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Investigating Dormancy and Germination Characteristics to Promote Restoration Success in the Northern Great Plains

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INVESTIGATING DORMANCY AND GERMINATION CHARACTERISTICS TO PROMOTE RESTORATION SUCCESS IN THE NORTHERN GREAT PLAINS

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This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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INVESTIGATING DORMANCY AND GERMINATION CHARACTERISTICS TO PROMOTE RESTORATION SUCCESS IN THE NORTHERN GREAT PLAINS

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Failures in seed-based restoration can be partially attributed to a lack of knowledge on seed dormancy. Dormancy is beneficial for plant establishment in unpredictable environmental conditions, but a lack of uniform germination can hinder restoration efforts. The purpose of this research was to gain a better understanding of dormancy displayed by select forbs of the northern Great Plains. The first data chapter attempted to identify and break dormancy class. Seeds were treated with scarification, smoke, and fertilizer pretreatments in an attempt to break seed dormancy. Seeds were also placed in spring and summer temperatures to identify how seasonal phenology influenced final germination and germination rate. The second data chapter attempted to identify how dormancy percentage varies between different seed sources of the same species. This chapter also investigates how short-term storage temperature and seed cleaning methods may influence physical dormancy levels. From the first data chapter, I determined that *Erysimum asperum*, *Gaillardia aristata*, and *Ipomopsis aggregata* all display physiological dormancy. Although the remaining species were nondormant, temperature did influence germination. From the second data chapter, I determined that levels of dormancy can vary drastically between different sources for the same species. Although I could not conclude why dormancy varied, I determined that short-term storage temperature and cleaning methods likely did not influence dormancy levels. From

a management perspective, *Dalea villosa* seeds can be stored at room temperature or refrigerated, and can be dehulled with a hand debearder without concern of impacting dormancy levels. Temperatures and pretreatments found to be influential can be used to effect final germination and germination rate of these native species prior to seeding in restoration. Breaking dormancy of *E. asperum*, *G. aristata,* and *I. aggregata* before seeding will increase final germination of these species in the field, and increase restoration success in the northern Great Plains.

CHAPTER 1: INTRODUCTION

Grasslands are one of the most extensive biomes in the world (Samson and Knopf 1994, Török et al. 2021), and ecologically important on many levels. Excluding Antarctica and Greenland, approximately 40% of earth's land is classified as grassland (White et al. 2000). Grasslands provide ecological functions such as reducing soil erosion, storing carbon (Carlier et al. 2009), and supporting biodiversity (Samson and Knopf 1994, Török et al. 2021). In the ecoregion of the northern Great Plains (NGP) alone, there are more than 1,600 species of native plants (Perkins et al. 2019).

However, grasslands are one of the most anthropogenically transformed and at-risk environments (Hoekstra et al. 2005) threatening the survival of these native plants. Only a fraction of historical grasslands remain and many are in isolated remnants. Today, grasslands continue to face constant pressures of fragmentation, conversion to agriculture (Samson et al. 2004), development (Török et al. 2021), and the introduction of non-native species (Tallamy et al. 2021), among other stressors. The continuous loss of the native plants in these grasslands poses a serious threat to the extinction of floral and faunal species along with the reduction of other critical ecological roles (Samson and Knopf 1994). The competition alone from non-natives has resulted in the decline of insect populations (Tallamy et al. 2021) and a lower reproductive success of native plants due to less frequent pollinator visitation (Morales and Traveset 2009).

Seed-based restoration is a promising solution to repair and recover degraded grasslands, however the lack of knowledge on seed germination makes restoration difficult. The United Nations has declared the years 2021-2030 as the Decade of

Ecosystem Restoration (United Nations Environmental Agency 2019). Still, by the start of this decade, research on grassland restoration has received less focus than restoration studies of other habitats such as wetlands and forests (Török et al. 2021). A lack of knowledge of plant germination (Jiménez-Alfaro et al. 2016) can partially be attributed to the underrepresentation of grasslands studies. Without knowledge on germination, seed-based restorations can be expected to fail. Many studies that have focused on seed-based restoration have cited the difficulty of establishing native seeds (James et al. 2011, Pedrini et al. 2020); with some resulting in germination percentages of <20% (Ceccon et al. 2016) and establishment of <2% (Chambers 2000). With the lack of advanced knowledge on the limitations of seed establishment being a limiting factor in restoration (Török et al. 2021), it is vital to understand the germination requirements of the seeds before beginning large field-based projects.

One issue with ecosystem restoration is the lack of research on seed dormancy, which is estimated to be present in nearly 70% of plant species (Baskin and Baskin 2004a). Seed dormancy is a biological mechanism that prevents germination even when conditions like water and temperature are deemed sufficient (Bewley 1997). Seed dormancy can be advantageous by reducing sibling competition (Philippi 1993) or bet-hedging in variable environmental conditions (Venable 2007). Although dormancy could be bred out of native species, like it is with agricultural seeds, this would result in a loss of the wild populations' genetic diversity which is critical for survival in changing climates (Pedrini and Dixon 2020).

An option for dormant seeds to germinate quickly for restoration, while still retaining genetic diversity, is to artificially break dormancy before seeding in the field.

There are many methods of breaking dormancy including scarification (Baskin and Baskin 2004b), chemical cues (Kildisheva et al. 2020), and stratification (Ruiz-Talonia et al. 2022). Although not all seeds display dormancy, treatments may influence germination characteristics of nondormant seeds. The goal of this research was to gain a better understanding of dormancy and germination characteristics of native forbs of the NGP. Knowledge gained through this research can lead to higher species-specific success in seed-based restoration, along with a better understanding of dormancy variation and the effects storage conditions may have on dormancy.

Therefore, in my first data chapter I examine nine native forbs for potential dormancy and identify dormancy breaking techniques. Seeds were put through an adapted move-along experiment (Baskin and Baskin 2003a) with potential dormancy breaking pretreatments (scarification, smoke, fertilizer). I determined if any treatment influenced final germination or germination rate, and identified which class of dormancy was present, if appliable.

The objective of my second data chapter was to elucidate the variation in dormancy percentage that can be expressed within a species between two different sources, and to determine if storage temperature and cleaning methods influence dormancy. Three species of *Dalea* were tested in this experiment. For each species, dormancy was compared between a commercial source and a seed production field source. Afterwards, a short-term storage experiment was tested to determine if refrigerating and dehulling (both common storage practices) seeds influenced dormancy.

CHAPTER 2: USING SEED PRETREATMENTS TO DETERMINE BEST GERMINATION CONDITIONS FOR GRASSLAND FORBS

Abstract

Seed-based ecological restoration often has two contradictory goals: wanting to establish native plant populations with appropriate genetic diversity, and wanting seeds to germinate and establish quickly. We have the capability to breed native seeds for preferred characteristics, such as quick and uniform germination, which could hasten the restoration process. However, in doing so, seeds may lose additional characteristics, such as dormancy, which would reduce the likelihood of survival in future generations as seeds experience more environmental stochasticity through the impacts of climate change. By breaking seed dormancy and pretreating seeds prior to seeding in grassland restoration, practitioners have the ability to control germination characteristics without compromising the genetic diversity of the seeds in future generations. Our goal is to determine which environmental conditions improve final germination and germination rate through investigating temperature effects, pretreatment effects, and dormancy class. Seeds of nine native forbs were pretreated with scarification, smoke, and fertilizer, and put through different temperature regimes. We analyzed the effects of treatments and their interaction on germination rate and final germination. We found that temperature affected germination variables of most study species and could be used to influence germination rate for eight of the study species. Temperature did not break dormancy of any species, but we identified pretreatments that can break dormancy for three species. Two of these species benefited from more than one dormancy breaking treatment. Understanding the germination requirements of native species will increase the success of

grassland restoration. Seeds can be treated prior to seeding to gain preferred germination characteristics. This will quicken the restoration process without compromising the functionality of seeds in future generations.

Introduction

Restoration practitioners and land managers have two seemingly contradictory goals for seed-based restoration. First, they want to establish populations with appropriate genetic diversity (Leger and Espeland 2010, Reynolds et al. 2012) as low genetic diversity is known to negatively influence germination characteristics (Capblancq et al 2021). Second, they want seeds to germinate and establish plants quickly. Quick germination is beneficial during restoration for many reasons including to reduce the risk of granivory (Linabury et al. 2019), reduce competition for resource uptake (Ross and Harper 1972), and to provide priority effects to help the desired vegetation compete with invasive plants (Vaughn and Young 2015). Restoration has been called the "testing ground" for ecological questions (Harris and Hobbs 2001) and these two conflicting goals present an opportunity where fundamental ecology can improve boots-on-the-ground restoration.

