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Investigating Effect of Seed Source and Developing Germination Protocols to Improve Success in Restoration of Arkansas Tallgrass Prairies

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Investigating Effect of Seed Source and Developing Germination Protocols to Improve Success in Restoration of Arkansas Tallgrass Prairies

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Abstract

American tallgrass prairie has faced losses estimated at 87-98% of original land area since European settlement. Native seeds are often used to supplement struggling or extinct plant populations in tallgrass prairie restoration and establishment sites. Two main considerations in restoration and establishment are from where to source seed and how to obtain high germination rates. In order to determine the effect of seed source, a common garden experiment was performed in Fayetteville, Arkansas in 2022 with five prairie species: *Andropogon gerardii* (big bluestem), *Bouteloua curtipendula* (sideoats grama), *Panicum virgatum* (switchgrass), *Schizachyrium scoparium* (little bluestem), and *Sorghastrum nutans* (Indiangrass). Seed was sourced from Oklahoma, Kentucky, Illinois, South Dakota, North Dakota, Minnesota, and Ontario, Canada. These sources represented a variety of latitudinal distances from the common garden. Differences in date of anthesis of first culm, mature height of tallest growing point, annual aboveground biomass production, and annual seed production of the individuals were analyzed among sources. The preliminary conclusion was that seed source does not affect success of a restoration or establishment, as there were very few significant differences in the measured characteristics among sources, and only one instance of the closer source being significantly taller with more biomass than the further source. Germination trials were conducted to investigate how germination pretreatments would affect germination rate for tallgrass prairie species, including fourteen common grasses and forbs found in Arkansas prairies. Seeds for each species were obtained from multiple sources across the USA and Canada. Pretreatments in the study included sterilization with hydrogen peroxide, dry and moist stratification of varying durations (1, 2, or 3 months), mechanical scarification with sandpaper, thermal scarification with boiling water, chemical scarification with hydrogen peroxide, and hormonal treatment with

gibberellic acid. Across all species, there were minimal instances where a pretreatment significantly improved germination rate. One consistent finding was that thermal scarification with boiling water should be avoided unless specifically prescribed.

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While a great many additional people made this thesis possible, more than would fit on this page, I'll have to generalize and simply say thank you to the members of the USDA Plant Materials Centers, the Arkansas Natural Heritage Commission, the Association of Official Seed Analysts, and the seed laboratories who kindly offered advice and aid for my various experiments.

Dedication

This thesis is wholeheartedly dedicated to my husband Mark, who helped and motivated me through the tough times throughout this process. Without him, I truly believe this thesis would not have been possible.

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Introduction and Literature Review

An Introduction to Tallgrass Prairies

In North America, the loss of native tallgrass prairie has been estimated at 87-98% since European settlement (Samson, Knopf, & Ostlie, 2004). The remaining area of once-continuous prairie tract is now divided into smaller, less valuable habitats. The size of a prairie directly determines the amount of carbon sequestration and the biodiversity it can support. Small prairies are also susceptible to colonization by invasive species. Thus, it has long been recognized that these remnants must be protected, and efforts must be initiated to restore areas which were previously tallgrass prairie. This is especially important when the site to be restored is adjacent to an existing remnant in order to increase its size and productivity. Restoration itself is defined as "the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed," (Society for Ecological Restoration, 2004).

It is critical to determine what species to remove, preserve, and supplement in order to meet the goals of a restoration or establishment project. While invasive species are often eradicated and endangered species are protected, there is still the question of how to increase individuals of a struggling or extinct population in the ecosystem. There are several ways to accomplish this, including transplanting prairie sod onto the restoration site, transplanting individual plants, and seeding the site (Christiansen & Landers, 1969; Sullivan, 1998). For most restoration efforts, seeding is the most accessible method in feasibility and total cost.

Seed Sourcing

Seed source refers to the origin of the seed sown at a restoration site. While it may seem reasonable to obtain or collect seed from a different population where seed is readily available, regardless of its location, this may not always be the best option for a successful restoration. Populations may differ greatly and may not be suited to establishment in a new site (McKay et al., 2005).

Within a species, there are many differing genetic traits such as bloom time, vigor, and environmental adaptations that can influence suitability of a population in various regions. These traits originate from mutations which travel within and across populations over time via gene flow, recombination, genetic drift, and natural selection (McKay et al., 2005). Due to these mechanisms, species naturally differentiate into ecotypes, which are distinct genotypes based on local adaptation (Hufford and Mazer, 2003). If desirable traits are artificially selected and bred into stable genotypes by humans, they are considered cultivars. These differences within species can have a long-term impact on a restoration project, especially when using seed sources or cultivars that may be poorly suited to a specific restoration site.

Environment—biotic and abiotic—plays a defining role in the creation of distinct ecotypes. Climate may be the first aspect to come to mind, but there is a litany of other environmental factors to which plants may adapt. Linhart and Grant (1996) reviewed research on plant adaption to soil composition, elevation, moisture, light availability, and competition with other plants as well as biotic interactions including pollination vectors, predators, and parasites, concluding that all these factors are important in local adaptation, and the development of local ecotypes. The complex and diverse microbiomes of each ecosystem have also been found to influence local adaptation, especially beneficial nitrogen-fixing bacteria and mycorrhizal fungi in

the soil with which host plants have adapted to form specialized relationships (Schultz et al., 2001; Bever, 2015; Rúa et al., 2016). Conversely, disease resistance can also be dependent on local adaptation, as summarized by Lesica and Allendorf (1999), due to the fact that a resistant trait against a certain disease would likely be less frequent, if present at all, in populations where the disease-causing pathogen is not present.

With the research discussed above concerning the topics of ecotypes and local adaptation, there is no doubt that a population adapted to a unique ecosystem would have a different genetic makeup than a population of the same species growing in a different ecosystem. Populations do not adapt and evolve in a vacuum—the entire ecosystem affects and is affected by the plant species which are an instrumental part of that ecosystem.

Commonly referred to as local seed sourcing, or local sourcing, the use of local ecotypes is a strategy for restoration efforts based on the assumption that populations found within a certain geographic distance from the restoration site are more appropriate for sourcing seed. Further improving this idea to incorporate climate and environment inspired the concept of seed transfer zones (STZs). These zones are defined as areas within which plant materials can be transplanted while lowering risk of being poorly suited to the destination site, and are usually most applicable when species-specific research has been applied (Hufford & Mazer, 2003).

However, the issue with local sourcing is that there is no commonly-agreed upon definition for “local”. There are not STZs created for every species, and a set geographic distance or general-purpose STZs cannot be relied upon to provide accurate source guidelines in most cases, especially when photoperiod is considered for differing latitudes as reviewed by Aitken and Whitlock (2013). Further complicating the issue, while the existence of ecotypes is indisputable, researchers tend to either encourage (McKay et al., 2005; Vander Mijnsbrugge,

Bischoff, & Smith, 2010) or discourage (Broadhurst et al., 2008; Herman et al., 2014) the use of local ecotypes in restoration. For the purposes of this review, these two opinions will be referred to as the "Local is best" and "Diversity is best" groups.

Restoration of an ecosystem is an infinitely complex task, with the decision of where to source seed no less complicated an undertaking. Effects of local or nonlocal sourcing may be found from the success of the individual plant to the success of the restoration site ecosystem. These effects are commonly discussed and researched by the two sides of the issue and include survivability of individuals, inbreeding and outbreeding at the population level, population response to climate change, and overall effect on the ecosystem.

The success of outsourced individuals is the cornerstone of a restoration effort. If the introduced plant material does not survive or reproduce, then further steps and goals in the restoration such as persistence of the population, slowing spread of invasive species, and reintroduction of animal life may not be accomplished. In relation to individual fitness, the "Local is best" opinion often values the concept of a "homesite advantage", which simply suggests that plants have a higher ability to survive and reproduce (fitness) in the environment where they have adapted than in a foreign environment. There have been data supporting homesite advantage, including publications by Montalvo and Ellstrand (2000), Schultz et al. (2001), Gustafson, Gibson, and Nickrent (2004a), and Wilson et al. (2016). The conclusions from these studies give rise to the concern that seed from nonlocal sources may not establish well in the restoration site.

However, there have also been studies where local adaptation did not notably influence plant productivity or, results have been so varied among species, that a general conclusion could not be made to support or refute homesite advantage (Carter & Blair, 2013; Leimu & Fischer,

2008; Hereford, 2009). Furthermore, certain populations have been demonstrated to not be specifically adapted to their own home environment (Rapson & Wilson, 1988; Gómez et al. 2009). Aspects such as biomass production and attractiveness to local pollinators, which can contribute to fitness of a plant in an environment, can be found to be no better or even worse in a home environment than when transplanted into a foreign environment—invasive species have proven this fact. Populations that are more fit in foreign environments than their home environments could even be considered maladapted to their home environment. Crespi (2000) outlined a definition of maladaptation as the prevalence of an expressed trait in a population which does not support the highest possible fitness in its environment. If an individual or population contains a trait which allows it to acclimate well to new environments, it may be as successful or even more so than in its original environment (Clausen, Keck, & Kiesey, 1940; Havens et al. 2015). With such conflicting results from studies designed to prove or disprove homesite advantage, the concept should not be used to advocate for the use of local seed only, especially for species and populations that have not yet been adequately studied.

Protection of population-specific traits and locally adapted genes is another factor considered when choosing seed source. Advocates of the "Local is best" approach place high priority in the prevention of outbreeding depression, or the reduction in fitness of offspring due to the parents being genetically dissimilar (Heiser & Shaw, 2006). For example, if a population has a specific set of alleles that are well-suited to their home environment and foreign alleles were to be introduced, these new alleles may take place of the locally adapted alleles and disrupt the ecotype's fitness (Templeton, 1986).

While outbreeding is a concern when using nonlocal sources, there is also the potential for inbreeding depression, the eventual loss of fitness due to related individuals in a population

mating amongst themselves. Genetic diversity within and in proximity of a restoration site (the local area) has likely been limited by long-term habitat fragmentation and loss. This loss of diversity in alleles can result in a lower fitness due to increasing homozygosity in genotypes, and, in turn, deleterious recessive alleles which are expressed in homozygous recessive individuals, as reviewed by Charlesworth & Charlesworth (1999). For sites such as these, avoidance of inbreeding depression and encouragement of outbreeding enhancement (increased fitness in hybrid offspring which lessens prevalence of homozygous recessive individuals) would be accomplished by sourcing seed from nonlocal populations containing novel and beneficial alleles.

Care must be taken, however, to avoid the assumption that a small population is automatically inbred. There have been populations that were assumed to be inbred due to their size and distance from other populations that did in fact house high amounts of genetic diversity (Gustafson, Gibson, & Nickrent, 2004b; Mutegi et al. 2014). The risk of both inbreeding and outbreeding must be balanced and investigated for each species and even population, as traits such as pollination vector, self-compatibility, and ploidy (the number of chromosome sets in an individual that can vary across populations) can affect population dynamics (Wagenius et al., 2009; Durka et al. 2017).

