to those in our study. Optimal temperature and soil moisture contribute significantly to seed germination and seedling emergence (James et al. 2019). Our results support findings of other studies that showed increased emergence of grasses and Lewis flax (*Linum Lewisii* Pursh) when planted in deep, U-shaped furrows (Anderson et al. 2023, Camp et al. In Review). Additionally, the U-shaped furrows were designed with a wider bottom than traditional V-shaped furrows to decrease the amount of soil that collapses onto the seed, which may bury seeds too deeply (Anderson et al. 2023, Camp et al. In Review). We did not see the decreases in penstemon emergence that we would expect if the sides of the furrows were collapsing and burying seeds too deeply. By creating a favorable microsite, the deep, U-shaped furrowing technique may improve restoration success of small-seeded forbs, especially in areas where moisture is limited, and re-establishment of native forbs is essential.

Future work

Although these treatments improved the emergence of penstemon substantially, the percentage of seeds that emerged was quite low across our study, highlighting the complexity of restoration. Seedling emergence was absent at Sage Valley and severely limited at Enterprise, Utah, USA. Both Sage Valley and Enterprise are drier sites and received below average precipitation from January through June 2022 (PRISM Climate Group 2022). These sites may require years of higher precipitation to establish the penstemon species tested in this study. Thus, this research illustrates how the possibility of below-average precipitation should be considered when planning rangeland restoration projects (Madsen et al. 2016, Kildisheva et al. 2019b, Lewandrowski et al. 2016). Soil conditions at a restoration site may also be a concern (Madsen et al. 2019). At Sage Valley, thick soil crusts (>5 cm) developed, which likely prevented the emergence of small seedlings. Thus, when mineral soil crusts are present, additional treatments may be needed to restore an area (Laker and Nortjé 2019).

The low establishment rate in our study may also be due, in part, to the small seed sizes of the species we tested. At our Santaquin site, where we had the greatest success, only 0.1% of all seeds emerged. In addition to low precipitation, the low emergence of our seeds may be partially explained by the tradeoff between energy investment per seed and the quantity of seeds produced (Smith and Fretwell 1974). Plants that produce small seeds tend to yield greater quantities of seeds with less investment into individual seeds (Smith and Fretwell 1974). With this variation in parental investment, larger seeds correlate with improved germination and fitness, and smaller seeds tend to have lower success rates (Smith and Fretwell 1974, Silvertown 1984, Temme 1986). Like many native forb species, the penstemon seeds we planted were quite small (~ 1-3 mm). Successful restoration of disturbed, dry areas, such as our sites, will likely

require a multifaceted approach that addresses environmental, soil, and seed characteristics (Madsen et al. 2016).

With a broad range of factors limiting restoration success (e.g., dormancy, pathogens, drought, and soil crusting), compounding several treatments may be the most effective way to establish native forbs in disturbed and water-limited environments. In our study, we saw additive effects with GA_3 coating, fall planting, and deep, U-shaped furrows. For Palmer's and thickleaf penstemon, the highest emergence and establishment was consistently GA_3-coated seed planted in deep furrows in the fall. A combination of seed treatments (e.g., GA_3 seed coating) and planting techniques (e.g., deep, U-shaped furrows) may increase restoration success more than any of those factors alone. Using a combination of treatments and planting techniques to address multiple limiting factors may allow restoration practitioners to use and establish more native forbs in their restoration efforts.

LITERATURE CITED

Anderson R.M., Anderson, V.J., Phillips, K.C., Hansen, N.C., Stringham, T.K. and Madsen, M.D. 2023. Furrows and, to a lesser extent, seed priming improve restoration success in the sagebrush steppe. *Rangeland Ecology & Management* 87: 167-176.

Baskin C.C. and Baskin, J.M. 2014. Seeds: ecology, biogeography and evolution of dormancy and germination. 2nd ed. Academic Press, San Diego, CA, USA.

Bates D., Mächler, M., Bolker, B. and Walker, S. 2015. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software* 67(1):1.

Boyd C.S. and Lemos, J.A. 2015. Evaluating Winter/Spring Seeding of a Native Perennial Bunchgrass in the Sagebrush Steppe. *Rangeland Ecology & Management* 68(6):494-500.

Bujak C.M. and Dougher, T.A. 2017. Classification of seed dormancy and treatment of gibberellic acid to improve germination of arrowleaf balsamroot. *Native Plants Journal* 18(1):32-41.

Camp S. C. 2021. Improving Perennial Bunchgrass Seeding Success in Annual Grass Invaded Areas Using Pre-Emergent Herbicide and Furrowing Techniques. Dissertation / Thesis. Brigham Young University.

Chambers J.C. 2000. Seed movements and seedling fates in disturbed sagebrush steppe ecosystems: implications for restoration. *Ecological Applications* 10(5):1400-1413.

Davies K.W., Boyd, C.S., Madsen, M.D., Kerby, J. and Hulet, A. 2018. Evaluating a Seed Technology for Sagebrush Restoration Across an Elevation Gradient: Support for Bet Hedging. *Rangeland Ecology & Management* 71(1):19-24.

Finch-Savage W. and Leubner-Metzger, G. 2006. Seed dormancy and the control of germination. *New Phytologist* 171(3):501-523.

Fund A.J., Hulvey, K.B., Jensen, S.L., Johnson, D.A., Madsen, M.D., Monaco, T.A., Tilley, D.J., Arora, E. and Teller, B. 2019. Basalt Milkvetch Responses to Novel Restoration Treatments in the Great Basin. *Rangeland Ecology & Management* 72(3):492-500.

Gornish E.S., Aanderud, Z.T., Sheley, R.L., Rinella, M.J., Svejcar, T., Englund, S.D. and James, J.J. 2015. Altered snowfall and soil disturbance influence the early life stage transitions and recruitment of a native and invasive grass in a cold desert. *Oecologia* 177(2):595-606.

Hardegree S.P., Jones, T.A., Roundy, B.A., Shaw, N.L. and Monaco, T.A. 2016. Assessment of Range Planting as a Conservation Practice. *Rangeland Ecology & Management* 69(4):237-247.

Herman J. and Sultan, S. 2011. Adaptive Transgenerational Plasticity in Plants: Case Studies, Mechanisms, and Implications for Natural Populations. *Frontiers in Plant Science* 2:102.

Hoose B.W., Geary, B.D., Richardson, W.C., Petersen, S.L. and Madsen, M.D. 2022. Improving dryland seedling recruitment using fungicide seed coatings. *Ecological Solutions and Evidence* 3(1):e12132.

James J.J., Sheley, R.L., Leger, E.A., Adler, P.B., Hardegree, S.P., Gornish, E.S. and Rinella, M.J. 2019. Increased soil temperature and decreased precipitation during early life stages constrain grass seedling recruitment in cold desert restoration. *Journal of Applied Ecology* 56(12):2609-2619.

James J.J., Svejcar, T.J. and Rinella, M.J. 2011. Demographic processes limiting seedling recruitment in arid grassland restoration. *Journal of Applied Ecology* 48(4):961-969.

Jensen S., Christensen, W.F., Roundy, B., Anderson, V.J., Kitchen, S.G. and Allphin, L. 2022. Temporal and Spatial Factors Influence Native Forb Emergence More Than Sowing Depth. *Rangeland Ecology & Management* 83:41-49.

Kildisheva O.A., Erickson, T.E., Madsen, M.D., Dixon, K.W. and Merritt, D.J. 2019a. Seed germination and dormancy traits of forbs and shrubs important for restoration of North American dryland ecosystems. *Plant Biology* 21(3):458-469.

Kildisheva O.A., Dixon, K.W., Silveira, F.A.O., Chapman, T., Di Sacco, A., Mondoni, A., Turner, S.R. and Cross, A.T. 2020. Dormancy and germination: making every seed count in restoration. *Restoration Ecology* 28:S256-S265.

Kildisheva O.A., Erickson, T.E., Kramer, A.T., Zeldin, J. and Merritt, D.J. 2019b. Optimizing physiological dormancy break of understudied cold desert perennials to improve seed-based restoration. *Journal of Arid Environments* 170:104001.

Kitchen S.G. and Meyer, S.E. 1991. Seed Germination of Intermountain Penstemons as Influenced by Stratification and GA_3 Treatments. *Journal of Environmental Horticulture* 9(1):51-56.

Kramer A.T., Fant, J.B. and Ashley, M.V. 2011. Influences of landscape and pollinators on population genetic structure: Examples from three *Penstemon* (Plantaginaceae) species in the Great Basin. *American Journal of Botany* 98(1):109-121.

Kucera K.F., Fant, J.B., Jensen, S., Landeen, M., Orr, E., and Kramer, A.T. 2022. Genetic variation and structure change when producing and using mixed-source seed lots for restoration. *Restoration Ecology* 30(3):e13521.

Kuhnert R., Oberkofler, I. and Peintner, U. 2012. Fungal Growth and Biomass Development is Boosted by Plants in Snow-Covered Soil. *Microbial Ecology* 64(1):79-90.