Seeds found in a natural system (wild seeds) have unique germination characteristics developed over evolutionary time to ensure survival of the species. For example, germination timing can be asynchronous to spread germination across a season or among seasons to reduce the risk of all seeds experiencing unfavorable conditions (Evans and Dennehy 2005). Dormancy, another characteristic, is a mechanism that prevents germination from occurring even in environmentally favorable conditions (Baskin and Baskin 2004b). Although such characteristics are beneficial in the natural system, they make planting difficult for anthropogenic purposes (Miller et al. 2017). During ecological restoration or land management, the goal of practitioners is to have wild seeds behave more like domesticated crop seeds. Domesticated crop seeds ideally

germinate quickly and synchronously (Finch-Savage and Bassel 2016), and have been bred so as not to have dormancy (Pedrini and Dixon 2020). This breeding also reduces genetic diversity (Bhandari et al. 2017). Although breeding dormancy out of wild seed is possible (Witcombe and Whittington 1972, Ensslin et al. 2023), it would then be disadvantageous for future generations, putting the long-term success of restoration in peril. These future, nondormant seeds would be unable to deploy a bet-hedging strategy in unfavorable environments (Pausas et al. 2022).

This balance of wanting seeds from wildland species to behave as those from crops (i.e., germinate and establish quickly) while maintaining the full suite of germination characteristics of the wild seed, may be attained by influencing germination characteristics of seeds through environmental cues or seed treatments. However, dormancy can be difficult to identify and overcome. There are five classifications of dormancy (morphological dormancy, morphophysiological dormancy, physical dormancy, combinational dormancy, and physiological dormancy) all with different dormancy breaking requirements (Baskin and Baskin 2004b). In a natural system, dormancy is broken through a number of biotic and abiotic factors (Mousavi et al. 2011). By simulating these natural events, we can break dormancy prior to seeding. This allows for higher final germination in restoration efforts, without compromising survival of future generations. Dormancy breaking treatments, such as scarification, chemical cues, and altering temperature regimes can be performed in the laboratory to simulate these natural events (Kildisheva et al. 2020). Scarification can break dormancy by weaking the seed coat. In natural systems, this may occur through processes including fire, breakdown by microorganisms, and passage through the digestive tracts of animals (Gogue and

Emino 1979). These processes allow seeds to uptake water by breaking the hard seed coat (Kildisheva et al. 2020). Scarification can also weaken a restricting structure, allowing an embryo with low growth potential to germinate (Baskin and Baskin 2004b). In natural systems, chemical cues include the presence of smoke from fires and the introduction of nitrates onto the landscape (Kildisheva et al. 2020). These cues can set off a chain reaction of different physiological processes which lead to breaking dormancy (Bethke et al. 2006, Duermeyer et al. 2018, Lamont and Pausas 2023). Finally, laboratory stratification can simulate changing temperature conditions that are needed to break dormancy in nature (Kildisheva et al. 2020).

In order to expand knowledge on germination requirements and to improve future success in seed-based restoration, we studied the unique conditions needed to improve germination variables (final germination and germination rate) of nine forbs native to the northern Great Plains (NGP), a functional group that lacks diversity in restored grasslands (Augustine et al. 2021) and is threatened by invasive species (DeKeyser et al. 2009). The U.S. Bureau of Land Management has provided these species to us as 'high-priority' species (Table 1) for restoration in the NGP, an area of critical habitat for many species (Perkins et al. 2019, Niemuth et al. 2021). Our goal is to determine which conditions improve germination variables compared to untreated seeds. We (1) identify the class of dormancy expressed by each species, and (2) investigate if temperature and seed treatments influence germination variables.

Methods

Seed Quality

All seeds for our experiment were commercially sourced because restoration practitioners often purchase seeds (National Academies 2023). Eight species were sourced from Western Native Seed (Coaldale, CO), and *Erysimum asperum* was sourced from the Native Plant Initiative seed production field at South Dakota State University (Brookings, SD). All seeds were stored in a dry, refrigerated environment at 5°C. Seeds of *E. asperum* were stored for <10 months after harvest and the remaining seeds <4 months after delivery before beginning the germination experiment.

Three procedures were performed to assure seed quality and evaluate seed coat and embryo characteristics prior to the germination experiment. (1) A viability test was performed using Tetrazolium Chloride Testing guidelines according to the ISTA/AOSA (Miller 2010) with two replicates of 100 seeds. We required seed samples to have a viability ≥60% to assure quality seed, or else a new sample would be acquired. (2) Seed and embryo length were measured using a dissecting microscope, to calculate the E:S ratio (embryo length : seed length) for each species (Zhang et al. 2023). An E:S <0.5 would indicate an underdeveloped embryo (Baskin and Baskin 2007). (3) An imbibition test was performed to determine the permeability of the seed coat. The test used four replicates of 25 seeds, which were weighed before the start of the test (Baskin and Baskin 2014) and then placed in a 6 cm aluminum weigh dish. Enough deionized (DI) water was added to cover the base of the tin. After soaking for 24 hours, seeds were removed from the tin, blotted dry, and then reweighed (Baskin and Baskin 2014). To be defined as permeable, or capable of imbibition, the seed needed to increase in mass by at least 20% (Baskin and Baskin 2003a). If mass increase did not surpass this threshold, the sample was considered impermeable.

Germination Experiment

To determine germination requirements, each species was put through a move-along experiment (adapted from Baskin and Baskin 2003a). Seeds were treated in a factorial design with four pretreatments and nine temperature regimes. Each treatment consisted of five replicates of 50 seeds.

The four pretreatments were scarification, fertilizer, smoke, and a control. Pretreatments were chosen as scarification and chemical cues are often associated with breaking dormancy and promoting germination (Kildisheva et al. 2020). Seeds that were scarified were placed in an electric scarifier (Fred Forsberg & Sons Inc.) which was lined with 600 grit sandpaper (3M Co.). The scarifier was run in 10-second intervals, between which seeds were examined under a dissecting microscope for signs of scratching or damage to the seed coat. Seeds were then arrayed on germination paper (Anchor Paper Co.) moistened with DI water. For the fertilizer pretreatment, seeds were initially exposed to a 20mM solution of KNO_3 which was used to moisten the germination paper before seeds were arrayed. The smoke pretreatment required seeds to be placed, single-layer, into a 77-quart container. A Breville Smoking Gun burned 1.5g of wood (*Juniperus virginiana*); smoke of which was pumped into the container. The seeds were exposed to the smoke for 20 minutes, until the container was unsealed. Control seeds were left untreated. Seeds of the smoke and control treatments were then arrayed on germination paper moistened with DI water. Once seeds were arrayed, germination paper was placed into a 6mil 4”x 6” reclosable bag (Uline Inc.). After placement in the germination

chamber, replicates were rotated randomly and remoistened with DI water, regardless of pretreatment, when needed.

The move-along portion of the experiment exposed seeds to cyclical seasonal temperatures in a laboratory setting to determine temperature requirements for germination (Baskin and Baskin 2003a). Three germination chambers were used (Seedburo Equipment Co., Sheldon Mfg. Inc.), each set to represent the seasonal temperatures of the NGP across a 30-year average: a summer chamber (29/16°C), winter chamber (5/2°C) and spring/autumn chamber (18/6°C). Temperature data were gathered from NOAA National Centers for Environmental Information in 2021. Although temperatures of the NGP often drop below 2°C, the minimum temperature for the winter chamber had to be set at 2°C because the chambers were not capable of functioning below this temperature. Chambers were set to 12-hour diurnal periods, with high and low temperatures assigned to light and dark periods respectively.

Nine temperature regimes were used in this experiment: six stratified treatments and three controls. We defined a stratified treatment as any treatment that rotated through chambers to experience different seasons. Seeds began in either the winter chamber to represent cold stratification, or the summer chamber for warm stratification. Treatments were stratified in respective chambers for either 2, 4, or 8 weeks. After stratification, treatments rotated among chambers in seasonal progression. From here on, seeds rotated among chambers every 8 weeks regardless of initial stratification length. The three remaining temperature regimes were controls, which remained in assigned chambers throughout the entirety of the experiment: a summer, winter, and spring/autumn control.

Treatments were observed for germination daily for the first week in a new chamber, and approximately every three days thereafter. Germination was defined as any radicle emergence from the seed coat (Baskin et al. 2002). The experiment concluded after one complete seasonal cycle or until all seeds had germinated.

Dormancy Classification

Seed quality and treatment effects were used to identify which class of dormancy each species expressed (Fig 1). Dormancy was categorized into five classes: morphological dormancy, morphophysiological dormancy, physical dormancy, combinational dormancy, and physiological dormancy (Baskin and Baskin 2004b). Seeds with underdeveloped embryos (also have permeable seed coats) were considered to have either morphological (MD) or morphophysiological dormancy (MPD) (Kildisheva et al. 2020). If seeds with underdeveloped embryos germinated within four weeks, they were considered to have MD (Baskin and Baskin 2004a); if germination took longer than four weeks they were considered to have MPD (Kildisheva et al. 2020). Seeds with MD need time for the embryo to develop and to induce germination, whereas seeds with MPD need time in addition to a dormancy breaking treatment such as stratification (Kildisheva et al. 2020).