Introducing beneficial alleles to populations to combat climate change is a strong argument made by the “Diversity is best” group. The Intergovernmental Panel on Climate Change (2021) reports temperatures are warming, precipitation patterns are changing, and atmospheric carbon dioxide levels are rising. Populations that have already been reduced by anthropological activity are especially at risk for extinction due to lessened ability to adapt to these changing conditions (Rice & Emery, 2003; Hereford, 2009). This lessened ability to adapt

to changes, or plasticity, is directly connected to the loss of genetic diversity in the population. The use of nonlocal sourcing in restoration sites is an opportunity to not only aid in increasing genetic diversity, but actively select source material containing traits such as heat tolerance to increase the sustainability of the population (Harris et al., 2006). Yet this does not necessarily mean that no local seed should be used in restoration sites. Many of the "Diversity is best" authors cited call for a focus on incorporating genetic diversity-preserving methods into both local and nonlocal seed collection protocols—collecting from individuals across the entire source area rather than only the easy-to-reach portions, for instance. The captured traits from these methods may give populations the tools they need to adapt to changing climates.

Finally, the large-scale impact to the ecosystem must be kept in mind when sourcing seed. The "Local is best" approach attempts to alleviate any potentially negative effects of nonlocal ecotypes or cultivars on the ecosystem as a whole. Nonlocal genotypes may have increased vigor which could crowd out native ecotypes or other species. They could also have different bloom times that impact concurrent insect and bird life cycles, and may be less fit as a host and food source by the native fauna (Vander Mijnsbrugge, Bischoff, & Smith, 2010; Bucharova et al. 2016). Studies documenting these possible negative effects are limited, so these concerns are hypothetical for the most part, though no less vital to consider in the restoration process. Further research must be undertaken to form a more complete understanding of nonlocal ecotypes and cultivars in restoration ecosystems.

A common misconception is that the goal of a restoration is to return a damaged or destroyed ecosystem to its exact state before disturbance. However, this is almost impossible and, with global climate and biotic stressors rapidly changing, impractical. The return to a working, sustainable ecosystem with the ability to adapt is a preferable and much more practical

goal (Society for Ecological Restoration, 2004). When supplementing the resident plant population, the objective should be to find a balance of fitness and genetic diversity, to find a happy medium between the “Local is best” and “Diversity is best” ideologies.

Seed Germination

Once seed has been identified and obtained, a common obstacle in the restoration process is germination rate of that seed. Whether direct sowing into a field or being started in a greenhouse and transplanted, if germination rates are low, the restoration project may struggle. Low germination rates may be caused by a range of factors, but a factor that can be manipulated is seed dormancy and the breaking thereof. It is proposed that seed dormancy is an evolutionary trait to allow a seed to survive long periods of unfavorable germination conditions (Bentsink & Koornneef, 2008). However, a dormant seed is defined as a viable seed that does not germinate under usually favorable conditions for its germination (Baskin and Baskin, 2004; Loch et al., 2004).

The mechanisms of dormancy are not fully understood, but there are several main descriptions of dormancy that have been defined and categorized. Adkins, Bellairs, and Loch (2002) separated the mechanisms of dormancy into those that are outside the embryo (such as the seed coat) and within the embryo. The mechanisms in the seed coat, as outlined by Adkins, Bellairs, and Loch (2002), are the physical constriction of the embryo to prevent growth, a barrier against gas exchange and water infiltration, and the presence of chemical germination inhibitors within tissues surrounding the seed coat. There is much less understanding of mechanisms of dormancy within the embryo itself, but most hypotheses include the role of chemicals including gibberellic acid, other plant growth regulators, and certain nitrogen-containing compounds (Adkins, Bellairs, and Loch, 2002). Baskin and Baskin (2004)

recommended a different categorization system of seed dormancy, separating mechanisms of dormancy not by location but by type: physiological (overcome by temperature), morphological (overcome by time), physical (overcome by disruption of the seed coat), and combinations among the three.

Native seeds dormancy is often an obstacle in restoration projects due to two main factors. The first is that, while commercial seed producers often provide generalized germination instructions to consumers, there is often a lack of species-specific protocols that might increase germination rates. Secondly, most native seeds, with the exception of cultivars, have not been domesticated to germinate as easily as crop species. Therefore, wild type native seeds are likely to have lower germination rates or higher degrees of seed dormancy than developed cultivars (Schröder & Prasse, 2013).

In order to increase germination rates, a variety of seed pretreatments may be used to break the dormancy of the seed. The two main categories of seed pretreatments are stratification and scarification. Scarification is generally defined as breaking physical dormancy, where water permeability of the seed coat is addressed, while stratification is generally defined as breaking a type of physiological dormancy which is not well understood in all species (Knapp, 2000; Baskin & Baskin, 2004; Loch et al., 2004).

Stratification is broadly defined as a pretreatment which replicates temperature and moisture conditions in which the seed is naturally dormant throughout, thus fulfilling physiological requirements for germination after the inconducive conditions are over (Baskin & Baskin, 2004). When broadly applied to North American prairie restoration, the dormant conditions occur during winter, and so cold stratification is used.

Two approaches in cold stratification are dry and moist stratification. Dry stratification, as the name suggests, keeps seed cold and free of moisture while moist stratification involves keeping the seed cold in wetted media such as sand, vermiculite, or paper throughout the chilling period (Nuzzo, 1976; Eckberg et al., 2015). It is likely that species/varieties that are adapted to wetter environments react better to moist stratification, and species/varieties that are adapted to drier environments react better to dry stratification.

There are several types of commonly used scarification methods. Mechanical scarification refers to physical damage of the seed coat to allow moisture and air to permeate the seed; this is typically accomplished by rubbing the seed with sandpaper, nicking with a sharp instrument, or processing through a thresher/other mechanical implement (Jensen & Boe, 1991; Kaye & Kuykendall, 2001; Deng et al., 2021). Thermal scarification weakens the seed coat with heat, most often in an oven/hot air chamber or by soaking seed in hot water (Valbuena & Vera, 2002; Ren & Tao, 2004). Chemical scarification damages the seed coat with caustic chemicals such as strong acids or bases. Kindinger (1994) found promising results with *Tripsacum dactyloides* (eastern gamagrass) soaking in 30% hydrogen peroxide for 2 hours. Hydrogen peroxide has also been used to surface sterilize seeds at lower concentrations such as 3-5% (Walker & Erdman, 1926; Landis, 1998). However, further research is required for specific time amounts, concentrations, and species.

Gibberellic acid stimulation may also be used as a hormonal pretreatment separately from stratification or scarification, from concentrations anywhere from 10 to 1,000 ppm (Chisha-Kasumu, Woodward, & Price, 2007; Su et al., 2011). While the mechanism of gibberellic acid's effect is unknown, some have speculated that gibberellic acid treatment may encourage the

movement of stored food reserves and advance phytochrome responses to light, thus beginning the early stages of germination (Thomas, 1992; Plummer & Bell, 1995; Loch et al., 2004).

Commercial seed producers often provide generalized germination instructions to consumers, but species-specific protocols could increase germination rates and therefore increase the chance of success at a restoration site. Overall, the two goals of this research are to provide insight on seed sourcing and germination protocols for species that are native to remnant prairies in Arkansas. There are large gaps in the literature of species-specific research regarding both topics, and results are often conflicting.

The objective of the seed sourcing experiment was to determine the effect of seed source in restoration and establishment projects, and to identify an appropriate balance of locality and diversity. The hypothesis was that seed source effect would differ among species: i.e. seed source would have significant effects in one species but not another.

The objective of the germination experiments was to determine if scarification and stratification methods could improve germination of native prairie species. It was hypothesized that pretreatment would have a significant effect on germination rates, and that the most effective pretreatment would differ among species.

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Determining the Effect of Seed Source on Native Prairie Grasses

Abstract

American tallgrass prairie has faced losses estimated at 87-98% of original land area since European settlement. Native seeds are often used to supplement struggling or extinct plant populations in tallgrass prairie restoration and establishment sites. In order to determine the effect of seed source, a common garden experiment was established in Fayetteville, Arkansas in 2022 with five prairie species: *Andropogon gerardii* (big bluestem), *Bouteloua curtipendula* (sideoats grama), *Panicum virgatum* (switchgrass), *Schizachyrium scoparium* (little bluestem), and *Sorghastrum nutans* (Indiangrass). Seeds were sourced from Oklahoma, Kentucky, Illinois, South Dakota, North Dakota, Minnesota, and Ontario, Canada. These sources represented plant material from a variety of latitudinal distances from the common garden. Differences in date of anthesis of first culm, mature height of tallest growing point, annual aboveground biomass production, and annual seed production of the individuals were analyzed among sources. Preliminary conclusions were that seed source would likely not affect success of a restoration or establishment, as there were very few significant differences in the measured characteristics among sources. In only one instance did a closer source have significantly higher measurements than the further source; in *Bouteloua curtipendula*, individuals from Oklahoma grew significantly taller with more aboveground biomass than individuals from North and South Dakota (and produced more aboveground biomass than individuals from Ontario, Canada).

Introduction

American tallgrass prairie has faced losses estimated at 87-98% of original land area since European settlement. Native seeds are often used to supplement struggling or extinct plant populations in tallgrass prairie restoration and establishment sites. Seed source refers to the origin of the seed sown at a restoration site. While it may seem reasonable to obtain or collect seed from a different population where seed is readily available, regardless of its location, this may not always be the best option in a restoration project. Populations may differ greatly and may not be suited to establishment in a new site (McKay et al., 2005).

Within a species, there are many differing genetic traits such as bloom time, vigor, and environmental adaptations that can influence suitability of a population in various regions. These traits originate from mutations which travel within and across populations over time via gene flow, recombination, genetic drift, and natural selection (McKay et al., 2005). Due to these mechanisms, species naturally differentiate into ecotypes, which are distinct genotypes based on local adaptation as defined by Hufford and Mazer (2003). If desirable traits are artificially selected and bred into stable genotypes by humans, they are considered cultivars. It is important to recognize these differences within species, especially when considering seed sources or cultivars that may have traits poorly suited to the environment of the restoration site.

Environment—biotic and abiotic—plays a defining role in the creation of distinct ecotypes. Climate may be the first aspect to come to mind, but there is a litany of other environmental factors to which plants may adapt. Linhart and Grant (1996) reviewed research on plant adaption to soil composition, elevation, moisture, light availability, and competition with other plants as well as biotic interactions including pollination vectors, predators, and parasites,

concluding that all these factors are important in local adaptation, and therefore development of ecotypes.

Commonly referred to as local seed sourcing, the use of local ecotypes is a strategy for restoration efforts based on the assumption that populations found within a certain geographic distance from the restoration site are more appropriate for sourcing seed. Further improving this idea to incorporate climate and environment inspired the concept of seed transfer zones (STZs). These zones are defined as areas within which plant materials can be transplanted while lowering risk of being poorly suited to the destination site, and are usually most applicable when species-specific research has been applied. These zones are defined as areas within which plant materials can be transplanted while lowering risk of being poorly suited to the destination site, and are usually most applicable when species-specific research has been applied (Hufford & Mazer, 2003).