Laker M.C. and Nortjé, G.P. 2019. Chapter Five - Review of existing knowledge on soil crusting in South Africa. *Advances in Agronomy* 155:189-242.

Larson, A., D.J. Michaelis, and Madsen. M.D. 2023. Development and Use of a Slow-Release Polymer Seed Coating System to Deliver Growth Hormones for Enhancing Seed Germination and Early Plant Growth. Provisional US Patent Application No. 63451061.

Larson J.E., Ebinger, K.R. and Suding, K.N. 2021. Water the odds? Spring rainfall and emergence-related seed traits drive plant recruitment. *Oikos* 130(10):1665-1678.

Lenth R.V., Buerkner, P., Herve, M., Love, J., Miguez, F., Riebl, H. and Singmann, H. 2022. emmeans: Estimated Marginal Means, aka Least-Squares Means
. R package version 1.7.2.

Lewandrowski W., Erickson, T.E., Dixon, K.W. and Stevens, J.C. 2017. Increasing the germination envelope under water stress improves seedling emergence in two dominant grass species across different pulse rainfall events. *Journal of Applied Ecology* 54(3):997-1007.

Meyer S.E., Kitchen, S.G. and Carlson, S.L. 1995. Seed Germination Timing Patterns in Intermountain *Penstemon* (Scrophulariaceae). *American Journal of Botany* 82(3):377-389.

Meyer S.E. and Kitchen, S.G. 1994. Habitat-Correlated Variation in Seed Germination Response to Chilling in *Penstemon* Section Glabri (Scrophulariaceae). *The American Midland Naturalist* 132(2):349-365.

Madsen M.D., Davies, K.W., Boyd, C.S., Kerby, J.D. and Svejcar, T.J. 2016. Emerging seed enhancement technologies for overcoming barriers to restoration. *Restoration Ecology* 24:S77-S84.

Madsen M.D., Svejcar, L., Radke, J. and Hulet, A. 2018. Inducing rapid seed germination of native cool season grasses with solid matrix priming and seed extrusion technology. *PLOS ONE* 13(10):e0204380.

Monsen S.B. and Stevens, R. 2004. Seedbed preparation and seeding practices. *Restoring Western Ranges and Wildlands* 1:121-154.

Ogle D., Peterson, S. and St. John, L. 2013a. Plant Guide for Palmer's penstemon (*Penstemon palmeri*). USDA-Natural Resources Conservation Service, Plant Materials Center. Aberdeen, Idaho 83210.

Ogle D., Peterson, S. and St. John, L. 2013b. Plant Guide for thickleaf penstemon (*Penstemon pachyphyllus*). USDA-Natural Resources Conservation Service, Plant Materials Center. Aberdeen, Idaho 83210.

Ott J.E., Cox, R.D., Shaw, N.L., Newingham, B.A., Ganguli, A.C., Pellant, M., Roundy, B.A. and Eggett, D.L. 2016. Postfire Drill-Seeding of Great Basin Plants: Effects of Contrasting Drills on Seeded and Nonseeded Species. *Rangeland Ecology & Management* 69(5):373-385.

Palzkill D.A., DePaul, L. and Sivilli, C. 1988. Seed Germination Response of *Penstemon* spp. To Gibberellic Acid. *Turfgrass and Ornamentals Research Summary* 75:81-82

Payal K., Maikhuri, R.K., Rao, K.S. and Kandari, L.S. 2014. Effect of gibberellic acid- and water-based pre-soaking treatments under different temperatures and photoperiods on the seed germination of *Allium stracheyi* Baker: An endangered alpine species of Central Himalaya, India. *Plant Biosystems* 148(6):1075-1084.

Penfield S. and MacGregor, D.R. 2017. Effects of environmental variation during seed production on seed dormancy and germination. *Journal Experimental Botany* 68(4):819-825.

PRISM Climate Group. 2022. PRISM Climate Data. Oregon State University. https://prism.oregonstate.edu, data created 11 July 2022 (Accessed 11 July 2022).

R Core Team. 2022. R: A language and environment for statistical computing.

Rawlins J.K., Anderson, V.J., Johnson, R. and Krebs, T. 2009. Optimal seeding depth of five forb species from the Great Basin. *Native Plants Journal* 10(1):32-42.

Rogis C., Gibson, L.R., Knapp, A.D. and Horton, R. 2004. Enhancing Germination of Eastern Gamagrass Seed with Stratification and Gibberellic Acid. *Crop Science* 44(2):549-552.

Shaw N.L. 2005. Increasing Native Forb Seed Supplies for. National Proceedings: Forest and Conservation Nursery Associations 2004:94.

Silvertown J.W. 1984. Phenotypic Variety in Seed Germination Behavior: The Ontogeny and Evolution of Somatic Polymorphism in Seeds. *American Naturalist* 124(1):1-16.

Smith C.C. and Fretwell, S.D. 1974. The Optimal Balance between Size and Number of Offspring. *American Naturalist* 108(962):499-506.

Soil Survey Staff. 2021. Web Soil Survey. Natural Resources Conservation Service, United States Department of Agriculture. Available online at http://websoilsurvey.nrcs.usda.gov/ (Accessed 6 July 2021).

St. John L., Tilley, D. and Ogle, D. 2011. Plant Guide for Firecracker Penstemon (*Penstemon eatonii*). USDA-Natural Resources Conservation Service, Plant Materials Center. Aberdeen, Idaho 83210.

Svejcar T., Boyd, C., Davies, K., Hamerlynck, E. and Svejcar, L. 2017. Challenges and limitations to native species restoration in the Great Basin, USA. *Plant Ecology* 218(1):81-94.

Temme D.H. 1986. Seed size variability: a consequence of variable genetic quality among offspring?. *Evolution* 40(2):414-417.

Tilley D., Wolf, M., Jolley, D. and Hirning, G. 2021. Seedling Emergence and Seed Production of Curlycup Gumweed. *Native Plants Journal* 23(3):299-308.

Walker S.C. and Shaw N.L. 2005. Current and potential use of broadleaf herbs for reestablishing native communities. Sage-grouse habitat restoration symposium proceedings; 2001 June

4-7, Boise, ID. Proc. RMRS-P-38. Fort Collins, CO: US Department of Agriculture, Forest Service, Rocky Mountain Research Station. p. 56-61.

FIGURES

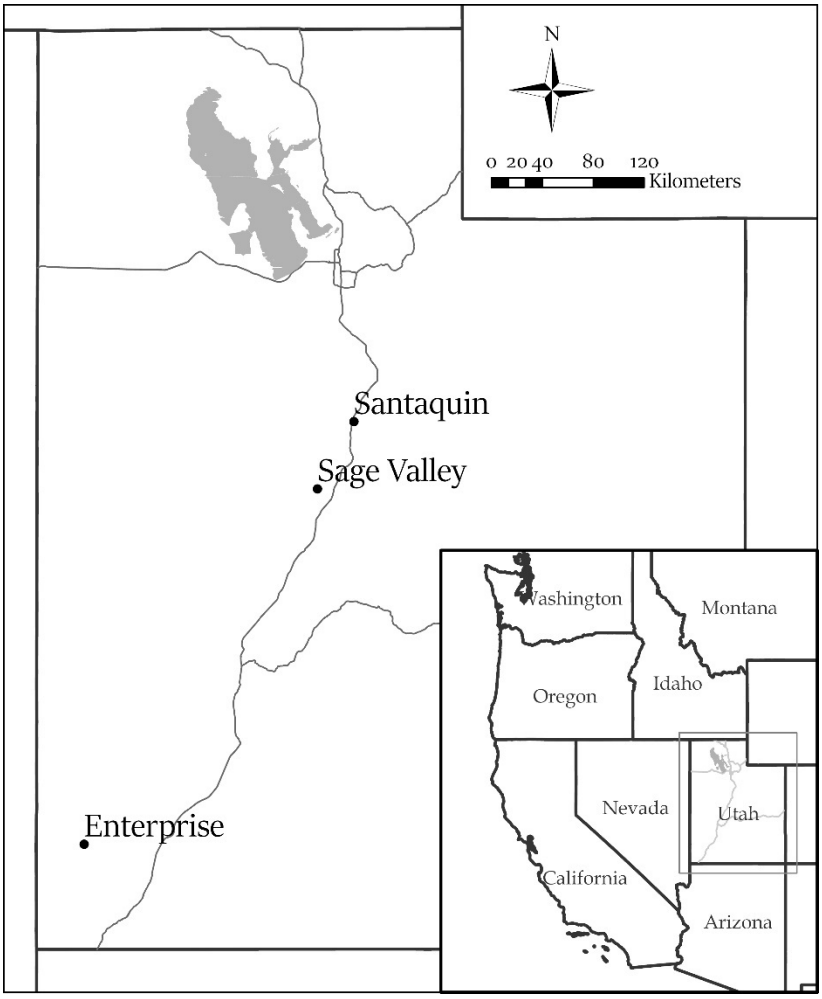

Figure 1-1. Locations of planting sites at Santaquin, Sage Valley, and Enterprise, Utah, USA.