Seeds with developed embryos and impermeable seed coats were considered to have either physical (PY) or combinational dormancy (PY+PD) (Finch-Savage and Leubner-Metzger 2006). If seeds with developed embryos and impermeable seed coats germinated within four weeks after scarification, they were considered to have PY; if germination took longer than four weeks they were considered to have PY+PD (Ruiz-

Talonia et al. 2022). Seeds with PY need scarification to break dormancy, whereas seeds with PY+PD need scarification in addition to another dormancy breaking treatment such as stratification (Kildisheva et al. 2020).

Seeds were classified as nondormant (ND) if untreated seeds germinated ≥70% in four weeks (Ruiz-Talonia et al. 2022) over a range of conditions (Baskin and Baskin 2004a); seeds also had developed embryos and permeable seed coats (Kildisheva et al. 2020). Untreated seeds sown in spring and summer temperatures were considered as the ideal conditions for classifying ND seeds. Dormant seeds with developed embryos and permeable seed coats were considered to have physiological dormancy (PD) (Kildisheva et al. 2020, Ruiz-Talonia et al. 2022). Seeds with PD need a dormancy breaking treatment such as scarification (Baskin and Baskin 2004b), chemical cues (Kildisheva et al. 2020), or stratification (Ruiz-Talonia et al. 2022).

Statistical Analysis

Our cold stratification treatment was designed to mimic standard seed pretreatments with seeds in moist, cool conditions. However, we found substantial germination during cold stratification; temperatures of 5°C are sufficient for forbs to germinate >60% (Richardson et al. 2018). Unfortunately, our methods did not create a satisfactory pretreatment and left too few seeds to move along to the spring conditions. Therefore, cold stratification was excluded from further analysis. Stratification length of untreated seeds was not significant for warm treatments for any species (analysis of variance, $p > 0.05$; Table 2). Thus, different lengths were nested and reclassified as

summer temperatures throughout the remainder of the experiment. From here on, the predictor variable of temperature has two levels: spring and summer.

Viability was calculated as the number of viable seeds out of total seeds tested. Response variables, final germination and germination rate, were calculated with Auto-Germ (Richardson et al. 2018). Final germination was determined at four weeks for untreated seeds at each temperature (to be used in determining dormancy class; Fig 1), and at the termination of the experiment for all treatments. All data were analyzed with JMP® (Version 16.1.0, SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) was used to test if main effects or the interaction was significant ($\alpha = 0.05$). The goal of the experiment was to determine if a treatment would improve germination compared to sowing untreated seeds in the spring. Thus, a Dunnett's test ($\alpha = 0.05$) was used to compare treatment effects to the controls. Seeds in spring temperatures were used as the control for the main effect of temperature. Untreated seeds were used as the control for the main effect of pretreatment. Untreated seeds sown in spring temperatures was the control for the interaction.

Results

Three procedures were performed to assure seed quality and evaluate seed coat and embryo characteristics (Table 3). Viability varied among species but ranged from 72-93%. No species were found to have low viability (<60%), assuring seed quality for the experiment. The E:S ratio ranged from 0.69-0.95 among species, indicating all species had a developed embryo. All species imbibed >20% in mass after 24 hours, indicating all samples had permeable seed coats.

Final germination was significantly impacted by the main effect of temperature for two species (*E. asperum* and *Linum lewisii*), by the main effect of pretreatment for five species (*Dalea candida*, *E. asperum*, *Gaillardia aristata*, *Ipomopsis aggregata*, and *L. lewisii*), and by their interaction for two species (*D. purpurea* and *Ratibida columnifera*; Table 4). Final germination of two species (*Achillea millefolium occ.* and *Liatris punctata*) was not affected by temperature, pretreatment, nor their interaction (Table 4).

Final germination was influenced by both temperature and pretreatment (Table 4). Final germination was higher in spring temperatures compared to summer temperatures for *E. asperum* and *L. lewisii*. All pretreatments increased final germination for *E. asperum*. Final germination was increased by scarification for *G. aristata*; and scarification or fertilizer for *I. aggregata*. Final germination was decreased by smoke for *D. candida*; and scarification or fertilizer for *L. lewisii*. All pretreatments increased final germination when sown in summer temperatures for *D. purpurea*. Final germination was decreased by smoke treated seeds sown in spring temperatures for *R. columnifera*. No treatment increased final germination over the control for *A. millefolium occ.*, *D. candida*, *L. punctata* or *R. columnifera*.

Germination rate was significantly impacted by the main effect of temperature for four species (*A. millefolium occ*., *I. aggregata*, *L. lewisii,* and *R. columnifera*), by the main effect of pretreatment for three species (*A. millefolium occ*., *L. lewisii,* and *R. columnifera*), and by their interaction for four species (*D. candida*, *D. purpurea*, *G. aristata,* and *L. punctata*; Table 4). Germination rate of one species (*E. asperum*) was not affected by temperature, pretreatment, nor their interaction (Table 4).

Germination rate was influenced by both temperature and pretreatment (Table 4). Germination rate was higher in summer temperatures compared to spring temperatures for *A. millefolium occ*., *I. aggregata*, *L. lewisii* and *R. columnifera*. Germination rate was increased by scarification for *L. lewisii*; and scarification and smoke for *R. columnifera.* Germination rate was decreased by fertilizer for *A. millefolium occ*. Germination rate was increased by scarification and fertilizer when sown in summer temperatures for *D. candida*; untreated seeds, scarification and fertilizer when sown in summer temperatures for *D. purpurea*; and all treatments sown in summer temperatures for *G. aristata* and *L. punctata*. No treatment increased germination rate over the control for *E. asperum*.

Germination at four weeks was evaluated for untreated seeds in both spring and summer temperatures (Table 5). Six species demonstrated germination >70% for untreated seeds in these conditions. Seed of these species (*A. millefolium occ.*, *D. candida*, *D. purpurea*, *L. lewisii*, *L. punctata,* and *R. columnifera*) were determined to be nondormant (Table 6; Fig 1). Three species demonstrated germination <70% for untreated seeds in these conditions (Table 5). Seeds of these species (*E. asperum*, *G. aristata,* and *I. aggregata*) had developed embryos, permeable seed coats (Table 3), and demonstrated increased germination from treatment effects (Table 4). These treatment effects (scarification and chemical cues) are associated with PD (Finch-Savage and Leubner-Metzger 2006); thus these three species were determined to have PD (Table 6; Fig 1).

Discussion

The fundamental paradox of seed-based restoration wanting seed to germinate quickly while maintaining the full suite of genetic diversity in terms of germination

characteristics can be addressed with an increased understanding of the basic ecology of seeds and the effects of common seed treatments. By understanding seed dormancy and treating seeds to overcome inherent seed dormancy, we can expect wildland seeds to behave more like crop seeds (germinate and establish quickly) while maintaining the germination characteristics of the wild seed. We found that temperature influenced germination variables for all but one species. Pretreatments increased final germination for three species, which were determined to express PD. Through investigation of temperature and pretreatment effects, we were able to determine which conditions improve germination variables for our study species.

Temperature influenced germination variables for eight of the study species. Although changing temperature conditions did not break dormancy, it did elucidate optimal sowing temperatures for germination. The treatment of warm stratification (summer temperatures) only increased final germination for one species: *D. purpurea*. Our sample of *D. purpurea* was already determined to be ND (Fig 1). Temperature also influenced final germination for *E. asperum* and *L. lewisii*, which had higher germination in spring temperatures compared to summer. Other studies have found that *D. purpurea* germinates best at 20°C (Schellenberg and Biligetu 2015), which falls within our summer temperature range, and that *L. lewisii* germinates best at moderate temperatures (Rawlins et al. 2012, Richardson et al. 2018).

Faster germination can result in a competitive advantage in environmentally favorable conditions (Ludewig et al. 2014) and influence priority effects (Tan et al. 2012), as earlier germinants are first to access resources (Seiwa 1998) and have a longer growing season for reproduction (Donohue et al. 2010). Although early germination can

increase fitness (Verdú and Traveset 2005), it also increases exposure risk toward unpredictable, unfavorable environmental events (Donahue et al. 2010). In climates with unfavorable early spring conditions, slower germination would be beneficial (McArthur et al. 1987) as it would act as a bet-hedging strategy. Many species exhibit a positive relationship between germination rate and temperature (Aflakpui et al. 1998) up until a maximum allowable temperature (Bradford 2002, Phartyal et al. 2003, Qui et al. 2010). In our study, exposure to summer temperatures resulted in an increased germination rate for all species, except for *E. asperum*, when compared to spring temperatures.