However, the issue with local sourcing is that there is no commonly-agreed upon definition for "local". There are not STZs created for every species, and a set geographic distance or general-purpose STZs cannot be relied upon to provide accurate source guidelines in most cases (Aitken & Whitlock, 2013). Further complicating the issue, while the existence of ecotypes is indisputable, researchers tend to either encourage (McKay et al., 2005; Vander Mijnsbrugge, Bischoff, & Smith, 2010) or discourage (Broadhurst et al., 2008; Herman et al., 2014) the use of local ecotypes in restoration. For the purposes of this review, these two opinions will be referred to as the "Local is best" and "Diversity is best" groups.

Restoration of an ecosystem is an infinitely complex task, with the decision of where to source seed no less complicated an undertaking. Effects of local or nonlocal sourcing may be found from the success of the individual plant to the success of the restoration site ecosystem.

These effects are commonly discussed and researched by the two sides of the issue and include fitness (the ability to survive and reproduce) of individuals, inbreeding and outbreeding at the population level, and population response to climate change.

The success of outsourced individuals is the cornerstone of a restoration effort. If the introduced plant material does not survive or reproduce, then further steps and goals in the restoration such as persistence of the population, slowing spread of invasive species, and reintroduction of animal life may not be accomplished. In relation to individual ability to survive and reproduce, the "Local is best" opinion often values the concept of a "homesite advantage", which simply suggests that plants have a higher fitness in the environment where they have adapted than in a foreign environment. There have been data supporting homesite advantage, including recent publications by Montalvo and Ellstrand (2000), Schultz et al. (2001), Gustafson, Gibson, and Nickrent (2004a), and Wilson et al. (2016). The conclusions from these studies give rise to the concern that seed from nonlocal sources may not establish well in the restoration site.

Protection of population-specific traits and locally adapted genes is another factor considered when choosing seed source. Advocates of the "Local is best" approach place high priority in the prevention of outbreeding depression, or the reduction in fitness of offspring due to the parents being genetically dissimilar (Heiser & Shaw, 2006). While outbreeding is a concern for using nonlocal sources, there is also the potential for inbreeding depression, the eventual loss of fitness due to related individuals in a population mating amongst themselves.

Introducing beneficial alleles to populations to combat climate change is a strong argument made by the "Diversity is best" group. The Intergovernmental Panel on Climate Change (2021) unrefutably reports temperatures are warming, precipitation patterns are changing, and atmospheric carbon dioxide levels are rising. Populations that have already been

reduced by anthropological activity are especially at risk for extinction due to lessened ability to adapt to these changing conditions (Rice & Emery, 2003; Hereford, 2009). Yet this does not necessarily mean that no local seed should be used in restoration sites. Many of the "Diversity is best" authors cited thus far instead call for a focus on incorporating genetic diversity-preserving methods into both local and nonlocal seed collection protocols—collecting from individuals across the entire source area rather than only the easy-to-reach portions, for instance. The captured traits from these methods may give populations the tools they need to prepare for adaptation to changing climates.

A common misconception found in the restoration community is that the goal of a restoration is to return a damaged or destroyed ecosystem to its exact state before disturbance. However, this is almost impossible and, with global climate and biotic stressors rapidly changing, impractical. The return to a working, sustainable ecosystem with the ability to adapt is a preferable and much more practical goal (Society for Ecological Restoration, 2004). When supplementing the resident plant population, the objective should be to find a balance of fitness and genetic diversity, to find a happy medium between the "Local is best" and "Diversity is best" ideologies.

The overall goal of this research was to determine, for each of the species in the study, if seed source affected an individual's success in a restoration or establishment site. A common garden experiment was established for this purpose, where individuals from various sources were grown in the same environment (climate, photoperiod, and cultivation). If seed source determines plant growth and success in a population, the individuals of certain sources were expected to differ from other sources. However, if seed source was not important, all individuals

of the same species were expected to grow similarly. The overarching hypothesis of the experiment was that the effect of seed source would differ among species.

Materials and Methods

The basis of the experiment was a common garden study, where species varieties (ecotype, cultivar, etc.) were compared in a common site, where other variables such as nutrient availability, stressors, climate, photoperiod, and cultivation method were uniform. Therefore, any differences among sources could be attributed to the source itself rather than the growing conditions.

In the fall of 2020, seeds were collected from remnant Arkansas tallgrass prairies for a common garden study. The species of interest included two grasses, big bluestem (*Andropogon gerardii* Vitman) *and* Indiangrass (*Sorghastrum nutans* [L.] Nash) and two forbs, rattlesnake master (*Eryngium yuccifolium* L.) and prairie blazingstar (*Liatris pycnostachya* Michx.). Seeds were collected from four Arkansas prairies including Chesney Prairie (Benton County, 36.2187, -94.4821), Cherokee Prairie (Franklin County, 35.3356, -94.0385), Railroad Prairie (Prairie County, 34.7822, -91.7194), and Downs Prairie (Prairie County, 34.7827, -91.4965). However, several complications arose in the original study, including the discovery that some of the prairies had been seeded with foreign seed, which could have compromised the "source" assumptions. A wet spring in 2021 prevented planting until summer, and an extremely low rate of germination was observed for all species involved (data not shown), so a limited number of plants were available to adequately establish the common garden. Eventually, it was decided to completely restart the common garden in 2022, and attempt to address these issues.

For the 2022 trial, five grass species were included in the common garden study and up to six seed sources were tested for each species (Table 1, Figures 1-5): big bluestem (*Andropogon*

gerardii), sideoats grama (*Bouteloua curtipendula* [Michx.] Torr.), switchgrass (*Panicum virgatum* L.), little bluestem (*Schizachyrium scoparium* [Michx.] Nash), and Indiangrass (*Sorghastrum nutans*). Originally, there was also a selection of forb species in the study, reflecting those found in the germination chapter of this thesis. However, the forb individuals for the common garden study either did not germinate or survive the transplant to the field in large enough quantities to be considered for the final data analysis.

Seed were obtained from the target sources in 2021 and early 2022 with written confirmation about where the population was originally found and collected. Seeds were stored in an environmentally-controlled laboratory (average temperature = 20-22 °C) until being sown in germination pouches (CYG™ Germination Pouch, Mega International, Roseville, MN) in April of 2022. The germination pouches allowed for uniform moisture conditions throughout the germination period. The seeds were placed in a walk-in growth chamber (Model PGW36, Conviron, Winnipeg, Manitoba, Canada) set at 30 °C day and 20 °C night for 8-hour day and 16-hour night cycles, respectively, with approximately 450 μmol m^{-2} s^{-1} of light provided in the day cycle (AOSA Rules for Testing Seeds, 2016). After approximately three weeks, multiple seedlings of each source were transplanted into 10 cm diameter pots filled with a commercial potting media (PRO-MIX, FLX, Quakertown, PA). The seedlings were then placed in a greenhouse with an average of 60% relative humidity, 26 °C day temperature, and 23 °C night temperature until transplanting to the field on 14 June 2022.

The location of the trial was the Milo J. Shult Agricultural Research & Extension Center in Fayetteville, Arkansas (36.099760, -94.169291). The predominant soil type is a Captina silt loam (fine-silty, siliceous, active, mesic Typic Fragiudults) with an average pH of 6.2 based on the USDA Web Soil Survey (www.websoilsurvey.sc.egov.usda.gov). The plots were prepared by

tilling to a depth of 12-15 cm and then incorporating a slow-release nitrogen fertilizer (6-0-2, Milorganite, Milwaukee, WI) at approximately 700 kg ha^{-1}.

Since comparison of species was not of interest in this study, each species was established as a separate plot to minimize competition between species. As such, the common garden consisted of five species-specific plots. Within each species plot, seed source was considered the independent variable and five replications of each source were established in a completely randomized design within each species plot. At the time of planting, five seedlings were randomly selected from each seed source to avoid only selecting the most fit individuals in the population. The selected seedlings were then transplanted to the common garden plots, with individuals spaced 0.9 meters apart. A diagram of the general layout of the entire common garden can be found in Figure 6.

The plants were hand-watered daily (approximately 3 liters per plant, though amount varied according to size of plant and water penetration through soil) for approximately a month except during rainfall events. Black heavy duty weed barrier fabric (Lab Work Auto Parts, Chino, CA) was placed around the plants to reduce weed competition. In September 2022, the landscape fabric was removed to allow for further growth of the plants, while the plots were weeded by hand afterward.

Effect of seed source was assessed by collecting data on anthesis date, height of tallest/longest growing point at maturity, annual seed production, and annual aboveground biomass production. Starting in August 2022, the plots were checked weekly and the first date of anthesis was recorded for each individual, with anthesis defined as the appearance of the first flowers. At the end of November 2022, maximum height/length was recorded for each plant, including culms that were collapsed onto the ground. Seed and aboveground biomass were

harvested at the end of November 2022 as well. Seed and biomass were harvested and processed by hand before being dried at 70˚C for 48 hours and weighed separately (Bucharova et al., 2017).

All statistical analyses and resulting graphs were completed using R statistical software (R Core Team, 2021) and RStudio (RStudio Team, 2022), as well as the packages discussed in the Appendix. Differences in the week of anthesis, tallest growing point, seed production, and biomass production among sources were compared within species using a one-way analysis of variance (ANOVA) test. In preliminary analysis of the data, type I and type III ANOVA tests both output the same results for every comparison of measurements despite occasions of unbalanced data, and therefore type I was used for all further analyses. A significant difference would support the hypothesis that source did affect restoration efforts. If there was a significant difference, Tukey's honestly significant difference (HSD) test was utilized to determine which sources were different. Assumptions of the ANOVA test, homogeneity of variables and normal distribution, were confirmed by plotting a histogram of residuals and Bartlett's test, respectively. If either assumption was violated, then a nonparametric ANOVA test called the Kruskal-Wallis test by ranks was utilized instead of the standard ANOVA test. Dunn's test for multiple comparisons would then be used to determine pretreatments with significantly different results.

While statistics were run on the first year data, the results from this trial should not be fully considered until data from a second year is collected.

Results and Discussion

Assumptions of the ANOVA test were assessed for all data to determine if a standard ANOVA test or the Kruskal-Wallis test would be used for analysis (Table 2).

For *A*. *gerardii*, there were no significant differences among sources in all four measurements (Figure 7). In *B*. *curtipendula*, there were no significant differences in anthesis

date and seed production. However, Roundstone Seed was significantly taller than Minnesota Native Landscapes and the Plant Materials Center while also producing significantly more aboveground biomass than all other sources (Figure 8). The source of Roundstone Seed was about 350 km from the common garden while the PMC came from a source 1,060 km away and Minnesota Native Landscapes came from a source 1,100 km away. Within *P. virgatum*, all measurements but aboveground biomass production technically had significant differences, but the p-value was so close to 0.05 in anthesis date (0.049) and seed production (0.042) that Dunn's test could not determine which source was significantly different. In height, Wildflower Farm (source 1,560 km away) and Roundstone Seed (source 560 km away) were significantly taller than the PMC B (source 1,270 km away) (Figure 9). The data for *S. scoparium* contained no significant differences in any of the four measurements (Figure 10). While there were no significant differences in biomass or seed production in *S. nutans*, Minnesota Native Landscapes (source 1,020 km away) had a significantly earlier anthesis date than Roundstone Seed (source 740 km away), and Wildflower Farm (source 1,560 km away) was significantly taller than the PMC (source 1,170 km away) (Figure 11).