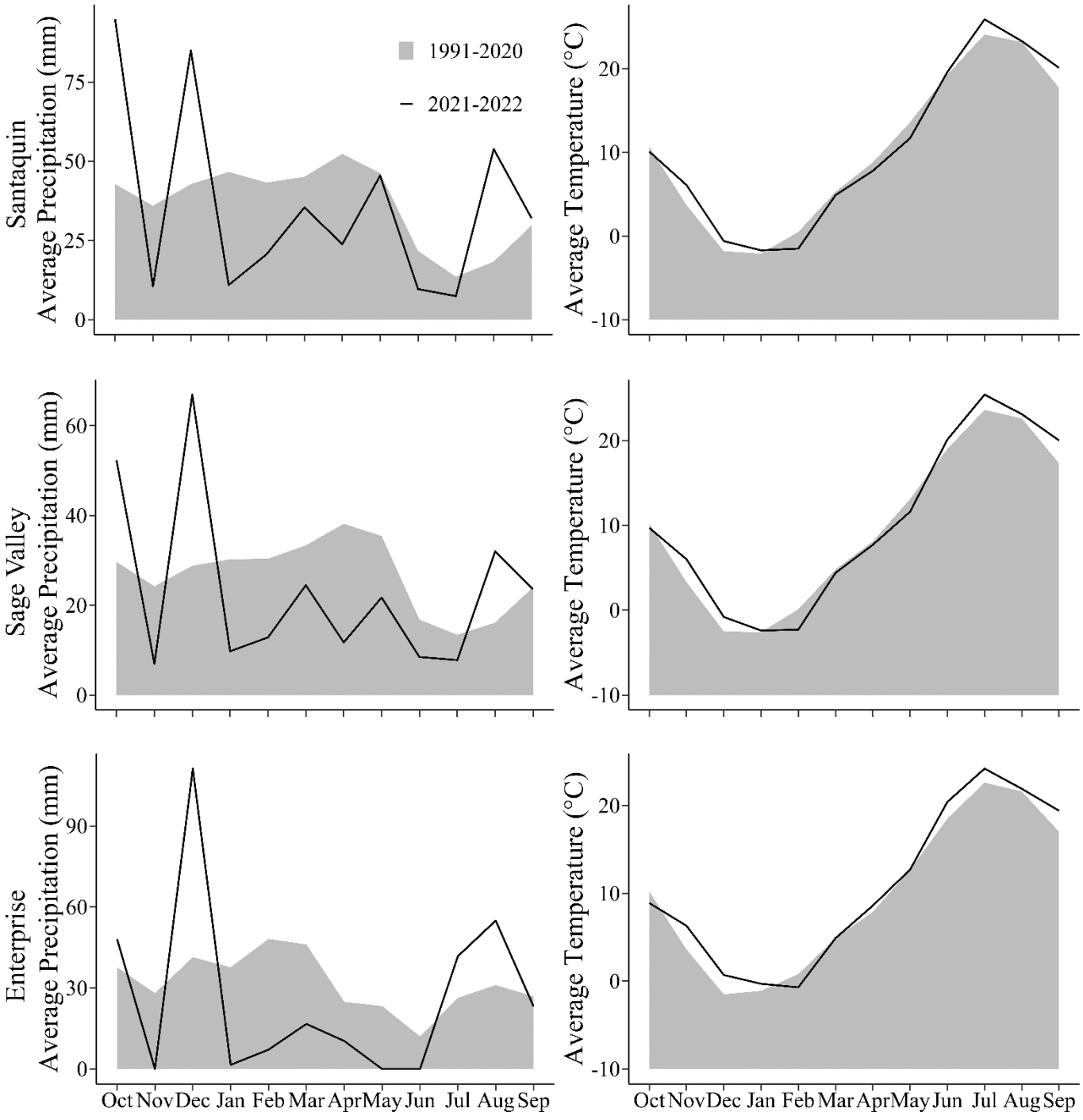

Figure 1-2. Precipitation and temperature data for Santaquin, Sage Valley, and Enterprise, Utah, USA from October 2021 through September 2022 compared to 30-year averages (1991 to 2020).

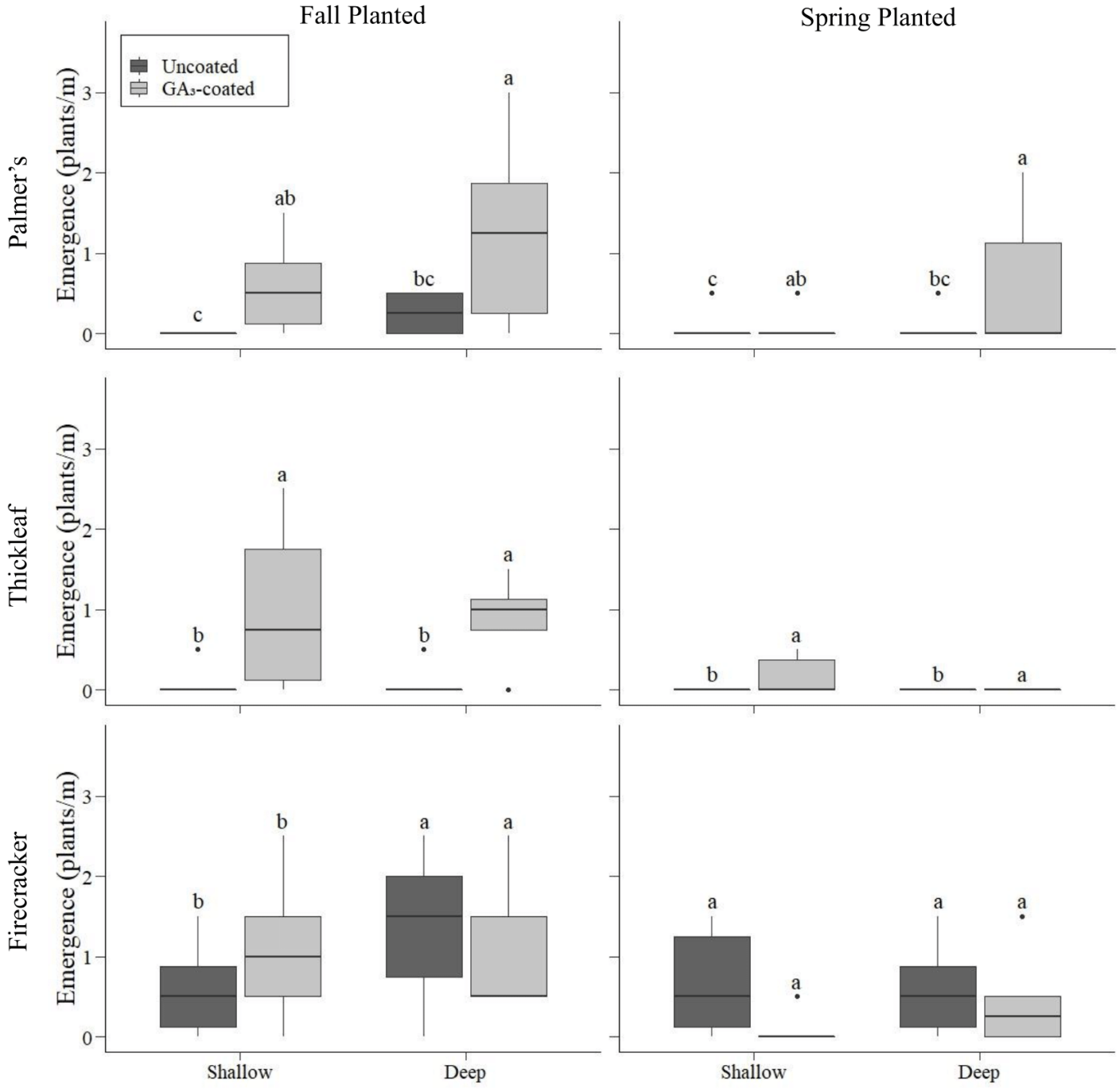

Figure 1-3. Emergence of penstemon species at Santaquin, Utah, USA by season of planting, planting depth, and seed treatment. Differences at the $p < 0.05$ level are indicated by different letters within species and seasons.