Scarification, as a seed pretreatment, increased final germination of three of our species. Scarification is attributed to breaking dormancy in species with impermeable seed coats (Kimura and Islam 2012), such as many species in the Fabaceae family (Jayasuriya et al. 2013). However, it is often overlooked that scarification can break dormancy in permeable seeds as well (Baskin and Baskin 2004b). Scarification can break PD by increasing oxygen uptake, stimulating hormonal change (Mousavi et al. 2011), and weakening external structures to allow more potential for the radicle to protrude (Baskin and Baskin 2004b). Our species that responded to scarification (*E. asperum*, *G. aristata,* and *I. aggregata)* are from the Brassicaceae, Asteraceae, and Polemoniaceae families; families not associated with impermeable seed coats (Kildisheva et al. 2020). Two study species of the Fabaceae family, *D. candida* and *D. purpurea*, unexpectedly did not require scarification for germination. Both *D. candida* and *D. purpurea* have previously been identified as having impermeable seed coats and requiring scarification (Baskin and Baskin 2014), but we found no treatment necessary. Moreover, germination was >90% for untreated seeds of both *Dalea* species.

Only three study species expressed dormancy. Seeds of *E. asperum*, *G. aristata,* and *I. aggregata* all had the characteristics of the treatment effects associated with PD (Baskin and Baskin 2004b, Mousavi et al. 2011, Kildisheva et al. 2020, Ruiz-Talonia et al. 2022). Scarification broke dormancy for all three species, and chemical cues broke dormancy for *E. asperum* and *I. aggregata*. Some research has identified phylogenetic trends in dormancy (Baskin and Baskin 2003b, Carta et al. 2016), although it should be noted that dormancy expression can vary at the genus level (Baskin and Baskin 2014). Nonetheless, other members of the *Gaillardia* and *Erysimum* genera express PD (Baskin and Baskin 2014), yet to our knowledge this dormancy class has not been previously defined for *G. aristata* and *E. asperum*. Another study on *I. aggregata* suggested this species expresses PD (Kildisheva et al. 2019), consistent with our result.

Enhanced knowledge on germination requirements has implications for increasing grassland restoration success. Restoration failures are partly attributed to a lack of species-specific knowledge on germination requirements (Broadhurst et al. 2016), and better comprehension of germination requirements will lead to increased restoration success (Seglias et al. 2018). Although we determined temperature influenced germination variables of most study species, planting seeds at the optimal temperature may not always be cost-effective. For example, *D. purpurea* had optimal germination in summer temperatures. However, sowing untreated seeds in summer temperatures only increased germination by 4 percentage points (Table 6). Because direct seeding is typically performed in spring or autumn (Zajicek et al. 1986, Boyd and James 2013), it may not be cost effective to independently seed *D. purpurea* in summer for a 4 percentage point increase in final germination. Other considerations on emergence and

establishment in summer would also have to be considered. The cost-effectiveness should be considered before implementing treatments on any species.

Additionally, because slower and faster germination rates can both be advantageous (Ludewig et al. 2014, García et al. 2023), preferred germination rate should be decided by the objective of the restoration. However, final germination should be considered before implementing treatments to increase germination rate. For example, *E. asperum* and *L. lewisii* had lower final germination when sown in summer temperatures. Although summer temperature may speed up germination, it would also decrease final germination for these species, negatively impacting restoration success.

By breaking dormancy, we bypass the need to wait for favorable environmental conditions and instead promote germination on the timeline of the restoration. All of this is done without breeding out dormancy, thus the genetic diversity and seed functionality of the next generation is not compromised. To break dormancy of *E. asperum*, *G. aristata,* and *I. aggregata*, we encourage treatment prior to seeding. Gaining a further understanding of germination requirements allows for greater success of native forbs in restoration plantings. By breaking dormancy prior to seeding, we can expect wild seeds to germinate quickly, without compromising genetic diversity.

Table 1. List of study species tested.

scientific name	family	common name
Achillea millefolium occidentalis	Asteraceae	western yarrow
Dalea candida	Fabaceae	white prairie clover
Dalea purpurea	Fabaceae	purple prairie clover
Erysimum asperum	Brassicaceae	western wallflower
Gaillardia aristata	Asteraceae	blanketflower
Ipomopsis aggregata	Polemoniaceae	scarlet gilia
Linum lewisii	Linaceae	Lewis flax
Liatris punctata	Asteraceae	dotted blazing star
Ratibida columnifera	Asteraceae	upright prairie coneflower

Table 2. Significance of warm stratification length. Untreated seeds (controls) were tested with ANOVA to determine significance ($\alpha = 0.05$) of warm stratification length (2, 4, 8 weeks, and summer control) by species. Denominator *df*=16.

species	*df*	*F*	*p*
A. millefolium occ.	3	2.03	0.15
D. candida	3	1.03	0.41
D. purpurea	3	0.11	0.95
E. asperum	3	0.51	0.68
G. aristata	3	2.74	0.08
I. aggregata	3	2.04	0.15
L. lewisii	3	1.08	0.38
L. punctata	3	1.29	0.31
R. columnifera	3	1.10	0.38

Table 3. Viability, embryo development, and seed coat permeability. Viability percentage was calculated from the Tetrazolium Chloride Test. An E:S ratio <0.5 indicates an embryo is likely underdeveloped (Baskin and Baskin 2007). Mass increase percentage was calculated from the imbibition test. Standard error in parentheses.

species	viability (%)	E:S	mass increase (%)
A. millefolium occ.	76 (2.1)	0.84 (0.02)	57 (14)
D. candida	91 (1.2)	0.95 (0.01)	67 (10)
D. purpurea	72 (2.8)	0.91 (0.01)	104 (5.9)
E. asperum	93 (1.0)	0.92 (0.01)	181 (14)
G. aristata	76 (2.5)	0.69 (0.02)	67 (2.5)
I. aggregata	91 (1.5)	0.85 (0.02)	930 (36)
L. lewisii	80 (2.7)	0.84 (0.01)	181 (5.3)
L. punctata	91 (<0.1)	0.74 (0.03)	93 (1.2)
R. columnifera	89 (0.4)	0.78 (0.02)	57 (2.3)

Table 4. Treatment effect by species. Source indicates explanatory variables temperature (T; *df* =1), pretreatment (P; *df* =3), and their interaction (T x P; *df* =3). Denominator *df*=92. Significant p-values (ANOVA, $\alpha = 0.05$) are in boldface. Treatment effect indicates which treatment was significantly (Dunnett's test, $\alpha = 0.05$) greater (+) or less (-) than the control (n.s. = not significant, n.a. = main effect not analyzed due to significant interaction). Pretreatments include control/untreated (C), scarification (SC), fertilizer (F), and smoke (SK). Temperatures include summer (sum) and spring (spr).

		final germination			germination rate		
species	source	*F*	*p*	treatment effect	*F*	*p*	treatment effect
A. millefolium occ.	T	0.83	0.36	n.s.	247	**<0.01**	sum (+)
	P	1.02	0.39	n.s.	7.92	**<0.01**	F (-)
	T x P	0.76	0.52	n.s.	1.68	0.18	n.s.
D. candida	T	3.49	0.06	n.s.	26.1	**<0.01**	n.a.
	P	7.56	**<0.01**	SK (-)	33.7	**<0.01**	n.a.
	T x P	0.64	0.59	n.s.	9.15	**<0.01**	SC, F in sum (+)
D. purpurea	T	80.9	**<0.01**	n.a.	168	**<0.01**	n.a.
	P	5.23	**<0.01**	n.a.	36.7	**<0.01**	n.a.
	T x P	5.26	**<0.01**	any in sum (+)	21.7	**<0.01**	C, SC, F in sum (+)
E. asperum	T	17.3	**<0.01**	sum (-)	0.02	0.90	n.s.
	P	46.4	**<0.01**	SC, F, SK (+)	1.80	0.15	n.s.
	T x P	0.33	0.80	n.s.	1.24	0.30	n.s.
G. aristata	T	0.09	0.77	n.s.	285	**<0.01**	n.a.
	P	34.1	**<0.01**	SC (+)	6.19	**<0.01**	n.a.
	T x P	0.95	0.42	n.s.	7.12	**<0.01**	any in sum (+)
I. aggregata	T	0.55	046	n.s.	4.46	**0.04**	sum (+)
	P	10.6	**<0.01**	SC, F (+)	2.50	0.06	n.s.
	T x P	0.46	0.71	n.s.	1.60	0.20	n.s.
L. lewisii	T	7.34	**<0.01**	sum (-)	103	**<0.01**	sum (+)
	P	3.58	**0.02**	SC, F (-)	6.81	**<0.01**	SC (+)
	T x P	2.39	0.07	n.s.	1.87	0.14	n.s.
L. punctata	T	2.55	0.11	n.s.	458	**<0.01**	n.a.
	P	0.34	0.80	n.s.	39.7	**<0.01**	n.a.
	T x P	2.19	0.09	n.s.	10.7	**<0.01**	any in sum (+)
R. columnifera	T	0.16	0.69	n.s.	568	**<0.01**	sum (+)
	P	4.09	**<0.01**	n.a.	36.5	**<0.01**	SC, SK (+)
	T x P	3.50	**0.02**	SK in spr (-)	1.92	0.13	n.s.