Straight-line distance between source and the common garden is not a perfect metric to use when investigating the effect of sourcing, especially when, for example, ecoregions, photoperiods, and climate are taken into consideration. However, the separate effect of ecoregion, photoperiod, and climate and other possible factors of seed source are not well-researched, and this study has been an overview of all these factors together. With further species-specific studies focusing on each factor of seed sourcing, a better understanding of seed sourcing as a whole and even an equation weighing certain factors more or less heavily may later be developed.

Therefore, straight-line distance may be used an approximation where in-depth analyses may not be possible at the current time. With that in mind, *B. curtipendula* was the only species where individuals from the closest source (Roundstone Seed at 350 km away) grew significantly taller than individuals from further sources (the Plant Materials Center at 1,090 km and Minnesota Native Landscapes at 1,360 km) while producing significantly more aboveground biomass than all sources, including those previously mentioned and Wildflower Farm at 1,550 km.

Some limitations of the experiment were the relatively small sample size, with only five individuals (sometimes less) representing each source, and the fact that only one year of data was collected. An additional possible limitation was the occurrence of stalks being completely broken off some plants throughout the growing season due to heavy winds and rains, resulting in aboveground biomass and seed weight measurements to represent less than the actual total.

Conclusion

The preliminary conclusions that may be drawn at the end of the first year of the study are that seed source does not have a great effect on individual success of native plants in restoration or establishment cites. However, there is further research that must be pursued, both to collect more data on the current common garden, create a better metric for differences among sources, and also to create more common gardens in different locations with different species and sources.

Furthermore, a better measurement of success at both the individual level and population level beyond individual anthesis date, height, biomass production, and seed production would benefit future research.

Tables and Figures

Table 1. Summary of individuals used in the 2022 common garden study. While production source denotes where the seed was purchased/received from, source material origin refers to the location where the plant material was found before human interference. The coordinates of the approximate center of the reported source city/county were used, with distance rounded to the nearest tens of kilometers to reflect this uncertainty. If multiple counties/states were listed, the approximate center of all locations was used.

Species and common name	Production Source	Cultivar	Source Material Origin	Source Material Latitude	Source Material Longitude	Approximate Distance (km)
Andropogon gerardii Vitman Big bluestem	Roundstone Seed		LaRue County, KY	37.5739	-85.7400	770
	Bismarck, ND Plant Materials Center A	'Bonilla'	Beadle County, SD	44.5898	-98.5062	1,010
	Bismarck, ND Plant Materials Center B	'Bison'	Oliver County, ND	47.0858	-100.9415	1,340
	Wildflower Farm		Coldwater, Ontario, Canada	44.6542	-79.5590	1,560
Bouteloua curtipendula (Michx.) Torr. Sideoats grama	Roundstone Seed	'El Reno'	Canadian County, OK	35.5323	-97.9550	350
	Bismarck, ND Plant Materials Center	'Pierre'	Stanley County, SD	44.3668	-100.3538	1,060
	Minnesota Native Landscapes		Douglas County, MN	45.9427	-95.4591	1,100
	Wildflower Farm		Coldwater, Ontario, Canada	44.6542	-79.5590	1,560
Panicum virgatum L. Switchgrass	Roundstone Seed	'Cave-in-Rock'	Hardin County, IL	37.4692	-88.1653	560
	Minnesota Native Landscapes		Houston County, MN	43.6749	-91.4892	870

Table 1. (cont.)

Species and common name	Production Source	Cultivar	Source Material Origin	Source Material Latitude	Source Material Longitude	Approximate Distance (km)
Switchgrass (cont.)	Bismarck, ND Plant Materials Center A	'Forestburg'	Sanborn County, SD	44.0222	-98.1081	940
	Bismarck, ND Plant Materials Center B	'Dacotah'	Morton County, ND	46.3800	-100.9424	1,270
	Wildflower Farm		Coldwater, Ontario, Canada	44.6542	-79.5590	1,560
Schizachyrium scoparium (Michx.) Nash Little bluestem	Bismarck, ND Plant Materials Center A	'Badlands Ecotype'	Badlands, SD	45.3697	-102.4896	1,240
	Bismarck, ND Plant Materials Center B	'Itasca'	ND, SD, & MN	47.0232	-98.1250	1,260
	Wildflower Farm		Coldwater, Ontario, Canada	44.6542	-79.5590	1,560
Sorghastrum nutans (L.) Nash Indiangrass	Roundstone Seed		Hart County, KY	37.3742	-85.9872	740
	Minnesota Native Landscapes		Benton, Sherburne, Mille Lacs, & McLeod County, MN	45.3032	-93.5547	1,020
	Bismarck, ND Plant Materials Center	'Tomahawk'	Dickey County, ND; Marshall County, SD; & Brown County, SD	46.1972	-98.3575	1,170
	Wildflower Farm		Coldwater, Ontario, Canada	44.6542	-79.5590	1,560

Table 2. For the common garden study, assumptions of the standard ANOVA test were confirmed by plotting a histogram of residuals and Bartlett's test. If the histogram did not show approximately normal distribution or Bartlett's test showed that the assumption of equal variances was violated, then a nonparametric Kruskal-Wallis test by ranks was utilized instead of the standard ANOVA test. For each test, p-values are reported.

Species	Standard	Kruskal-Wallis
	p-value	
Andropogon gerardii		
Anthesis		0.075
Height	0.089	
Biomass	0.075	
Seed	0.155	
Bouteloua curtipendula		
Anthesis		0.062
Height	0.001	
Biomass	<0.001	
Seed		0.058
Panicum virgatum		
Anthesis		0.049
Height	0.011	
Biomass		0.052
Seed		0.042
Schizachyrium scoparium		
Anthesis		0.601
Height	0.632	
Biomass		0.090
Seed	0.346	
Sorghastrum nutans		
Anthesis		0.039
Height	0.012	
Biomass	0.138	
Seed		0.118

Figure 1. Map detailing source material locations for *Andropogon gerardii* (big bluestem) in the 2022 common garden study. The name of each source denotes the production source where the seed was purchased/received from while the location denotes the origin source material where the plant material was found before human interference. If multiple counties/states were listed as source material, the approximate center of all locations was used. Coordinates were found and placed using the Google My Maps feature at www.google.com/maps/d/u/0/.

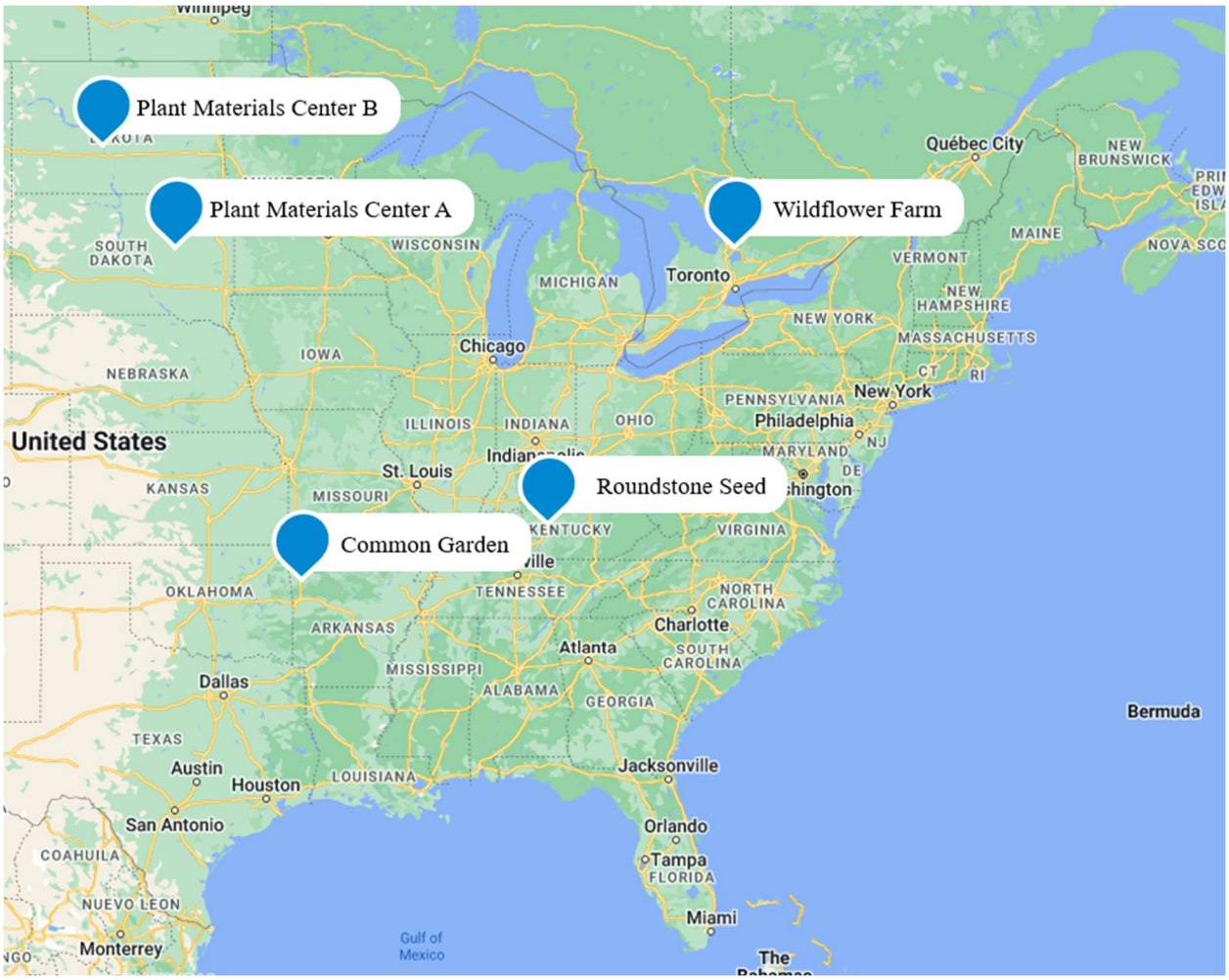


Figure 2. Map detailing source material locations for *Bouteloua curtipendula* (sideoats grama) in the 2022 common garden study. The name of each source denotes the production source where the seed was purchased/received from while the location denotes the origin source material where the plant material was found before human interference. If multiple counties/states were listed as source material, the approximate center of all locations was used. Coordinates were found and placed using the Google My Maps feature at www.google.com/maps/d/u/0/.