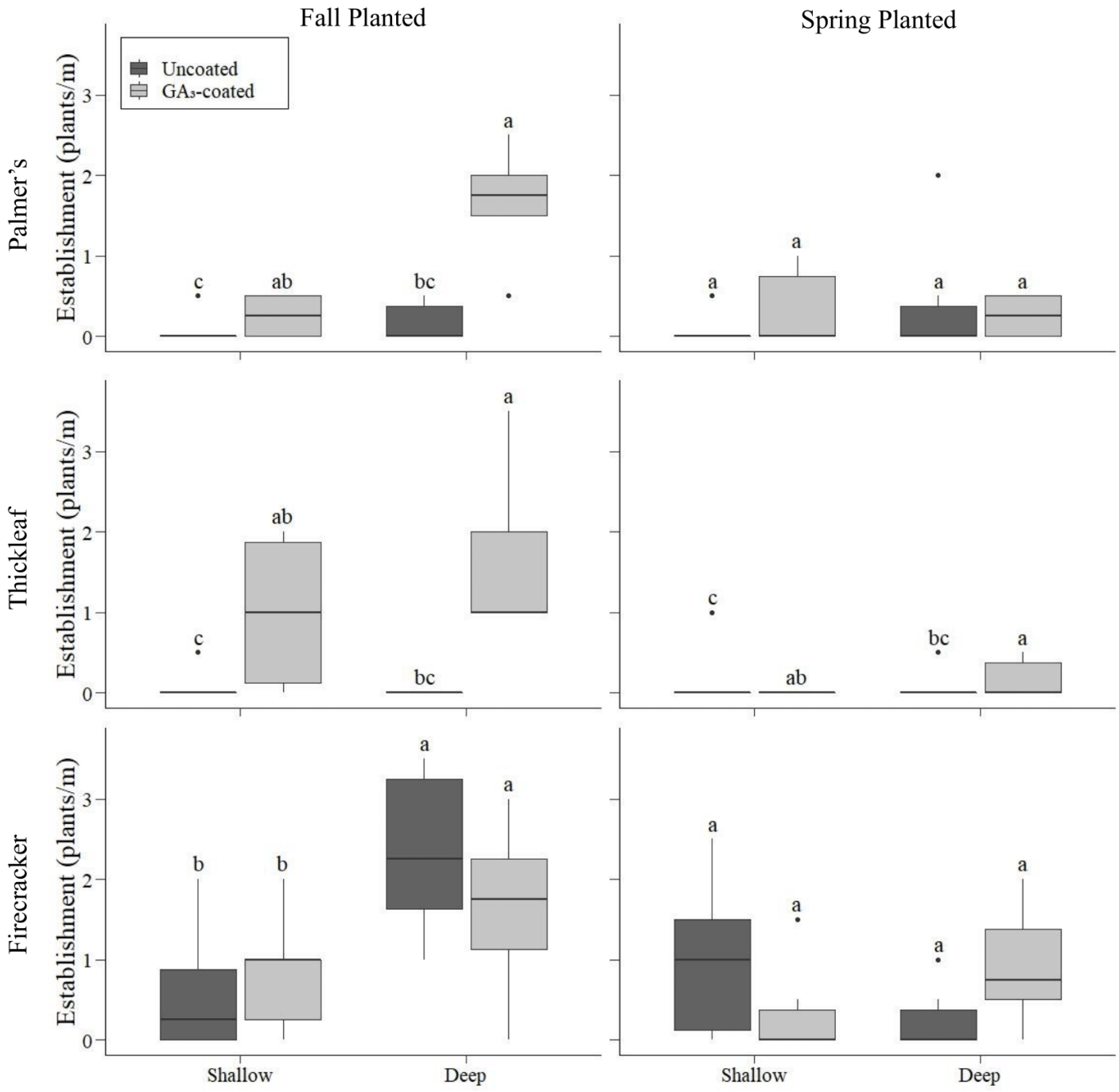

Figure 1-4. Establishment of penstemon species at Santaquin, Utah, USA by season of planting, planting depth, and seed treatment. Differences at the $p < 0.05$ level are indicated by different letters within species and seasons.

TABLES

Table 1-1. Descriptions of soil, temperature, and precipitation from Santaquin, Sage Valley, and Enterprise, Utah, USA. Climate data is based on 30-year averages from PRISM models (PRISM Climate Group 2022). Soil data is sourced from the web soil survey (Soil Survey Staff 2022).

Site	Soil Map Unit	Mean Annual Precipitation (mm)	Low Mean Monthly Temperature (ºC)	High Mean Monthly Temperature (ºC)	Elevation (m)
Santaquin	Donnardo Stony Loam	475	-2	23.8	1620
Sage Valley	Juab Loam	349	-2.9	23.3	1500
Enterprise	Checkett-rock Outcrop Complex	362	-1.4	22.6	1620

Table 1-2. Gibberellic acid coating recipes for the penstemon species used in this study.

Penstemon Species	45% Polyvinylpyrrolidone	Calcium carbonate	Gibberellic acid	Ethylcellulose	Acetone	Ethanol
	----------grams----------				----milliliters----	
Palmer's	47	200	0.382	4.62	50	10
Thickleaf	52	300	0.382	4.62	50	10
Firecracker	58	200	0.382	4.62	50	10

CHAPTER 2

Fungicide Seed Coating Increases Emergence of Bluebunch Wheatgrass Under High Fungal Conditions

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ABSTRACT

Pathogenesis from soil and seed-borne fungus can limit success when restoring native species. Soil and seed-borne pathogens can limit the survival and growth of native seeds and seedlings. Fungicides can combat fungal pathogens, but in some studies, fungicides treatments are ineffective at improving seedling emergence over untreated, control seed. These studies suggest low fungal presence due to dry conditions may be the cause of the ineffectiveness of fungicide treatments for some years and sites. This study tested if fungicide treatment effectiveness is indeed related to the amount of fungus in the soil. We analyzed the emergence and biomass of uncoated, blank-coated, and fungicide-coated bluebunch wheatgrass (*Pseudoroegneria spicata*) across five soil fungal levels (autoclaved, untreated, low, medium, and high). For both percent emergence and total biomass, uncoated seed performed best in autoclaved soil and declined with increasing level of fungus, but level of fungus did not impact fungicide-coated seed. When grown in autoclaved, untreated, or low fungal soils, percent emergence and total biomass from fungicide-coated seeds was not different from uncoated seeds. In medium and high fungal soils, percent emergence and total biomass from fungicide-coated seeds was more than two times greater than uncoated seed ($p < 0.05$). These results indicate fungicide coatings can be effective at increasing restoration success for bluebunch wheatgrass, but the effectiveness of this treatment depends on the microbial environment of the planting site.

INTRODUCTION

Restoring native species after disturbance is becoming increasingly important for countering widespread ecosystem degradation across the globe (Hardegree et al. 2018, Fund et al. 2019, Coban et al. 2022). Establishment of native species, however, can be negatively affected by various biotic and abiotic pressures (Madsen et al. 2016, James et al. 2019). In rangeland restoration efforts, typically fewer than 10% of seeds planted reach maturity (Kildesheva et al. 2019, Pedrini et al. 2020). Factors such as drought, pathogens, competition, soil crusting, and extreme temperatures may contribute to this low success (Madsen et al. 2016). These biotic and abiotic pressures limiting the establishment of native seeds need to be addressed to improve the success of restoration efforts (Fund et al. 2019, Kildisheva et al. 2019). A significant bottleneck impairing seeding success occurs between germination and emergence (James et al. 2011, Gornish et al. 2015, Hoose et al. 2022). One factor that may contribute to this bottleneck is pathogenesis (Wagner and Mitschunas 2008, Allen et al. 2018). Research indicates that soil and seed-borne pathogens have the potential to limit the survival and growth of native grass seeds and seedlings (Gornish et al. 2015, Perkins and Bennett 2018, Ehlert et al. 2019). As climatic conditions change, pathogens may be an increasing concern for restoration due to seeds being more stressed by variable environmental conditions (Gornish et al. 2015, James et al. 2019).

In the Great Basin region of Utah, native seeds are often planted in the autumn, which allows seed to be exposed to pathogens through the winter (Kuhnert et al. 2012, Hardegree et al. 2018, Tilley et al. 2018). Planting in the autumn ensures that seeds are in place in the spring to capitalize on available soil moisture from snow to allow for seed germination and plant growth (Tilley et al. 2018). This is important in the Great Basin since these areas are water-limited and

most of the precipitation comes as snow in the winter (Tilley et al. 2018). However, cool, moist soil conditions are highly conducive to pathogens, and seeds that overwinter in these environments are susceptible to pathogenesis (Kuhnert et al. 2012, Gornish et al. 2015, Hoose et al. 2022). In the Great Basin, seeds are exposed to these conditions for several months before they can emerge from the soil in the spring (James et al. 2011, Gornish et al. 2015, Hoose et al. 2022). A potential mechanism to increase the emergence and survival of native seeds is to protect seeds from these pathogens over winter. Seed coatings may help overcome this limitation by providing a protective shell with anti-pathogen ingredients close to the seed (Pedrini et al. 2017).

One method of combatting fungal pathogens is through chemical fungicides (Wagner and Mitschunas 2008, Baibakova et al. 2019). Because a variety of seed-borne and soil-borne pathogens can negatively impact native seeds (Crist and Friese 1993), a mix of different fungicides can be used to target the variety of known pathogens (Hoose et al. 2022). Research on bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) Á. Löve) has identified various pathogens frequently associated with that species (Gornish et al. 2015). Studies designed with those identified pathogens in mind showed that a mix of fungicides incorporated into a seed coating improved seed germination, seedling emergence, and plant growth (Hoose et al. 2022, Sowards et al. In Preparation). Increases in seedling emergence can be over 300% with fungicide seed coating, but success varies by site and year (Hoose et al. 2022, Sowards et al. In Preparation).