Table 5. Germination at four weeks for spring and summer treatments of untreated (control) seeds. Values above 70% germination, the threshold for nondormant seeds (Ruiz-Talonia et al. 2022), are in boldface. Standard error in parentheses.

	germination (%) at four weeks	
species	spring	summer
A. millefolium occ.	**88** (1.8)	**86** (1.4)
D. candida	**97** (1.0)	**97** (2.0)
D. purpurea	**90** (1.3)	**94** (0.7)
E. asperum	66 (3.5)	54 (2.1)
G. aristata	64 (3.5)	69 (1.8)
I. aggregata	29 (2.3)	29 (1.9)
L. lewisii	**80** (1.6)	**72** (1.5)
L. punctata	**97** (0.5)	**97** (0.5)
R. columnifera	**95** (1.6)	**89** (1.1)

Table 6. Final germination of spring and summer treatments for controls and pretreatments greater than the control ($p < 0.05$). Column labels: pretreat. = pretreatments; dorm. = dormancy class. Pretreatments include control/untreated (C), scarification (SC), fertilizer (F), and smoke (SK). Recommendations were determined from significant treatment effects (Table 4) on final germination. Recommended treatments are in boldface. ND = nondormant, PD = physiological dormancy. Standard error in parentheses.

		final germination (%)			
species	pretreat.	spring	summer	dorm.	recommendation
A. millefolium occ.	C	89 (1.8)	86 (1.5)	ND	plant untreated in spring or summer
D. candida	C	97 (0.8)	99 (0.6)	ND	plant untreated in spring or summer
D. purpurea	C	90 (1.3)	**94 (0.7)**	ND	plant untreated in summer
E. asperum	C	67 (3.3)	57 (2.1)	PD	pretreat (scarify or fertilizer recommended); plant in spring
	SC	**90 (3.1)**	84 (1.3)		
	F	**94 (1.7)**	87 (1.3)		
	SK	77 (2.8)	68 (2.2)		
G. aristata	C	64 (3.5)	69 (1.8)	PD	scarify; plant in spring or summer
	SC	**91 (2.4)**	**86 (2.6)**		
I. aggregata	C	37 (2.4)	40 (1.9)	PD	scarify or fertilize; plant in spring or summer
	SC	**51 (6.0)**	**52 (1.6)**		
	F	**49 (4.2)**	**53 (1.7)**		
L. lewisii	C	**81 (2.0)**	72 (1.5)	ND	plant untreated in spring
L. punctata	C	97 (0.5)	97 (0.5)	ND	plant untreated in spring or summer
R. columnifera	C	95 (1.6)	89 (1.1)	ND	plant untreated in spring or summer

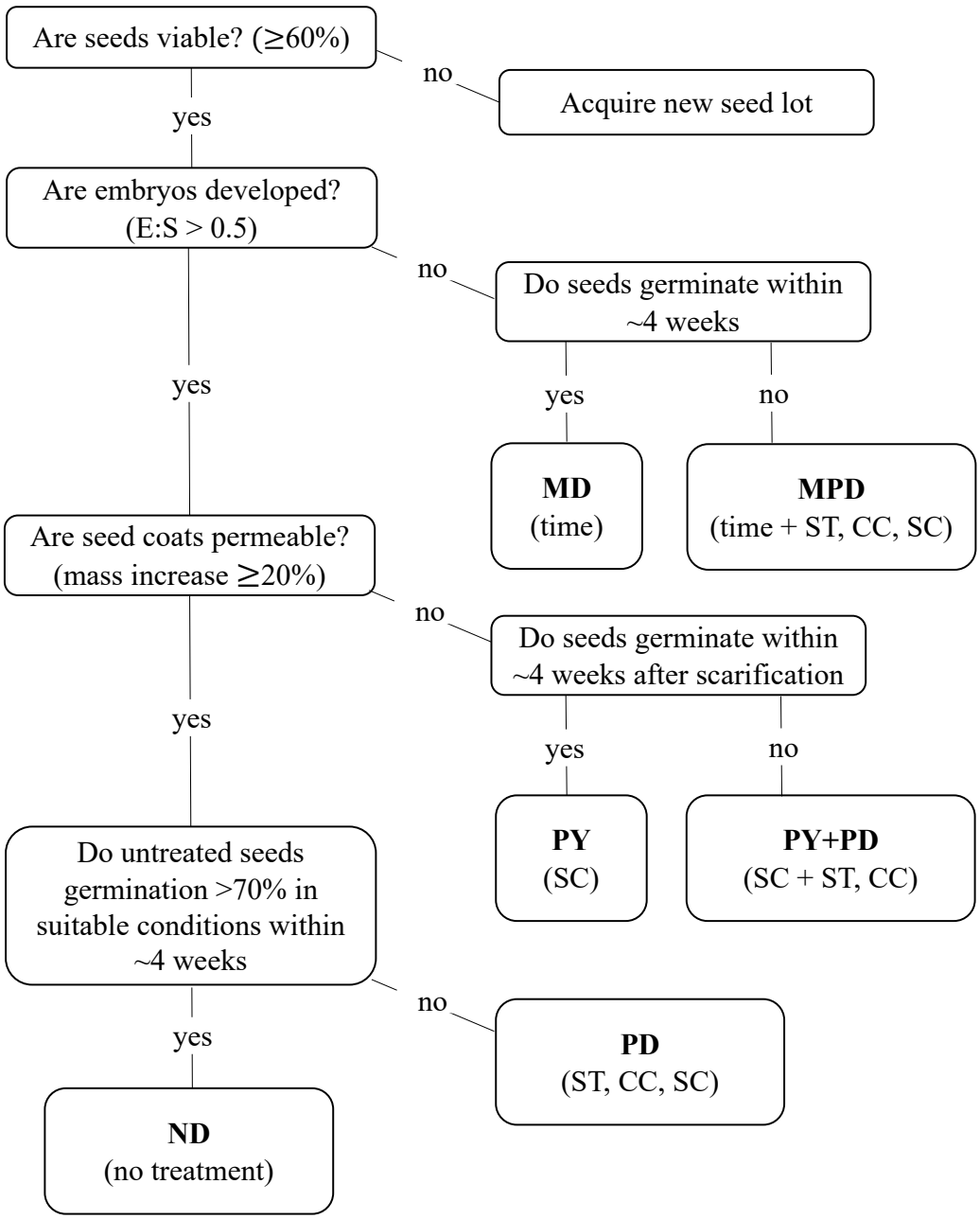

Figure 1. A decision tree used for determining seed dormancy. Only treatments used are expressed in the decision tree: ST = cold/warm stratification, CC = chemical cues (fertilizer and smoke), SC = scarification. Viability was first assessed using a Tetrazolium

Chloride Test. If viable, seeds were dissected and evaluated for embryo development. An E:S <0.5 indicated either morphological dormancy (MD) which required time to germinate or morphophysiological dormancy (MPD) which required time and additional treatments. Seed coat permeability was then evaluated with an imbibition test. Samples with impermeable seed coats indicated either physical dormancy (PY) which required scarification or combinational dormancy (PY+PD) which required scarification and additional treatments. Seeds with developed embryos and permeable seed coats indicated they were either nondormant (ND) which did not need treatments to germinate >70% or physiological dormancy (PD) which required treatments.

CHAPTER 3: LARGE INTRASPECIFIC VARIATION OF PHYSICAL DORMANCY FOUND IN *DALEA* SPECIES

Abstract

Seed sources for the same species can express different levels of dormancy. Many factors influence seed dormancy including environmental conditions that seeds experience during storage. Understanding why seed sources differ in dormancy percentage, and if storage conditions influence dormancy, can both affect how restoration practitioners choose to store and plant native seed. Two experiments were conducted to examine differences in dormancy between seed sources of the same species, and to determine if typical seed storage conditions influence physical dormancy. During the first experiment, we compared dormancy in three species (*Dalea candida*, *D. purpurea,* and *D. villosa*) between seeds sourced from a seed production field and a commercial supplier. Seeds were placed in deionized water for 48 hours and Imbibition Rate Index was calculated. After imbibition, seeds were dissected and evaluated for physical dormancy. During the second experiment, *D. villosa* from the seed production field was tested to determine if typical seed storage conditions influenced physical dormancy. Seeds were stored at room temperature (23°C) or refrigerated (5°C), and were either dehulled or stored with the hull on as a control. Seeds were stored for at least six months before testing for imbibition and dormancy. Large differences in dormancy between sources of the same species were recorded for all three *Dalea* species. Short-term storage conditions of *D. villosa* did not influence Imbibition Rate Index or physical dormancy. Knowledge of dormancy is important when planning native restorations. Short-term

storage conditions are likely not influencing dormancy of *Dalea* species, and concern over storage conditions should not be of top priority if seeds are to be planted within six months.