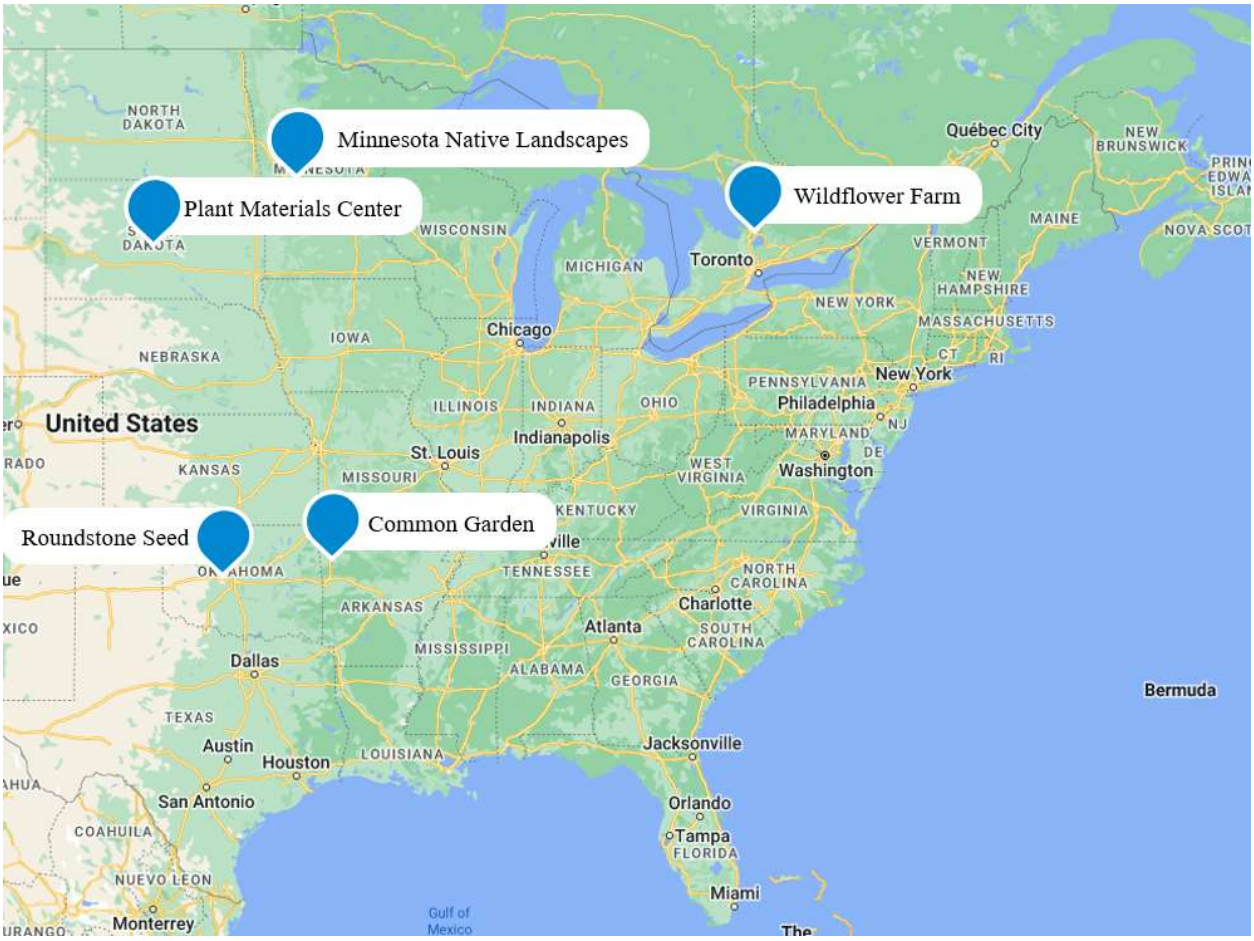


Figure 3. Map detailing source material locations for *Panicum virgatum* (switchgrass) in the 2022 common garden study. The name of each source denotes the production source where the seed was purchased/received from while the location denotes the origin source material where the plant material was found before human interference. If multiple counties/states were listed as source material, the approximate center of all locations was used. Coordinates were found and placed using the Google My Maps feature at www.google.com/maps/d/u/0/.

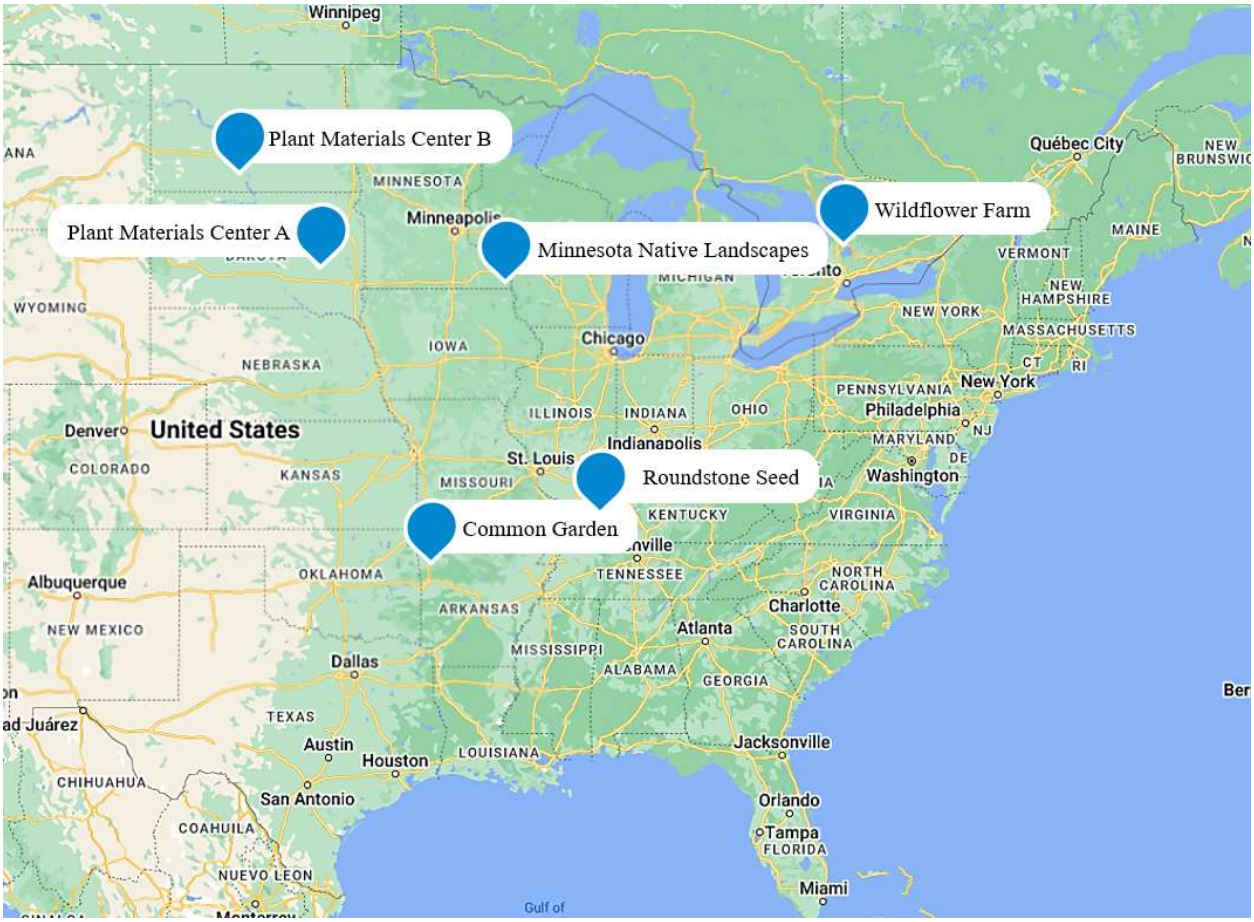


Figure 4. Map detailing source material locations for *Schizachyrium scoparium* (little bluestem) in the 2022 common garden study. The name of each source denotes the production source where the seed was purchased/received from while the location denotes the origin source material where the plant material was found before human interference. If multiple counties/states were listed as source material, the approximate center of all locations was used. Coordinates were found and placed using the Google My Maps feature at www.google.com/maps/d/u/0/.

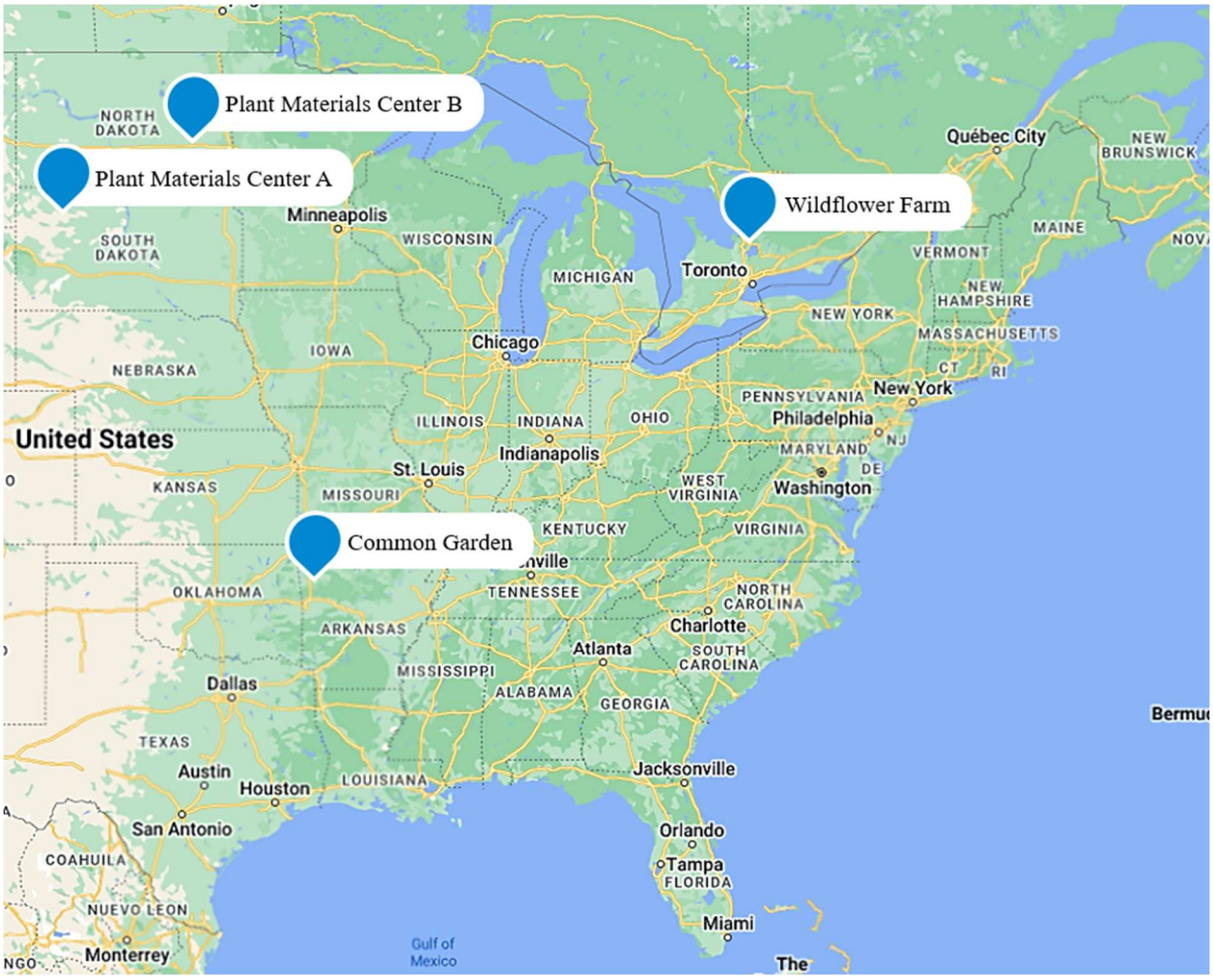


Figure 5. Map detailing source material locations for *Sorghastrum nutans* (Indiangrass) in the 2022 common garden study. The name of each source denotes the production source where the seed was purchased/received from while the location denotes the origin source material where the plant material was found before human interference. If multiple counties/states were listed as source material, the approximate center of all locations was used. Coordinates were found and placed using the Google My Maps feature at www.google.com/maps/d/u/0/.