Although fungicide treatments can be effective, for some sites and years fungicides do not improve seedling emergence over the untreated seed (Mordecai 2012, Hoose et al. 2022, Koutzoukis et al. In Press, Sowards et al. In Preparation). A theory postulated to explain why the

treatment was ineffective in some cases was that those sites and years were particularly dry, so there were fewer fungal pathogens in the soil (Hoose et al. 2022, Koutzoukis et al. In Press, Sowards et al. In Preparation). Moisture is a key influence on fungal occurrence (Griffin 1963, Christensen 1989, Krupinsky et al. 2002). Fungi, including pathogenic fungi, generally decrease as moisture decreases and increase with increasing moisture (Rogers 1939, Shields et al 1973). Rogers (1939) tracked mycelial strands across temperature and moisture gradients and observed the strands degraded rapidly under high temperatures or drying. If conditions are dry, the patterns explained in Rogers (1939), Griffin (1963), and Shields et al. (1973) suggest that there may be fewer fungi in the soil. Under less pathogen pressure, the need, and thus the efficacy of fungicide treatments, may be diminished. In wetter years, with more soil fungus, we expect fungicide treatments to be more effective than in drier years with less fungus.

The objective of this study was to test if the effectiveness of fungicide seed coating is indeed related to the amount of fungus in the soil. In this study, we examined the effect of fungal load on the performance of uncoated and fungicide-coated bluebunch wheatgrass seeds. We examined the emergence and biomass of seedlings grown in various fungal conditions, from autoclave-sterilized soil to untreated soil, to soils with increasing levels of fungal inoculation. We predicted that with increasing fungal loads, emergence and biomass from uncoated seed would decrease to a greater degree than fungicide-coated seed. Under these predictions, we also hypothesized that the emergence and plant biomass from fungicide-coated seed would only be higher than uncoated seed in soils with higher fungal loads.

METHODS

Model species

As fungicide seed coatings were previously shown to increase emergence for 'Anatone' bluebunch wheatgrass (Hoose et al. 2022), we used this cultivar to test the effectiveness of fungicide seed coatings under increasing fungal pathogen populations. Additionally, bluebunch wheatgrass is an important species due to its forage value for wildlife and livestock, and its ability to establish and grow under drought conditions (Ogle et al. 2010). This species is especially useful for restoration as seed can be successfully cultivated in plant production plots, harvested, and sown using rangeland seeding equipment (Ogle et al. 2010). Seed for this research was obtained from the Utah Division of Wildlife Resource's Great Basin Research Center (Ephraim, UT, USA). The seed had a 93% purity rate and a 93% germination rate.

Seed treatments

We evaluated two different seed coatings against uncoated seed. Seeds were either treated with a fungicide coating, coated without active ingredients (identified as "blank"), or left uncoated. In the fungicide coating, we applied a mixture of four different fungicides to target known pathogens of native seeds (Gornish et al. 2015). These fungicides were Apron, Dividend, Dynasty, and Maxim with active ingredients Mefenoxam, Difenoconazole and Mefenoxam, Azoxystrobin, and Fludioxonil, respectively (Syngenta, Basel, Switzerland). Mefenoxam targets oomycetes (e.g., Phytophthora, Pythium, etc.), while Difenoconazole, Azoxystrobin, and Fludioxonil are all broad-spectrum fungicides that target a variety of ascomycetes, basidiomycetes, deuteromycetes, and oomycetes (Munkvold 2009). All fungicides were applied at 167% label rate for cereal grasses (Hoose et al. 2022), but well below the maximum allowable amounts per unit area.

Fungicide coating was applied to 200-g batches of seed in a two-step process using a 31-cm rotary seed coater (Universal Coating Systems, Independence, OR, USA) following standard seed coating protocols (Halmer 2008, Pedrini et al. 2017). Fungicide was applied during the first step in a solution of Agrimer SCP II binder (Ashland Inc., Covington, KY, USA; Table 1). This liquid was applied directly to the seed using a syringe, with the liquid injected onto the seed coater's atomizer disk. In the second step, we gradually added calcium carbonate powder (Clayton Calcium, Parma, ID, USA) directly over the seed while pumping Agrimer SCP II binder onto the seed via the atomizer disk (Table 1). This second application provided a hardened coating around the seed to reduce leaching of the fungicide and protect this ingredient against environmental and climatic conditions.

We followed the same procedure as the fungicide coating to coat the blank treatment, but without adding fungicides (Table 1). The blank treatment served as a procedural control to observe the effects of the coating without active ingredients. All three seed treatments were dried at room temperature (~21˚C) on a forced-air dryer (Universal Coating Systems, Independence, OR, USA).

Soil inoculation

To examine the effectiveness of seed coatings under varying pathogen populations, an in-vitro laboratory trial was conducted under five soil fungal levels. Soil was collected from a degraded rangeland site near Santaquin, UT, USA (39.9073, -111.8163) and was classified as a stony loam (Soil Survey Staff 2022). Rocks and debris were removed from the soil by passing through a 4.75mm sieve. Soil was placed in 7x7x10 cm polycarbonate Magenta plant tissue culture boxes (Plantmedia, Dublin, OR, USA) that had four 4mm holes in the bottom for drainage. To maintain moisture and prevent the transfer of fungal spores, a lid was added by

placing a second Magenta box over the boxes containing soil. We treated the soil to both decrease and increase fungi to create five soil fungal levels. The lowest of the five fungal levels was created by autoclaving soil for 12 hours to kill most fungi (hereafter "autoclaved"). The second fungal level consisted of soil in which the natural fungal community was not manipulated (hereafter "untreated"). The final three fungal levels (low, medium, and high) were created by increasing fungal levels using five rounds of inoculation as described below.

We seeded 150 bluebunch wheatgrass seeds in each Magenta box, with the seeds mixed within the top 5 mm of soil. This allowed us to use the fungal inoculum naturally present on the seeds to inoculate the soil. Each box was watered by saturating the soil from the bottom up by placing them in trays filled with water. All trays containing the Magenta boxes were placed in a growth chamber (Environmental Growth Chambers, Chagrin Fall, Ohio, USA) programmed to alternate between 11ºC and 6ºC on a 12-hr light/dark cycle. After one week, germinated seeds were cut in half with scissors to kill the plants, and another 150 seeds were added to the surface of the soil. This process was repeated until we had seeded the boxes four times (600 seeds total). After the fourth addition of seeds, we continued to cut germinated seeds weekly for three weeks to kill all plants. Following inoculum build-up, a fifth round of bluebunch wheatgrass seeds were planted in the boxes, but at three levels to achieve a low, medium, and high fungal level. The low, medium, and high fungal boxes were seeded with 75, 150, and 231 seeds, respectively. These seeding rates were determined with preliminary studies that monitored the growth of bluebunch wheatgrass in inoculated boxes under different seeding rates. Boxes were again watered weekly as described above, and germinated seeds were cut in half each week for three weeks.

Study design

After building fungal inoculum in the Magenta boxes, 20 bluebunch wheatgrass seeds were planted in each box. We used toothpicks to mark where each seed was planted, in order to differentiate those seeds from the seeds used to build pathogen inoculum. We planted the study in a randomized complete block split-plot design with seven blocks. The blocks were randomly divided by fungal level to prevent the transfer of spores between fungal inoculum levels. The three seed treatments (uncoated, blank, and fungicide) were randomized within each fungal level (5 fungal levels X 3 seed treatments X 7 blocks = 105 pots). We then placed all boxes in a 4ºC cooler for three weeks to mimic winter field conditions and provide time for pathogens to attack the seeds. Boxes were then moved back to the growth chamber and watered weekly as described previously. After three weeks in the growth chamber, we counted the number of toothpick-marked seedlings per box and harvested the above-ground biomass. Above-ground biomass was dried for four days in a drier at 60ºC and then weighed.

Fungal biomass quantification

To check the assumption that fungal biomass varied across the different fungal levels, we used an i4 infinity microscope with a 40X objective lens (LW Scientific, Lawrenceville, GA, USA) to quantify fungal biomass per gram of soil (Ingham and Klein 1983, Seiter et al. 1999, Rygiewicz et al. 2010). For each fungal level in each block, three grams of soil from the top 10 mm of soil were collected from each box of a given fungal level (3 boxes per fungal level). These three samples were then mixed thoroughly. A 1 mL subsample of the soil mixture was then diluted with 9 mL of water. A drop of the diluted solution was placed on a microscope slide and covered with a coverslip. The entire slide was examined, and all fungal hyphae, length and width, were measured (Seiter et al. 1999). Hyphal lengths and widths were then used to calculate

fungal biomass (Scheu and Parkinson 1994, Seiter et al. 1999) using the biovolume to biomass conversion factor recommended by Van Veen and Paul (1979).

Statistical analysis

We analyzed the relationship between relative fungal biomass using and fungal level using linear regression in R (R Core Team 2022). We also analyzed the total above-ground biomass of the boxes and the percentage of seedlings that emerged using general linear mixed-effects models. For these models, seed treatment, soil fungal level, and the interaction between seed treatment and soil fungal level were defined as fixed effects. For all models, block was defined as a random effect to account for variation across blocks. We constructed models using the 'lmer' function of the 'lme4' package (Bates et al 2022) in R (R Core Team 2022). Residuals were checked for normality and equal variance. Pairwise comparisons were then conducted using the Tukey method with the 'emmeans' function of the 'emmeans' package in R (Lenth et al. 2022).