Introduction

Seed dormancy is a common characteristic among plants (Baskin and Baskin 2004a) and plays an important role in population persistence (Finkelstein et al. 2008). Dormancy enables a seed to withstand harsh environmental conditions and gives plants the best chance to establish in nature (Bentsink and Koornneef 2008, Kildisheva et al. 2020). Of the five common classifications of dormancy, two classes (physical and combinational dormancy) are characterized by an impermeable coat (Baskin and Baskin 2004b). Physically dormant seeds have at least one water-impermeable palisade layer of cells which contributes to the inability to uptake water (Turner et al. 2005, Mahadevan and Jayasuriya 2013). Such seed coat characteristics can protect the seed from harsh conditions (Jaganathan et al. 2016) and allow seeds to persists several years in the seed bank (Egley et al. 1983, Van Assche and Vandelook 2006).

Not all seeds of a source population will express dormancy (Batlla and Benech-Arnold 2010, Pedrini and Dixon 2020) and not all individual seeds will express dormancy. Seeds from a single species can express variation in dormancy percentages at the population (D'hondt et al. 2010, Kaye et al. 2018) and even the individual levels (Andersson and Milberg 1998). This variation in dormancy can be caused by environmental conditions both before and after dispersal of seeds from the maternal plant. Before dispersal, the environment in which the maternal plant experiences influences the level of dormant seeds produced (D'hondt et al. 2010). Whereas, post-dispersal environmental conditions, such as temperature, can break dormancy in a portion of seeds, leaving the remaining seeds in the seed bank physically dormant (Moreira and Pausas 2012). The loss of dormancy from a portion of seeds may act as a bet-hedging

strategy (Moreira and Pausas 2012) where seeds germinate over multiple years as a survival mechanism (Clauss and Venable 2000).

In nature, physical dormancy is overcome by different environmental factors. Fluctuating temperatures (Daibes et al. 2017), fires (Pausas and Lamont 2022), and animal digestion (Rolston 1978) break physical dormancy. In laboratory conditions, seeds are often mechanically scarified to break physical dormancy (Kildisheva et al. 2020); a process in which a rough surface is typically used to cause abrasion to the impermeable seed coat (Rolston 1978). However there are other laboratory treatments by which dormancy can be broken as well. Dry-storage can break physical dormancy in some species (Meisert 2002, Jayasuriya et al. 2009). Dehulling, the process in which the hull of a seed is removed, has been used to break physical dormancy (Spanò et al. 2017); this process could act as a form of mechanical scarification (Griffith and Booth 1988).

Members of the Fabaceae family, such as *Dalea*, are often characterized by physical dormancy (Baskin 2003). Mechanical scarification breaks dormancy and increases final germination in physically dormant seeds. Yet studies find dissimilarities in final germination results of Fabaceae species; results show large (>70 percentage points) to little (14 percentage points) improvement in germination after mechanical scarification treatments (Bushman et al. 2015, Jones et al. 2016, Kildisheva et al. 2018). The difference between these germination trials could be influenced by varying levels of dormancy among seed sources.

This research had two goals. The first goal was to examine differences in dormancy between seed sources of the same species. Seeds were harvested from a seed production field and paired with commercially sourced seeds. Because so many factors

can influence dormancy, we hypothesized that dormancy would differ significantly between sources of the same species. To understand the range of intraspecific dormancy percentage, we performed a source experiment on *Dalea candida* (white prairie clover), *D. purpurea* (purple prairie clover), and *D. villosa* (silky prairie clover). The second goal was to examine how cleaning methods (dehulling) and storage temperature influenced physical dormancy. Upon harvest, *Dalea* has a hull covering the seed, which is removed in processing by some commercial seed companies. This processing or dehulling may act as mechanical scarification (Griffith and Booth 1988). Because temperature can affect physical dormancy (Moreira and Pausas 2012) and the dehulling process can cause scarification (Griffith and Booth 1988), we hypothesized that storage temperature and cleaning methods would affect dormancy. Field-sourced seeds of *D. villosa* were either dehulled or left intact, and then stored at either room temperature or in cold storage. Dormancy was assessed after six months of storage.

Methods

Experiment 1: Source

To determine if dormancy percentage varies by source, three *Dalea* species (*D. candida*, *D. purpurea,* and *D. villosa*) were tested. Each species had seeds sourced from a seed production field and from a commercial supplier (Table 1). Field seeds came from the Native Plant Initiative seed production field at South Dakota State University (Brookings, SD). Seeds of *D. candida* and *D. purpurea* were sourced commercially from Western Native Seed (Coaldale, CO), and *D. villosa* from Millborn Seeds (Brookings, SD). At the time of the experiment, seeds sourced from the seed production field and

from Millborn Seeds had been stored at room temperature (23°C) for <2 months since harvest or delivery. Seeds of Western Native Seed had been stored <10 months in a dry, refrigerated environment (5°C). Seeds were analyzed and selected for individuals that were not visibly cracked or scratched. All sources were subject to a scarification treatment (vs. control), and dormancy was evaluated via imbibition and dissection.

First, seeds were dehulled (the sources from Western Native Seed came dehulled) using a hand debearder. After which, seeds were divided into control (unscarified) and scarified groups. Scarified seeds acted as a positive control and gave an upper limit to the amount of water the seed can imbibe and a standard for nondormant seeds. Scarified seeds were nicked with a blade just enough to cut through the seed coat. Experimental design consisted of three replicates of 20 seeds per group (Chia et al. 2016).

For imbibition, seeds were weighed to record dry weight (Baskin and Baskin 2014), and then placed in a 9 cm diameter petri dish. Deionized water was added to the petri dish where seeds soaked for five minutes, after which they were removed from the water, blotted dry, and reweighed as time = 0 (Turner et al. 2009). Seeds were placed back in the petri dish to soak. Replicates were removed, blotted and reweighed (Chia et al. 2016) at 1, 2, 4, 8, 24, and 48 hours. After 48 hours of imbibition, seeds were analyzed for physical dormancy via dissection. Seeds were determined to be physically dormant if their seed coat was still firm or hard; seeds were determined to be nondormant if their seed coat was soft and they had a water-filled embryo (Turner et al. 2009).

Experiment 2: Storage

Seeds of *D. villosa* from the seed production field were tested to determine if common seed storage conditions influence dormancy. Seeds of *D. villosa* were chosen due to a high dormancy percentage and seed availability. Seeds were analyzed and selected for individuals that were not visibly cracked or scratched. Seeds were separated into a factorial design with different storage temperature and cleaning methods. Storage conditions include dry storage in a refrigerator (5°C) or dry storage at room temperature (23°C) in a paper envelope. Cleaning methods include dehulling seeds before storage or storing seeds with hulls still on as a control. Seeds are often stored at either room temperature or in refrigerated conditions (Justice and Bass 1978) and can be dehulled by companies prior to purchase (Faborode et al. 2003). Seeds were stored for at least six months. After the storage period, seeds were dehulled if needed, and each treatment was divided into controls (unscarified) and scarified groups. Seeds were scarified and imbibition was conducted by the same process as with *Experiment 1: Source*.

Statistical Analysis

Response variables of Imbibition Rate Index (IRI) and dormancy percentage were used for both experiments. Because of its use as a variable in the IRI equation, (1) percent mass increase was calculated, where M_i is the mass of the replicate after imbibition, and M_d is the dry mass of the replicate (Turner et al. 2009). (2) IRI was calculated, where t is the time in hours, M_t is the mass increase percentage of the replicate after t hours, and M_{t-1} is the mass increase percentage of the replicate the t prior (Turner et al. 2009). (3) Percentage of physical dormancy present was calculated, where PY is the number of dormant seeds, and T is the total number of seeds.

$$(1)\ \%\ mass\ increase\ =\ \frac{(M_i - M_d)}{M_d} \cdot 100$$

$$(2)\ IRI\ (\%h^{-1}) = \sum \frac{(M_t - M_{t-1})}{t}$$

$$(3)\ dormancy\ (\%)\ =\ \frac{PY}{T} \cdot 100$$

For *Experiment 1: Source*, the explanatory variables were seed source and species. For *Experiment 2: Storage*, the explanatory variables were storage temperature and cleaning method. Only control (unscarified) seeds were used in analysis, unless otherwise specified. Data for both experiments was analyzed with Analysis of Variance (ANOVA; $\alpha = 0.05$) on JMP® (Version 16.1.0, SAS Institute Inc., Cary, NC, USA). Prior to running ANOVA, fractional data were transformed using an arcsine-square root transformation (Turner et al. 2009).

Results

Experiment 1: Source

Seed source influenced response variables (dormancy percentage and IRI) for each species, except for IRI of *D. candida* (Table 2). Source influenced response variables among species, where field and commercially supplied seeds differed in dormancy percentage and IRI (Tables 1, 3). Scarified treatments had a higher IRI than controls among species and increased in mass more than all respective controls (Table 3; Fig 1).

Experiment 2: Storage

Neither storage temperature nor cleaning method influenced the response variables of *D. villosa*. No significant interaction or main effects occurred for any of the response variables (Tables 4, 5). When storage treatments were compared to the results of field *D. villosa* from six months earlier (*Experiment 1: Source*), still no difference occurred (Table 6). Scarified treatments had a higher IRI and increased in mass more than all respective controls (Table 7; Fig 2).