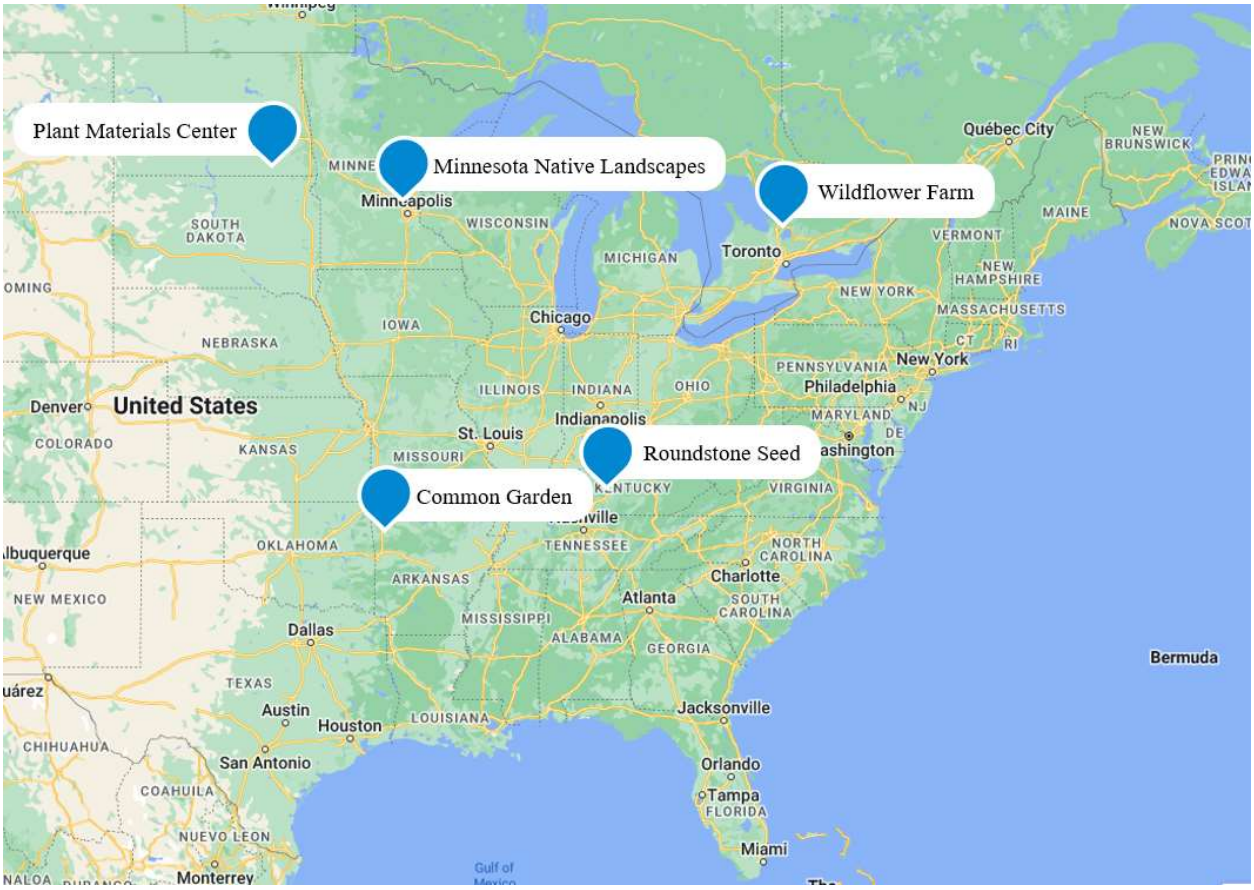


Figure 6. The general layout of the 2022 common garden plots comparing seed source effects. Each square represents an area of 0.91 x 0.91 m^2. Each individual from the denoted source was planted in the center of the square. Blank squares represent where an individual did not survive the transplant process or was removed from the study.

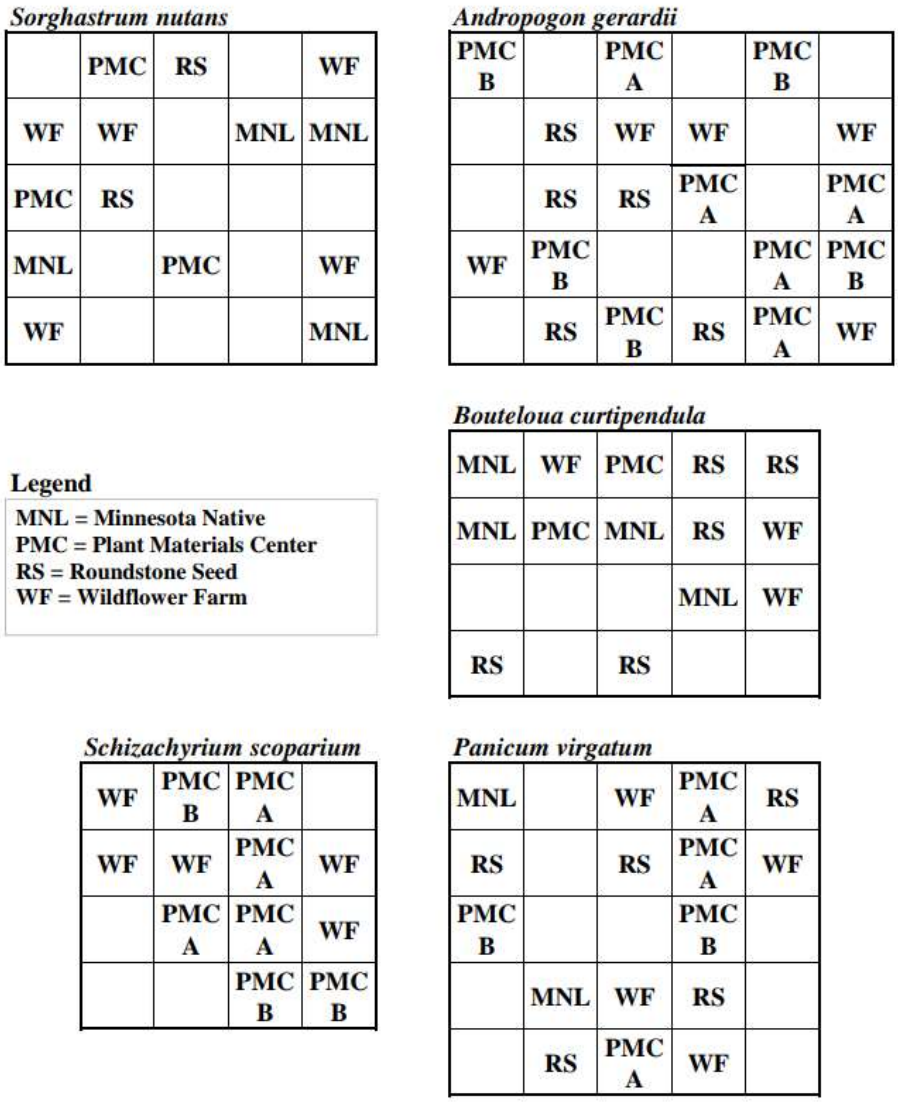


Figure 7. *Andropogon gerardii* (big bluestem) measurements of individual success in the 2022 common garden study, separated by source name. The letter at the top of each bar represents pairwise comparisons of least-squared means using either Tukey's HSD or Dunn's test with p = 0.05. The test used in each plot is indicated on the right side of the graphic. Bars sharing the same letter within each graph are not significantly different. "PMC" is an abbreviation for Plant Materials Center.

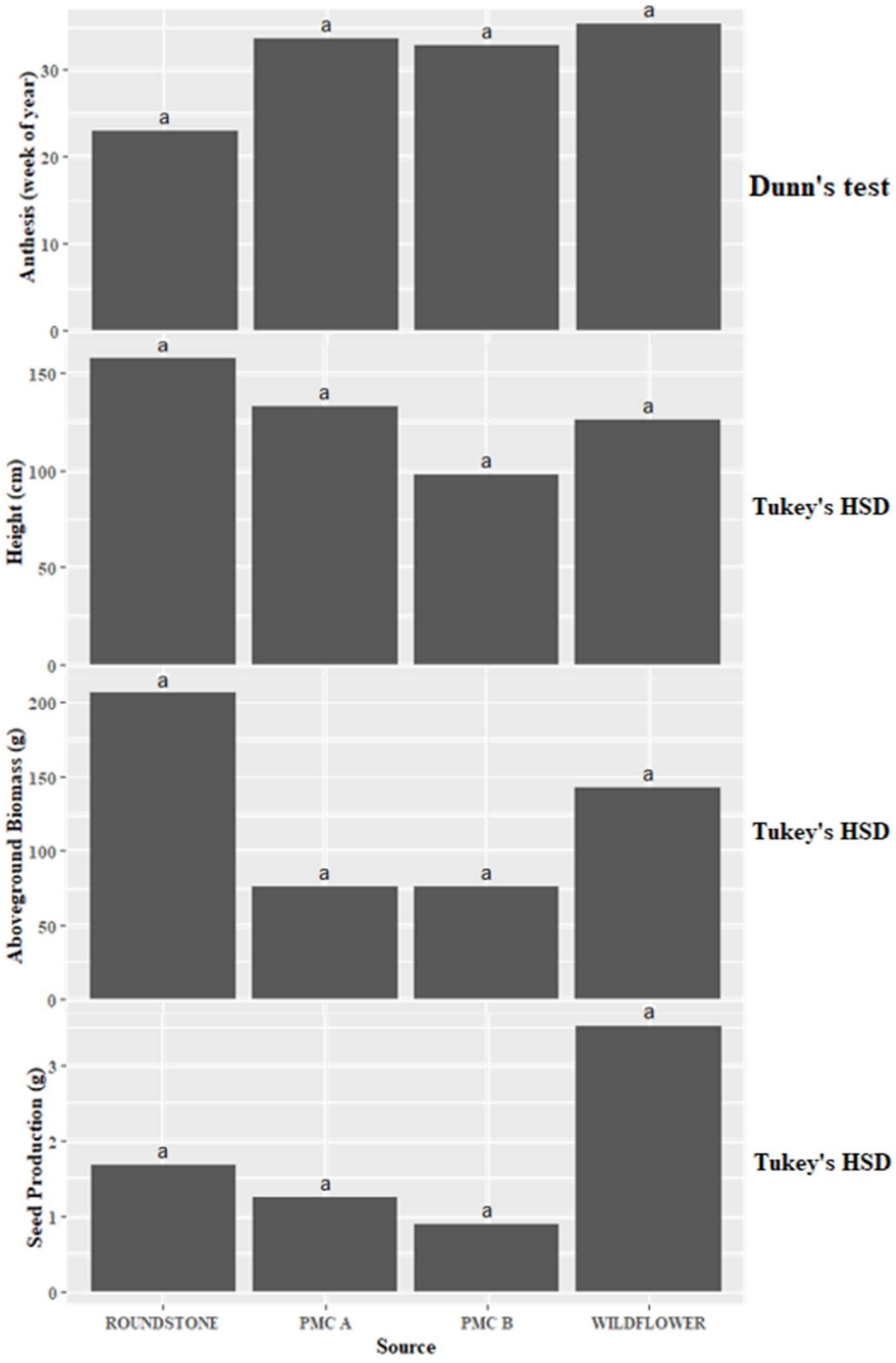


Figure 8. *Bouteloua curtipendula* (sideoats grama) measurements of individual success in the 2022 common garden study, separated by source name. The letter at the top of each bar represents pairwise comparisons of least-squared means using either Tukey's HSD or Dunn's test with p = 0.05. The test used in each plot is indicated on the right side of the graphic. Bars sharing the same letter within each graph are not significantly different. "PMC" is an abbreviation for Plant Materials Center.