RESULTS

Fungal biomass

There was a positive trend between fungal inoculation level and fungal biomass (p = 0.006; Fig. 2-1). Fungal biomass increased from the autoclaved soil to the untreated soil and through the increasing levels of fungal inoculated soils.

Seedling emergence

Percent emergence from uncoated seeds was greatest in autoclaved soil ($\bar{x}$ = 50.7%) with 2 times higher emergence than both untreated soil ($\bar{x}$ = 28.6%; p = 0.04) and medium fungus soil ($\bar{x}$ = 21.4%; $p < 0.001$; Fig. 2-2). Emergence was not different for uncoated seed between autoclaved soil and low fungus soil (p = 0.99) or high fungus soil (p = 0.17). Emergence was

also not different between uncoated seed and blank-coated seed at any level of inoculum (p > 0.27; Fig. 2-2). For fungicide-coated seeds, emergence did not vary across fungal levels (p > 0.27). In autoclaved, untreated, and low fungus soils, emergence from fungicide treatments was not different from emergence of uncoated seedlings (p > 0.91; Fig. 2-2). However, emergence of fungicide-coated seedlings was 8 and 2 times greater than uncoated seed in medium (uncoated $\bar{x}$ = 21.4%; fungicide-coated $\bar{x}$ = 52.1%; p < 0.001) and high fungus soils (uncoated $\bar{x}$ = 32.1%; fungicide-coated $\bar{x}$ = 54.3%; p = 0.04), respectively (Fig. 2-2).

Total above-ground biomass

Total biomass per box showed similar responses to percent emergence. Uncoated seedling biomass was greatest in autoclaved soil ($\bar{x}$ = 0.06 g), with 2 times greater biomass than produced from both medium fungus ($\bar{x}$ = 0.027 g; p = 0.003) and high fungus soils ($\bar{x}$ = 0.033 g; p = 0.05; Fig. 2-3). Uncoated seedlings had 130% greater biomass than blank-coated seedlings in autoclaved soil (uncoated $\bar{x}$ = 0.06 g; blank-coated $\bar{x}$ = 0.046 g; p = 0.04) but were not different from blank-coated seedlings grown in any other level of inoculum (p > 0.15; Fig. 2-3). For fungicide-coated seedlings biomass did not differ across levels of inoculum (p > 0.66; Fig. 2-3). In autoclaved, untreated, and low fungus soils, biomass from fungicide treatments was not different from uncoated seedlings (p = 1; 0.76; 0.53, respectively). However, biomass of fungicide-coated seedlings was 2 times greater than uncoated seed in both medium (uncoated $\bar{x}$ = 0.027 g; fungicide-coated $\bar{x}$ = 0.053 g; p = 0.001) and high fungus soils (uncoated $\bar{x}$ = 0.033 g; fungicide-coated $\bar{x}$ = 0.068 g; p = 0.05; Fig. 2-3).

DISCUSSION

We predicted that fungicide-coated bluebunch wheatgrass would perform better than uncoated seed at higher fungal levels but not at lower fungal levels. For both percent

emergence and total biomass, uncoated seed performed best in autoclaved soil and declined with increasing level of fungus, but level of fungus did not impact fungicide-coated seed. This led to the fungicide-coated seed performing better than uncoated seed at the medium and high levels of fungus. These results may help explain why previous research has reported variable success rates for fungicide seed treatments (Blaney and Kotanen 2001, Mordecai 2012, Hoose et al. 2022). Fungi generally decline under dry conditions (Rogers 1939, Griffin 1963, Shields et al. 1973), and those studies proposed low pathogen presence as the reason performance of fungicide treated seed did not differ from untreated seed (Blaney and Kotanen 2001, Mordecai 2012, Hoose et al. 2022). Based on the findings of this study, we expect fungicide seed coating to be most effective in years and locations with ample precipitation but not effective for extremely dry sites where fungal population declined due to desiccation (Rogers 1939).

Although blank-coated seed performance was similar to uncoated seed for most treatments, blank-coated seed had lower total biomass than uncoated seed in autoclaved soil. Emergence, however, was not different between blank-coated and uncoated seeds in autoclaved soil. As this coating had no active ingredient, this indicates that when little fungus is in the soil, seed coating alone may have a slightly negative effect on the growth of bluebunch wheatgrass. In most cases, however, seed coated with a blank coating performed the same as the uncoated seed. When more pathogens were present, coating without an active ingredient did not provide a benefit to seedling emergence and growth. For situations where higher levels of pathogens are present, blank coating is not an effective treatment, but coating with a fungicide is effective.

It is important to note that our low, medium, and high inoculated boxes had the added organic matter of the seeds used to inoculate the boxes with fungus, which may have affected the results we observed. The main effect of organic matter in our experiment was to act as an energy

source for the microbes (Shields et al. 1973), and our microscope work indicates there was an increase in fungus from our autoclaved and untreated soils through our inoculated soils. Similar research showed that fungicide treatment had a greater effect when levels of organic matter were higher (Sowards et al. In Preparation). The organic matter may have also influenced nutrient and moisture availability (Bot and Benites 2005, Kuhnert et al. 2012), but this study did not measure these factors.

With growing concern about the environmental impacts of synthetic fungicides and the development of fungicide resistance (Baibakova et al. 2019), interest in biological controls as alternatives to fungicides is increasing (Bonanomi et al. 2007, O'Callaghan 2016). A review of potential biological controls reported that microbes present in compost effectively suppressed pathogens in over 50% of studies (Bonanomi et al. 2007). Thus, seed coatings incorporating compost may be a viable option for introducing inoculum capable of promoting resistance to phytopathogens (Pane et al. 2012, Coban et al. 2022, Kavusi et al. 2023). Most research on these types of inoculum coatings, however, has been in commercial rather than restoration settings (O'Callaghan 2016, Pedrini et al. 2017). Future work could use the same methods as in this study to test biological control seed coatings to find effective alternatives to chemical fungicides.

Our study indicates fungicide coatings can be effective at increasing restoration success for bluebunch wheatgrass, but the effectiveness of this treatment depends on the microbial environment of the planting site. As the abiotic and biotic conditions at a restoration site greatly influence treatment and restoration success, these conditions should be considered when planning a restoration project (Dumroese et al. 2015, Lewandrowski et al. 2016, Madsen et al. 2016). Laboratory trials like ours can test how restoration technologies work under a variety of conditions (e.g., moisture, temperature, microbial community, etc.) (Mitschunas et al. 2006).

Under changing conditions (e.g., climate and disturbance) (Leishman et al. 2000, Fund et al. 2019, Coban et al. 2022), understanding how restoration techniques work under a variety of conditions is going to be increasingly important for effective restoration.

LITERATURE CITED

Allen P.S., Finch-Boekweg, H. and Meyer, S.E. 2018. A proposed mechanism for high pathogen-caused mortality in the seed bank of an invasive annual grass. *Fungal Ecology* 35:108-115.

Baibakova E.V., Nefedjeva, E.E., Suska-Malawska, M., Wilk, M., Sevriukova, G.A. and Zheltobriukhov, V.F. 2019. Modern fungicides: Mechanisms of action, fungal resistance and phytotoxic effects. *Annual Research & Review in Biology*:1-16.

Bates D., Mächler, M., Bolker, B. and Walker, S. 2015. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software* 67(1):1.

Blaney C.S. and Kotanen, P.M. 2001. Effects of Fungal Pathogens on Seeds of Native and Exotic Plants: A Test Using Congeneric Pairs. *Journal of Applied Ecology* 38(5):1104-1113.

Bonanomi G., Antignani, V., Pane, C. and Scala, F. 2007. Suppression of Soilborne Fungal Diseases with Organic Amendments. *Journal of Plant Pathology* 89(3):311-324.

Bot A. and J. Benites. 2005. The importance of soil organic matter: Key to drought-resistant soil and sustained food production. *Food & Agriculture Organization.*

Christensen M. 1989. A view of fungal ecology. *Mycologia* 81(1):1-19.

Coban O., Gerlinde B De, D. and Martine van, d.P. 2022. Soil microbiota as game-changers in restoration of degraded lands. *Science* 375(6584):0725.

Crist T.O. and Friese, C.F. 1993. The Impact of Fungi on Soil Seeds: Implications for Plants and Granivores in a Semiarid Shrub-Steppe. *Ecology* 74(8):2231-2239.

Dumroese R.K., Luna, T., Richardson, B.A., Kilkenny, F.F. and Runyon, J.B. 2015. Conserving and restoring habitat for Greater Sage-Grouse and other sagebrush-obligate wildlife: the crucial link of forbs and sagebrush diversity. *Native Plants Journal* 16(3):276-299.

Ehlert K.A., Mangold, J.M., Menalled, F., Miller, Z. and Dyer, A. 2019. Seeding, herbicide, and fungicide impact on perennial grass establishment in cheatgrass infested habitats. *Ecological Restoration* 37(2):67-70.