Discussion

Seed sources of the same *Dalea* species can express significantly different dormancy percentages. Understanding dormancy and variation of dormancy among seeds of a single species will provide guidance to restoration practitioners on how to properly source and store seeds. This research provides us a better understanding of the level of dormancy variation present in *Dalea* seeds and of suitable short-term storage conditions.

Our results revealed that all three *Dalea* species displayed physical dormancy. Physical dormancy has been previously reported in seeds of *D. candida* and *D. purpurea* (Dickerson et al. 1981, Sullivan and Daley 1981, McGraw et al. 2003, Baskin and Baskin 2014). Scarification has been deemed beneficial for *D. villosa* (Schellenberg and Biligetu 2015) alluding to physical dormancy and supporting our classification, although dormancy class was never specifically stated.

Our first hypothesis, that dormancy would differ significantly between sources of the same species, was supported. Individual plants are known to display variation in dormancy (Andersson and Milberg 1998). All three study species had drastic variation in dormancy, with the largest difference displayed by *D. purpurea*. Sources of *D. purpurea*

expressed 3% and 95% (Table 1) dormancy from commercial and field sources respectively. This is a prominent example of variation, with one source being almost completely nondormant whereas the other source is almost completely physically dormant. Dormancy percentage of seed source among species was also significant, where field seeds averaged 93% dormancy and commercial seeds averaged 21% dormancy. It has been suggested that cultivation of forbs selects for nondormant seeds (Qu et al. 2005, Ensslin et al. 2023) because of faster germination (Meyer and Kitchen 1994). However, we do not have enough information in our study to conclude if commercial sourcing is the cause of dormancy variation without further knowledge on where the commercial seeds were grown and the exact environmental and storage conditions of the seeds prior to shipment.

The hypothesis for our second experiment, that dormancy would be influenced by short-term storage temperature and cleaning methods, was not supported. Remarkably, dormancy of *D. villosa* was not different for either storage temperature or cleaning methods (Fig 2). Additionally, no difference in dormancy was found between these treated seeds and the results of field *D. villosa* from six months earlier in *Experiment 1: Source*. Although, storage temperature and cleaning methods have been effective at reducing physical dormancy for other species (Meisert 2002, Jayasuriya et al. 2009, Spanò et al. 2017), no impact of storage temperature nor cleaning methods lessened dormancy for our *D. villosa*. From these results, we elucidate that the large differences in dormancy percentage between sources are likely not due to short-term storage. Our commercially supplied seeds, which had low dormancy, could have been dehulled using alternative methods that might have scarified seeds more than the hand debearder

method. However, we find this hypothesis unlikely as all seeds used were examined under a microscope and no signs of scarification were present on seeds that were provided to us dehulled. We do not deny that long-term storage periods might have an effect on physical dormancy (Meisert 2002), but from our results we elucidate that the large differences in dormancy percentage as seen in *Experiment 1: Source*, are likely not due to short-term storage.

The research conducted on *Dalea* species provides ecological implications for future seed research and restoration. The difference in dormancy characteristics within a species, shows a possible disconnect that could occur between research and application. Studies breaking dormancy and writing germination protocol should use more than one source to determine results. Studying species from one source may not reveal the whole picture on germination characteristics. Due to dormancy variation, it would also be beneficial to diversify sources in a seed mix. Using multiple sources would likely result in a mixture of germination characteristics, artificially assuring a bet-hedging strategy (St. Clair et al. 2020).

We likely should not be concerned that short-term storage periods of *Dalea* seeds have dramatic effects on dormancy. Dehulling by suppliers is likely not breaking physical dormancy to large degrees, and it is safe to dehull seeds as long as done correctly to not damage the seed (Griffith and Booth 1988). Although longer or alternative storage conditions may influence dormancy (Meisert 2002), the lack of evidence on the influence of short-term results suggests that such differences in dormancy may more likely be influenced from pre-dispersal environmental effects in this instance.

Because seed availability is limited (Rowe 2010) it is crucial that we understand seed characteristics before using seed in the field. Lack of knowledge on germination characteristics, such as dormancy, is often an explanation for restoration failures (Broadhurst et al. 2016). Determining why there is such variation in dormancy and understanding what is causing these differences is important. Although purchasing seed with low dormancy can be desirable, it can also influence restoration success. These dormancy differences could be unrelated between field and commercial seeds, simply caused by genetic differences in individual plants (Copete et al. 2020), but it is worth continuing research with more sources to test if the commercial seed production process does cause some effect. If dormancy was determined to be bred out of native seeds, there may be concern for restoration success. Dormancy has been bred out of agricultural seeds (Pedrini and Dixon 2020) however, breeding dormancy out of native seeds, may change other critical survival characteristics over time, such as bet-hedging strategies (Pausas et al. 2022). However, at the moment, the cause of this drastic variation in *Dalea* is only speculation.

Dormancy differed between sources within a species, but it was likely not due to short-term storage effects. There is some correlation between commercially sourced seeds and dormancy loss, but we are unable to test if there was truly causation and what is the driving cause of these discrepancies. We are able to conclude that short-term storage has no effect on this dormancy discrepancy for *D. villosa*. This result suggests that the short periods of time we stored our seed before experimentation likely was not the cause of these variations in dormancy percentage, and differences in dormancy levels are more likely due to factors prior to purchase, such as genetic factors, environmental

conditions, or even producer handling. Furthering knowledge on dormancy would result in higher restoration success because it would provide practitioners a better understanding of how to best source and store seeds.

Table 1. *Dalea* seed source, and non-transformed mean dormancy % and mean IRI (Imbibition Rate Index) of control seeds in *Experiment 1: Source*. Seeds were sourced from Western Native Seed (WNS; Coaldale, CO), Millborn Seeds (MS; Brookings, SD) or the seed production field of the Native Plant Initiative (NPI) at South Dakota State University. Values in parentheses are standard errors.

species	seed source	dormancy (%)	IRI ($\%h^{-1}$)
D. candida	seed production field	90 (3.1)	4.03 (2.04)
	commercial (WNS)	3.5 (1.8)	12.8 (4.29)
D. purpurea	seed production field	95 (3.0)	1.79 (3.19)
	commercial (WNS)	3.4 (1.7)	30.4 (2.98)
D. villosa	seed production field	93 (1.5)	2.08 (2.47)
	commercial (MS)	56 (8.1)	27.9 (8.59)

Table 2. Significance of seed source (seed production field and commercially supplied seeds) of unscarified seeds by species, in *Experiment 1 Source*. Bolded values are significant (ANOVA, $\alpha = 0.05$). Denominator *df*=4.

		dormancy (%)			IRI (%h^{-1})		
species	test source	df	f-ratio	p-value	df	f-ratio	p-value
D. candida	seed source	1	138	**<0.01**	1	3.41	0.14
D. purpurea	seed source	1	98.1	**<0.01**	1	43.1	**<0.01**
D. villosa	seed source	1	27.9	**<0.01**	1	8.35	**0.04**

Table 3. *Experiment 1: Source* results among species. 'Seed source' includes only control (unscarified) seeds. 'Scarification treatment' includes scarified and control seeds. Bolded values are significant (ANOVA, $\alpha = 0.05$). 'Seed source' denominator *df*=16. 'Scarification treatment' denominator *df*=34.

	dormancy (%)			IRI ($\%h^{-1}$)		
test source	df	f-ratio	p-value	df	f-ratio	p-value
seed source	1	53.0	**<0.01**	1	24.9	**<0.01**
scarification treatment	1	40.9	**<0.01**	1	271	**<0.01**

Table 4. *Experiment 2: Storage* results of *D. villosa* from the seed production field (ANOVA, $\alpha = 0.05$). Denominator *df*=8.

	dormancy (%)			IRI (%h^{-1})		
test source	df	f-ratio	p-value	df	f-ratio	p-value
storage (S)	1	2.83	0.13	1	0.39	0.55
cleaning (C)	1	1.03	0.34	1	0.42	0.54
interaction (S x C)	1	0.98	0.35	1	1.04	0.34

Table 5. Storage conditions, and non-transformed mean dormancy % and mean IRI (Imbibition Rate Index) of control seeds in *Experiment 2: Storage*. Seeds used were *D. villosa* from the seed production field. Values in parentheses are standard errors.

storage	cleaning	dormancy (%)	IRI ($\%h^{-1}$)
5°C	dehulled	93 (4.4)	3.22 (1.43)
5°C	control	86 (3.7)	3.79 (1.69)
23°C	dehulled	97 (1.7)	3.82 (1.98)
23°C	control	97 (1.7)	1.30 (0.61)

Table 6. Comparing source and storage experiment results of *D. villosa* from the seed production field (ANOVA, $\alpha = 0.05$). Denominator *df*=28.