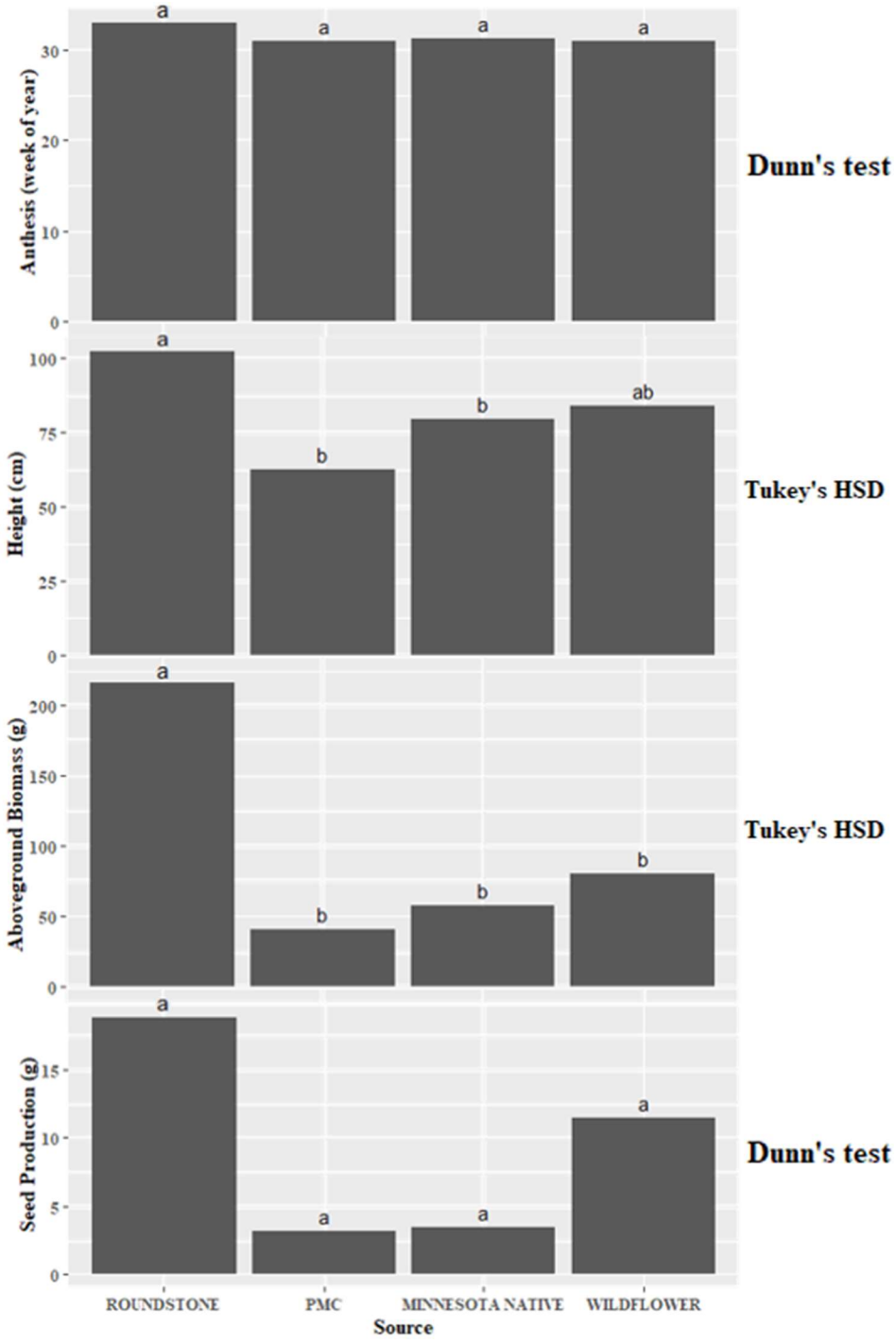


Figure 9. *Panicum virgatum* (switchgrass) measurements of individual success in the 2022 common garden study, separated by source name. The letter at the top of each bar represents pairwise comparisons of least-squared means using either Tukey's HSD or Dunn's test with p = 0.05. The test used in each plot is indicated on the right side of the graphic. Bars sharing the same letter within each graph are not significantly different. "PMC" is an abbreviation for Plant Materials Center.

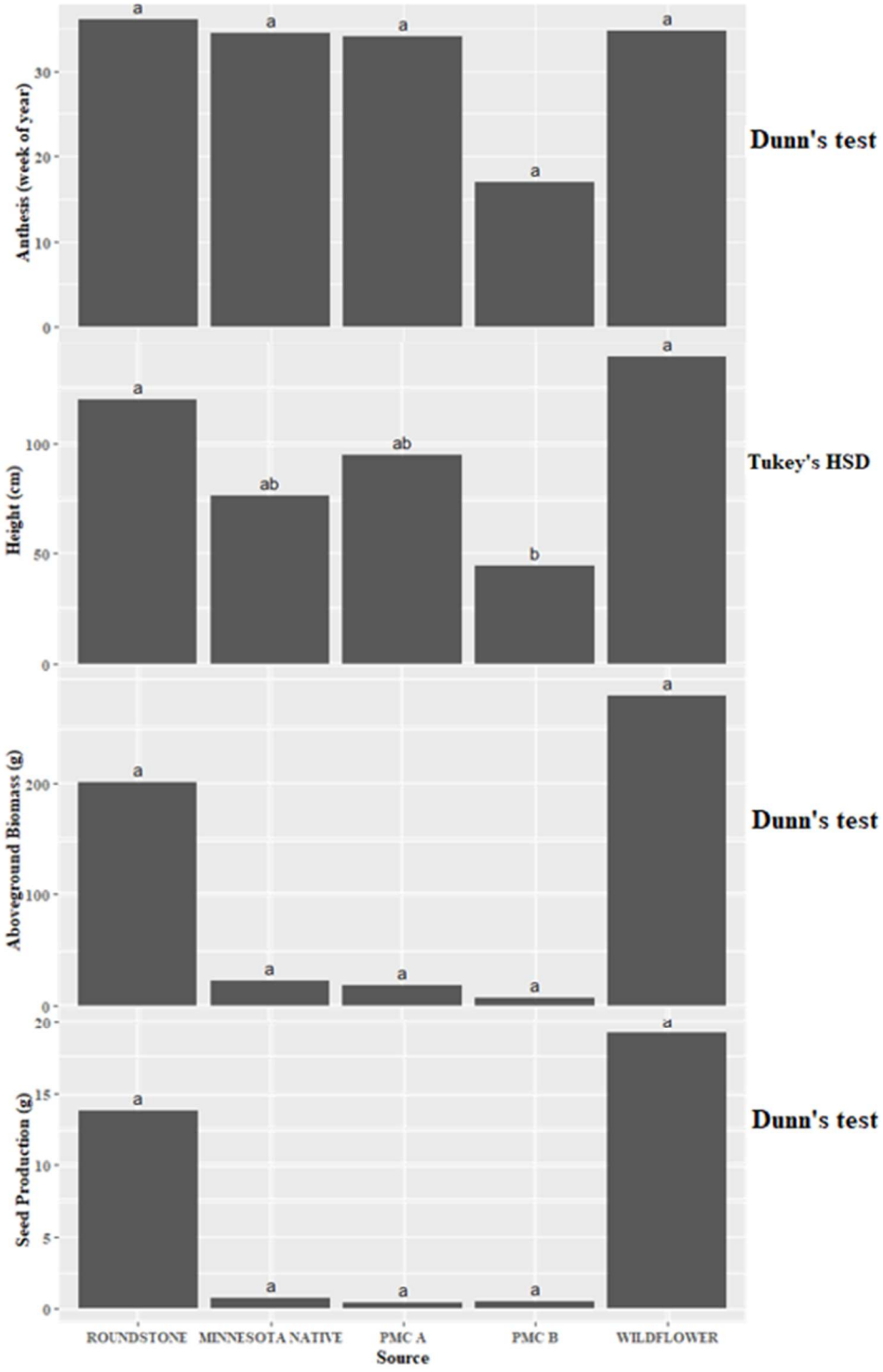


Figure 10. *Schizachyrium scoparium* (little bluestem) measurements of individual success in the 2022 common garden study, separated by source name. The letter at the top of each bar represents pairwise comparisons of least-squared means using either Tukey's HSD or Dunn's test with p = 0.05. The test used in each plot is indicated on the right side of the graphic. Bars sharing the same letter within each graph are not significantly different. "PMC" is an abbreviation for Plant Materials Center.