Fund A.J., Hulvey, K.B., Jensen, S.L., Johnson, D.A., Madsen, M.D., Monaco, T.A., Tilley, D.J., Arora, E. and Teller, B. 2019. Basalt Milkvetch Responses to Novel Restoration Treatments in the Great Basin. *Rangeland Ecology & Management* 72(3):492-500.

Gornish E.S., Aanderud, Z.T., Sheley, R.L., Rinella, M.J., Svejcar, T., Englund, S.D. and James, J.J. 2015. Altered snowfall and soil disturbance influence the early life stage transitions and recruitment of a native and invasive grass in a cold desert. *Oecologia* 177(2):595-606.

Griffin D.M. 1963. Soil Moisture and the Ecology of Soil Fungi. *Biological Reviews* 38(2):141-166.

Halmer P. 2006. Seed technology and seed enhancement. XXVII International Horticultural Congress-IHC2006: International Symposium on Seed Enhancement and Seedling Production 771.

Hardegree S.P., Abatzoglou, J.T., Brunson, M.W., Germino, M.J., Hegewisch, K.C., Moffet, C.A., Pilliod, D.S., Roundy, B.A., Boehm, A.R. and Meredith, G.R. 2018. Weather-Centric Rangeland Revegetation Planning. *Rangeland Ecology & Management* 71(1):1-11.

Hoose B.W., Geary, B.D., Richardson, W.C., Petersen, S.L. and Madsen, M.D. 2022. Improving dryland seedling recruitment using fungicide seed coatings. *Ecological Solutions and Evidence* 3(1):e12132.

Ingham E.R. and Klein, D.A. 1984. Soil fungi: measurement of hyphal length. *Soil biology & biochemistry* 16(3):279-280.

James J.J., Sheley, R.L., Leger, E.A., Adler, P.B., Hardegree, S.P., Gornish, E.S. and Rinella, M.J. 2019. Increased soil temperature and decreased precipitation during early life stages constrain grass seedling recruitment in cold desert restoration. *Journal Applied Ecology* 56(12):2609-2619.

James J.J., Svejcar, T.J. and Rinella, M.J. 2011. Demographic processes limiting seedling recruitment in arid grassland restoration. *Journal Applied Ecology* 48(4):961-969.

Kavusi E., Shahi Khalaf Ansar, B., Dehghanian, Z., Asgari Lajayer, B., Nobaharan, K., Ma, Y., and Glick, B.R. 2023. Delivery of Beneficial Microbes via Seed Coating for Medicinal and Aromatic Plant Production: A Critical Review. *Journal of Plant Growth Regulation* 42(2):575-597.

Kildisheva O.A., Erickson, T.E., Madsen, M.D., Dixon, K.W. and Merritt, D.J. 2019. Seed germination and dormancy traits of forbs and shrubs important for restoration of North American dryland ecosystems. *Plant Biology* 21(3):458-469.

Koutzoukis S, MD Madsen, KE Veblen. Under drought conditions, fungicide coating does not increase emergence of two native grass species in sagebrush stands of the Intermountain West.

Krupinsky J.M., Bailey, K.L., McMullen, M.P., Gossen, B.D. and Turkington, T.K. 2002. Managing Plant Disease Risk in Diversified Cropping Systems. *Agronomy Journal* 94(2):198-209.

Kuhnert R., Oberkofler, I. and Peintner, U. 2012. Fungal Growth and Biomass Development is Boosted by Plants in Snow-Covered Soil. *Microbial Ecology* 64(1):79-90.

Leishman M.R., Masters, G.J., Clarke, I.P. and Brown, V.K. 2000. Seed bank dynamics: the role of fungal pathogens and climate change. *Functional Ecology* 14(3):293-299.

Lenth R.V., Buerkner, P., Herve, M., Love, J., Miguez, F., Riebl, H. and Singmann, H. 2022. emmeans: Estimated Marginal Means, aka Least-Squares Means . R package version 1.7.2.

Lewandrowski W., Erickson, T.E., Dixon, K.W. and Stevens, J.C. 2017. Increasing the germination envelope under water stress improves seedling emergence in two dominant grass species across different pulse rainfall events. *Journal of Applied Ecology* 54(3):997-1007.

Madsen M.D., Davies, K.W., Boyd, C.S., Kerby, J.D. and Svejcar, T.J. 2016. Emerging seed enhancement technologies for overcoming barriers to restoration. *Restoration Ecology* 24:S77-S84.

Mitschunas N., Wagner, M. and Filser, J. 2006. Evidence for a positive influence of fungivorous soil invertebrates on the seed bank persistence of grassland species. *Journal of Ecology* 94(4):791-800.

Mordecai E.A. 2012. Soil moisture and fungi affect seed survival in California grassland annual plants. *PLoS One* **7**(6):e39083.

Munkvold G.P. 2009. Seed Pathology Progress in Academia and Industry. *Annu Rev Phytopathology* 47(1):285-311.

O'Callaghan M. 2016. Microbial inoculation of seed for improved crop performance: issues and opportunities. *Applied Microbiology and Biotechnology* 100(13):5729-5746.

Ogle D.G., St John, L., and Jones, T.A. 2010. Plant guide for bluebunch wheatgrass (*Pseudoroegneria spicata*). USDA-Natural Resources Conservation Service, Idaho and Washington Plant Materials Program:5.

Pane C., Villecco, D., Campanile, F. and Zaccardelli, M. 2012. Novel strains of Bacillus, isolated from compost and compost-amended soils, as biological control agents against soil-borne phytopathogenic fungi. *Biocontrol Science and Technology* 22(12):1373-1388.

Pedrini S., Balestrazzi, A., Madsen, M.D., Bhalsing, K., Hardegree, S.P., Dixon, K.W. and Kildisheva, O.A. 2020. Seed enhancement: getting seeds restoration-ready. *Restoration Ecology* 28:S266-S275.

Pedrini S., Merritt, D.J., Stevens, J. and Dixon, K. 2017. Seed Coating: Science or Marketing Spin?. *Trends in Plant Science* 22(2):106-116.

Perkins L.B. and Bennett, J.R. 2018. A field test of commercial soil microbial treatments on native grassland restoration. *Restoration Ecology* 26(5):851-857.

R Core Team. 2022. R: A language and environment for statistical computing.

Rogers C.H. 1939. The relation of moisture and temperature to growth of the cotton root rot fungus. *Journal of Agricultural Research* 58:701-709.

Rygiewicz P.T., Monleon, V.J., Ingham, E.R., Martin, K.J. and Johnson, M.G. 2010. Soil life in reconstructed ecosystems: Initial soil food web responses after rebuilding a forest soil profile for a climate change experiment. *Applied Soil Ecology* 45(1):26-38.

Scheu S. and Parkinson, D. 1994. Changes in bacterial and fungal biomass C, bacterial and fungal biovolume and ergosterol content after drying, remoistening and incubation of different layers of cool temperate forest soils. *Soil Biology and Biochemistry* 26(11):1515-1525.

Seiter S., Ingham, E.R. and William, R.D. 1999. Dynamics of soil fungal and bacterial biomass in a temperate climate alley cropping system. *Applied Soil Ecology* 12(2):139-147.

Shields J.A., Paul, E.A., Lowe, W.E. and Parkinson, D. 1973. Turnover of microbial tissue in soil under field conditions. *Soil Biology and Biochemistry* 5(6):753-764.

Soil Survey Staff. 2021. Web Soil Survey. Natural Resources Conservation Service, United States Department of Agriculture. Available online at http://websoilsurvey.nrcs.usda.gov/ (Accessed 6 July 2021).

Tilley D., Fund, A. and Pickett, T. 2018. A Review of Techniques and Technologies for Improving Seedling Establishment. Technical Note Plant Materials, USDA-Natural Resources Conservation Service No. 72.

van Veen Johannes A. and Paul Eldor, A. 1979. Conversion of Biovolume Measurements of Soil Organisms, Grown Under Various Moisture Tensions, to Biomass and Their Nutrient Content. *Applied and Environmental Microbiology* 37(4):686-692.

Wagner M. and Mitschunas, N. 2008. Fungal effects on seed bank persistence and potential applications in weed biocontrol: a review. *Basic and Applied Ecology* 9(3):191-203.