	dormancy (%)			IRI ($\%h^{-1}$)		
test source	df	f-ratio	p-value	df	f-ratio	p-value
experiment	1	<0.01	0.95	1	0.30	0.59

Table 7. *Experiment 2: Storage* results between scarified and control (unscarified) seeds, among treatments. Bolded values are significant (ANOVA, $\alpha = 0.05$). Denominator *df*=22.

	dormancy (%)			IRI ($\%h^{-1}$)		
test source	df	f-ratio	p-value	df	f-ratio	p-value
scarification treatment	1	912	**<0.01**	1	3650	**<0.01**

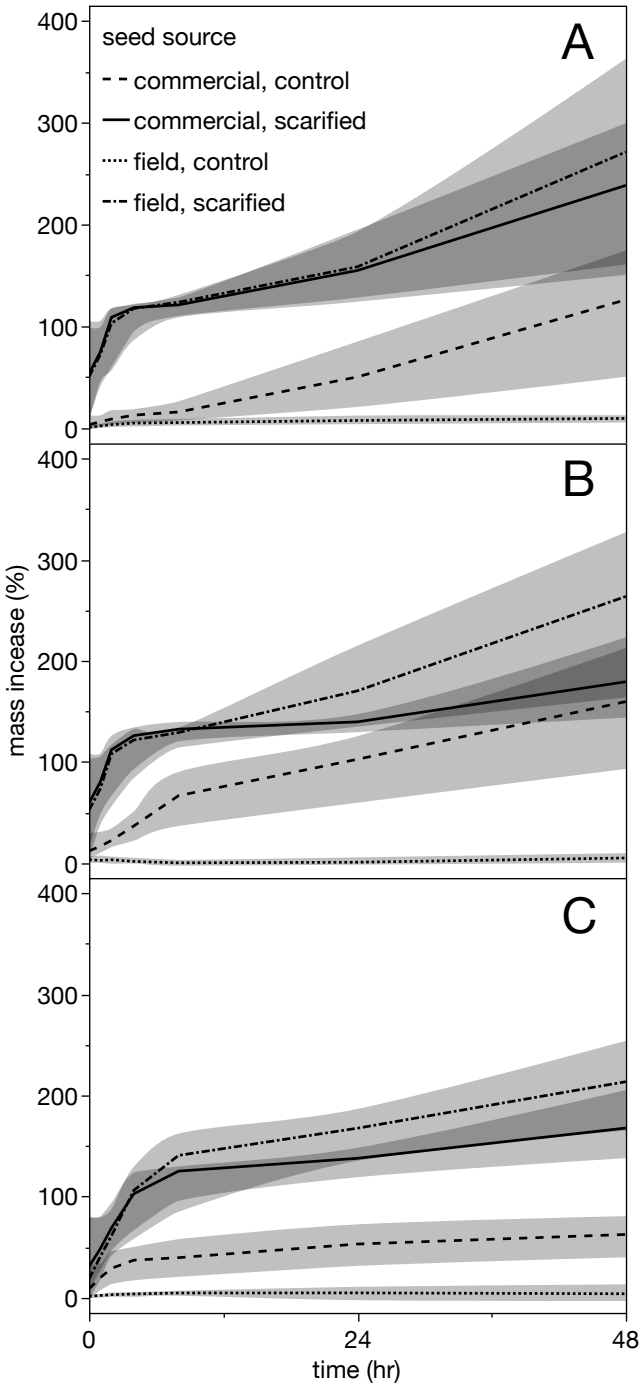

Figure 1. Mass increase (%) during imbibition of *D. candida* (A), *D. purpurea* (B), and *D. villosa* (C) from commercial and field sources with scarified and control (unscarified) treatments. Shaded polygons represent confidence of fit. Figures present moving average of mass increase (%) over time with non-transformed data.

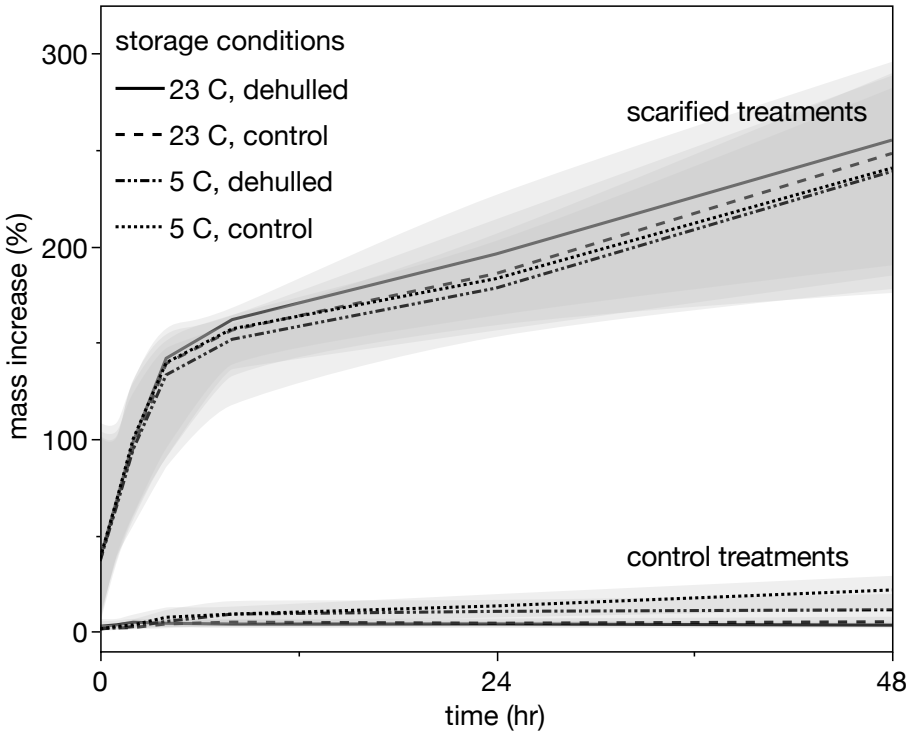

Figure 2. Mass increase (%) of scarified and control (unscarified) treatments of field sourced *D. villosa* at different storage conditions. Shaded polygons represent confidence of fit. Figure presents moving average of mass increase (%) over time with non-transformed data.

CHAPTER 4: SUMMARY

The goal of this research was to further the understanding of dormancy characteristics of native plants in the northern Great Plains. In doing so, information gained can produce higher species-specific success in seed-based restoration. The objective of the first data chapter was to identify the potential dormancy class and dormancy-breaking techniques of nine native forbs using a move-along experiment (adapted from Baskin and Baskin 2003a). The objective of the second data chapter was to elucidate the variation in dormancy percentage expressed between two seed sources of the same species, and to determine if storage temperature and cleaning methods influence dormancy.

In the first data chapter, I found that three of the nine study species expressed physiological dormancy. Dormancy of *Erysimum asperum*, *Gaillardia aristata*, and *Ipomopsis aggregata* was broken by a scarification treatment. Additionally, chemical cues were able to break dormancy for *E. asperum* and *I. aggregata*. The remaining species were classified as nondormant, but final germination and germination rate were still influenced by treatments. For example, although *Linum lewisii* did not need a dormancy-breaking treatment, I found that the final germination was higher when initially exposed to moderate spring temperatures rather than warmer summer temperatures.

In the second data chapter, I found that all three *Dalea* species expressed different levels of dormancy between seed production field sources and commercially supplied sources. Seeds from the seed production field had higher dormancy levels than

commercially supplied seeds, however we did not gather enough information to determine if this observation is significant. When testing storage temperature and cleaning methods of *Dalea villosa*, I determined that neither storage temperature condition, nor dehulling seeds influenced physical dormancy in storage periods up to six months.

Information gathered through this research has direct implications for seed-based restoration success in the northern Great Plains. The determination of dormancy class and dormancy breaking techniques for *E. asperum*, *G. aristata,* and *I. aggregata* will provide better species-specific success in restoration. Breaking dormancy before seeding will increase the final germination of these species and provide more opportunity for establishment. Treatments and temperature conditions were identified for the remaining nondormant species. I identified temperature conditions that promoted germination for select species, and treatments that influenced germination rate. Treating seeds to encourage a faster germination rate can improve restoration efforts by providing early germinating seeds first access to resources (Seiwa 1998).

This research also identified the difference in dormancy between different sources of the same species. Knowing dormancy levels can help plan the timing of emergence (Batlla and Benech-Arnold 2010) in a restoration, which can influence establishment as stated earlier with germination rate. It was also determined that for *D. villosa,* storage temperature and cleaning methods, up to six months, did not influence physical dormancy. As long as seeds are stored appropriately, and are planned for seeding within a few months, there should be no concern that storage techniques are impacting dormancy for *D. villosa*. The impacts of short-term storage should be tested on other species to

determine if this result is a trend among physically dormant seeds. The information gathered on seed storage and treatments furthers collective knowledge on seed dormancy, and contributes to the success of seed-based restoration in the northern Great Plains.

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