FIGURES

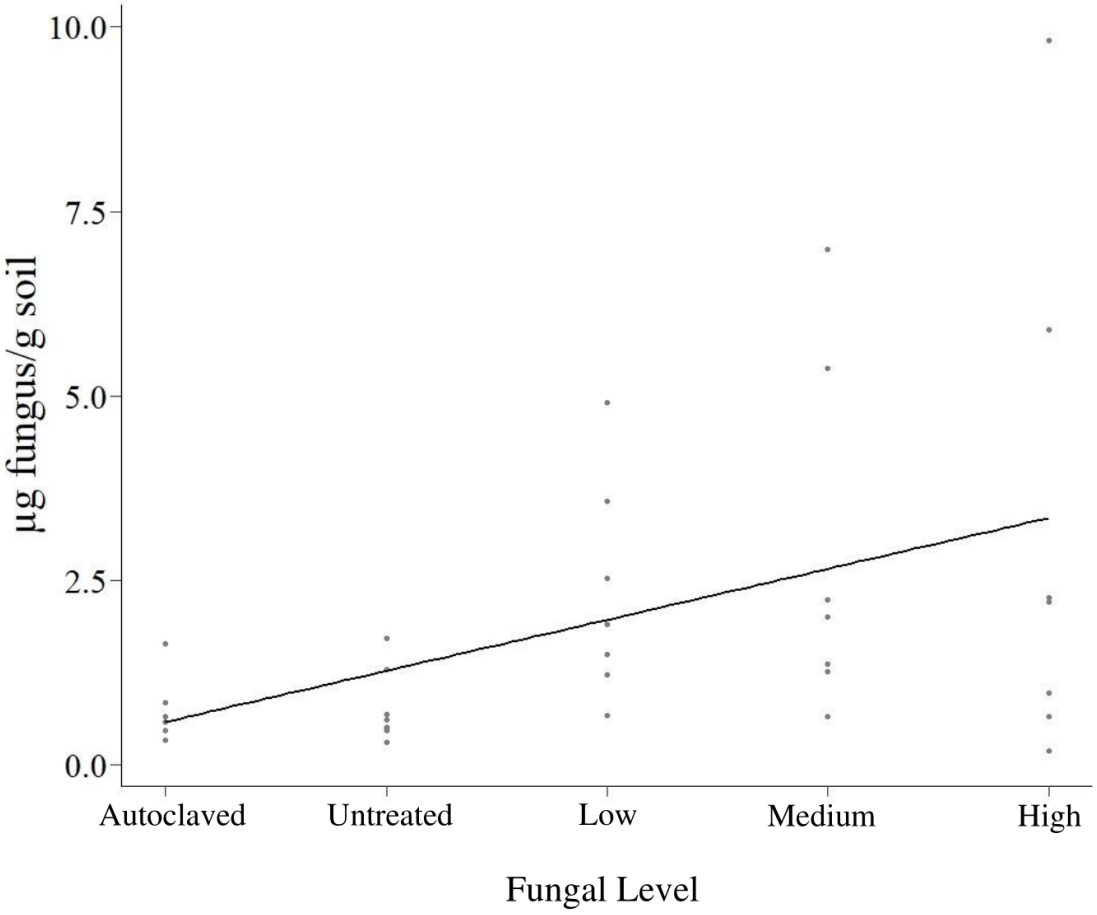

Figure 2-1. Fungal biomass in micrograms per gram of soil by fungal inoculation level.

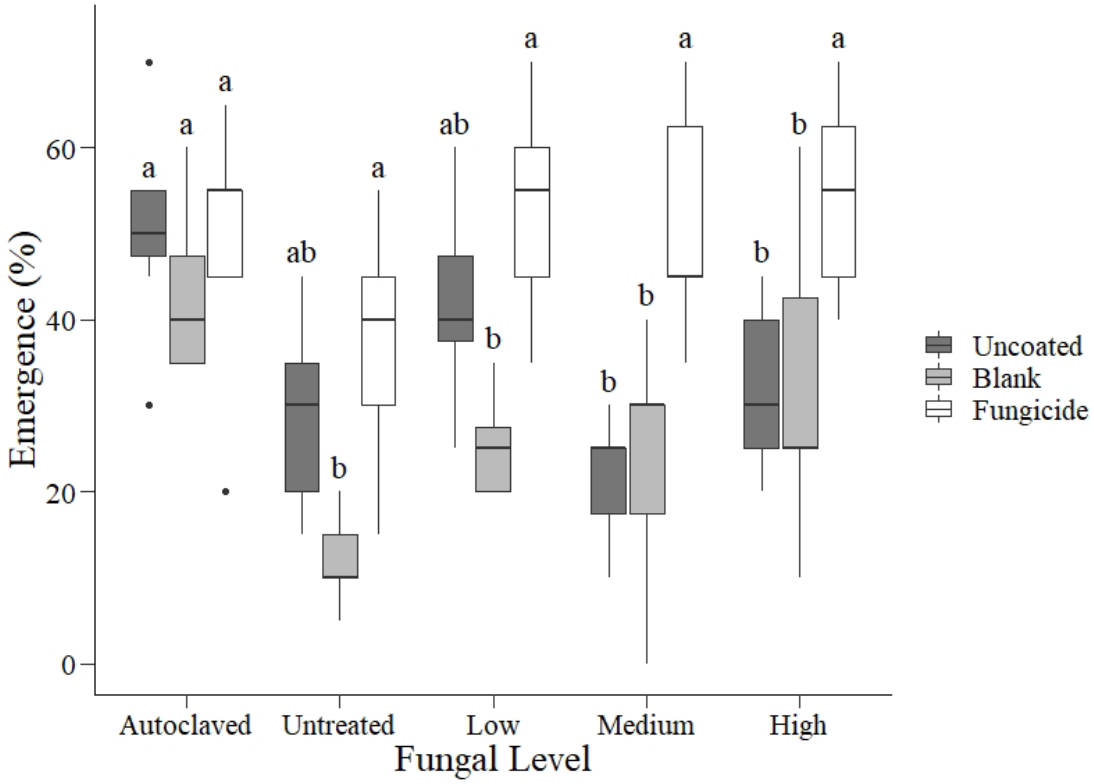

Figure 2-2. Percent emergence of uncoated, blank, and fungicide-coated seed grown in a range of fungal inoculum levels. Letters indicate significant difference at the $p < 0.05$ level within soil inoculum levels.

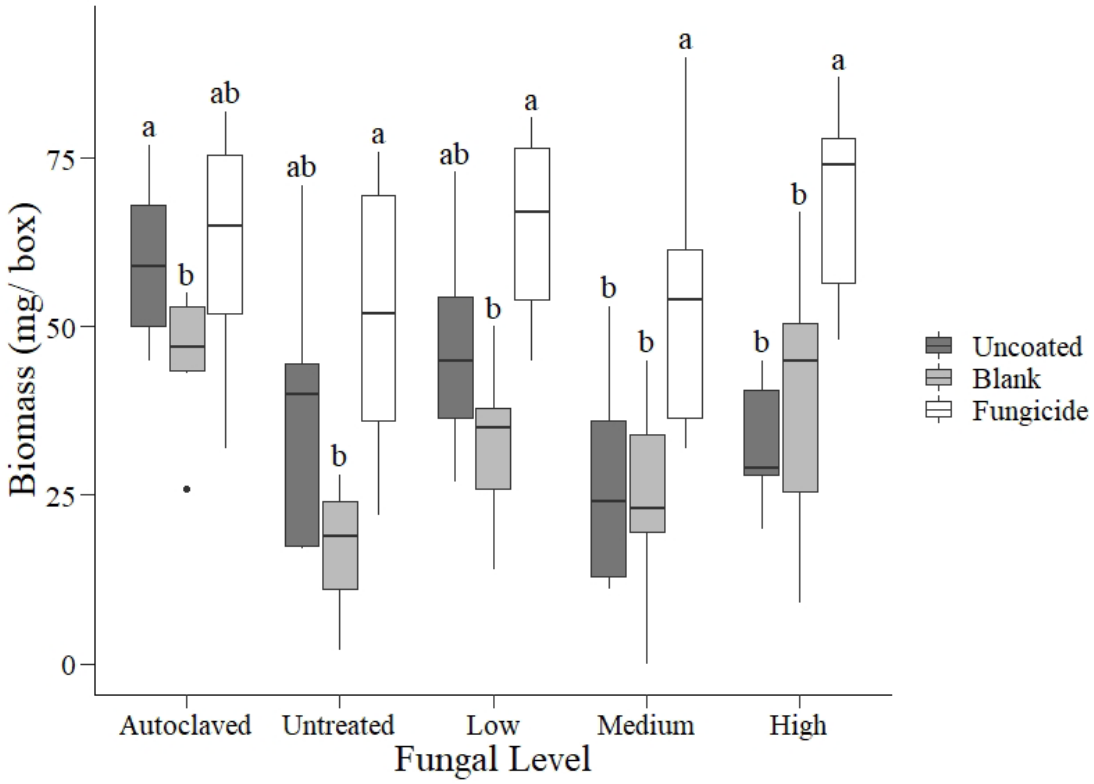

Figure 2-3. Total biomass per box for uncoated, blank, and fungicide-coated seed grown in a range of fungal inoculum levels. Letters indicate significant difference at the $p < 0.05$ level within soil inoculum levels.

TABLES

Table 2-1. Fungicide and blank seed coating recipes.

Treatment	Agrimer SCP II	Calcium Carbonate	Apron (Mefenoxam)	Dividend (Difenoconazole and Mefenoxam)	Dynasty (Azoxystrobin)	Maxim (Fludioxonil)
	---------- grams ----------					
Fungicide	130	350	0.1625	1.0883	0.09	0.0436
Blank	130	350				