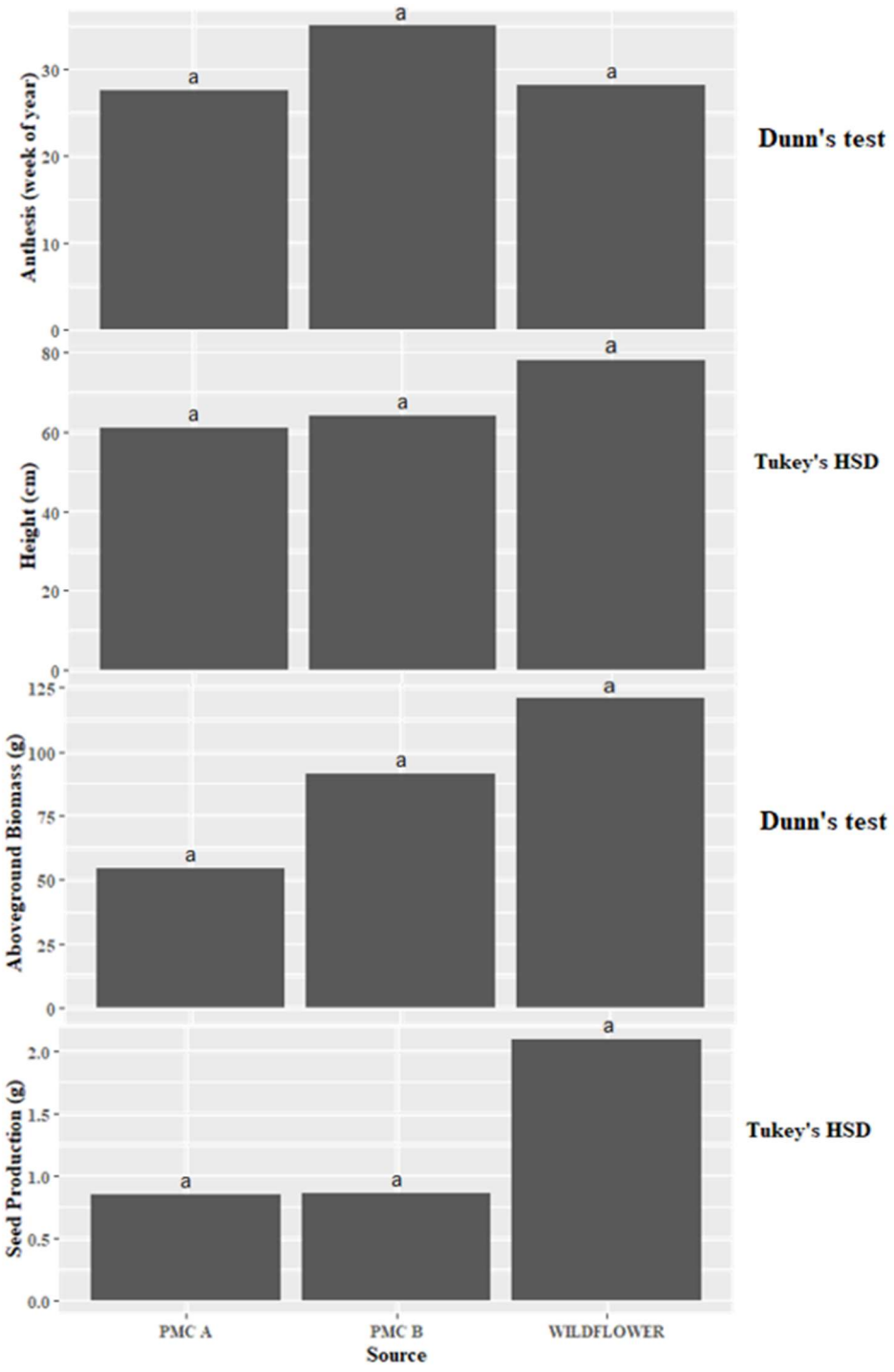
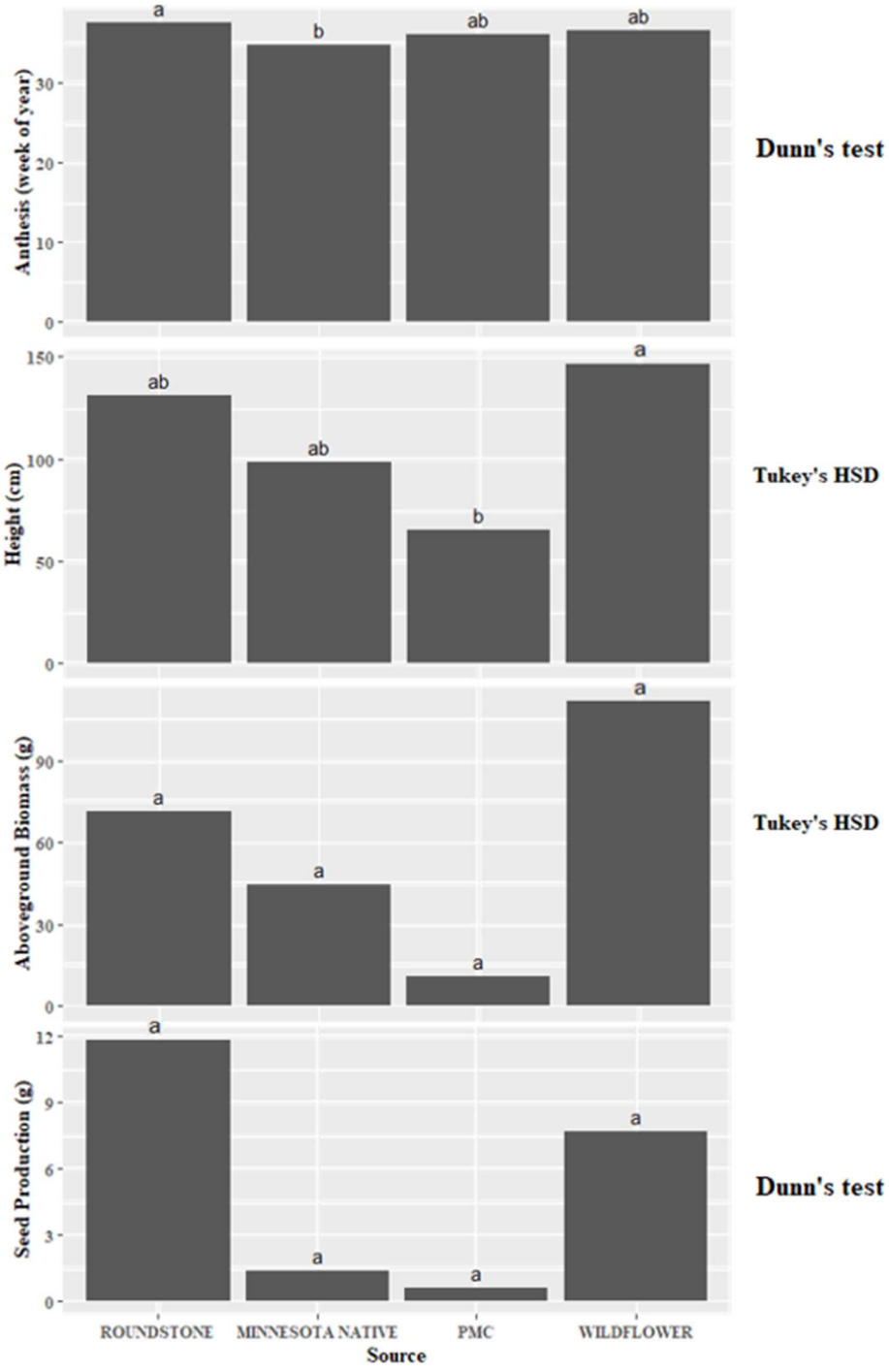


Figure 11. *Sorghastrum nutans* (Indiangrass) measurements of individual success in the 2022 common garden study, separated by source name. The letter at the top of each bar represents pairwise comparisons of least-squared means using either Tukey's HSD or Dunn's test with p = 0.05. The test used in each plot is indicated on the right side of the graphic. Bars sharing the same letter within each graph are not significantly different. "PMC" is an abbreviation for Plant Materials Center.



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Seed Pretreatment Effects on Germination of Native Prairie Grasses and Forbs

Abstract

Native seeds are often used to supplement struggling or extinct plant populations in tallgrass prairie restoration and establishment sites. However, germination of native seeds is often inconsistent and can be a major obstacle in planting success. Most native species are not domesticated, and therefore have not been selected for high germination rates. There is also a lack of research on germination conditions for native plants, and, subsequently, a lack of useful germination protocols for those species. This research was conducted to investigate how germination pretreatments would affect germination for tallgrass prairie species, including fourteen common grasses and forbs found in Arkansas prairies. Pretreatments in the study included sterilization with hydrogen peroxide, dry and moist stratification of varying durations (1, 2, or 3 months), mechanical scarification with sandpaper, thermal scarification with boiling water, chemical scarification with hydrogen peroxide, and hormonal treatment with gibberellic acid. Across all species, there were minimal instances where a pretreatment significantly improved germination rate. One consistent finding was that thermal scarification with boiling water should be avoided unless specifically prescribed. Overall, there was no "one-pretreatment-fits-all" approach to improving germination of native prairie species. Those wishing to restore or establish a native tallgrass prairie are recommended to first try germinating seed with no pretreatments, and if low germination rates are obtained to attempt dry stratification with moist stratification as the next step.

Introduction

A very common obstacle when working with native prairies species is germination rate of seed supplemented into various projects. Whether direct sowing into a field or starting in a greenhouse and transplanted, if germination rates are low, the restoration project may struggle. Low germination rates may be caused by a range of factors, but a factor that can be manipulated is seed dormancy and the breaking thereof. It is proposed that seed dormancy is an evolutionary trait to allow a seed to survive long periods of unfavorable germination conditions (Bentsink & Koornneef, 2008). However, a dormant seed is defined as a viable seed that does not germinate under usually favorable conditions for its germination (Baskin and Baskin, 2004; Loch et al., 2004).

The mechanisms of seed dormancy are not fully understood, but there are several main descriptions of dormancy that have been defined and categorized. Adkins, Bellairs, and Loch (2002) separated the mechanisms of dormancy into those that occur outside the embryo (such as the seed coat) and those that occur within the embryo. The mechanisms in the seed coat, as outlined by Adkins, Bellairs, and Loch (2002), are the physical constriction of the embryo to prevent growth, a barrier against gas exchange and water infiltration, or the presence of chemical germination inhibitors within tissues surrounding the seed coat. There is much less understanding of mechanisms of dormancy within the embryo itself, but most hypotheses include the role of chemicals that inhibit germination, including gibberellic acid, other plant growth regulators, and certain nitrogen-containing compounds (Adkins, Bellairs, and Loch, 2002). Baskin and Baskin (2004) recommended a different categorization system of seed dormancy, separating mechanisms of dormancy, not by location in relation to the embryo, but by type:

physiological (overcome by temperature), morphological (overcome by time), physical (overcome by disruption of the seed coat), and combinations among the three.

Native seeds dormancy is often an obstacle in restoration projects due to two main factors. The first is that, while commercial seed producers often provide generalized germination instructions to consumers, there is often a lack of species-specific protocols that might increase germination rates. Secondly, most native seeds, with the exception of cultivars, have not been domesticated to germinate as easily as crop species. Therefore, wild type native seeds are likely to have lower germination rates or higher degrees of seed dormancy than developed cultivars (Schröder & Prasse, 2013).

In order to increase germination rates, a variety of seed pretreatments may be used to break the dormancy of the seed. The two main categories of seed pretreatments are stratification and scarification. Scarification is generally defined as breaking physical dormancy, where water or air permeability of the seed coat is addressed, while stratification is generally defined as breaking a type of physiological dormancy which is not well understood in all species (Knapp, 2000; Baskin & Baskin, 2004; Loch et al., 2004).

Stratification is broadly described as a pretreatment which replicates temperature and moisture conditions in which the seed is naturally dormant throughout, thus fulfilling physiological requirements for germination after the inconducive conditions are over (Baskin & Baskin, 2004). When broadly applied to North American prairie restoration, the dormant conditions occur during winter, and so cold stratification is used.

Two approaches in cold stratification are dry and moist stratification. Dry stratification, as the name suggests, keeps seed cold and free of moisture while moist stratification involves keeping the seed cold in wetted media such as sand, vermiculite, or paper throughout the chilling

period (Nuzzo, 1976; Eckberg et al., 2015). It is assumed that species/varieties that are adapted to wetter environments react better to moist stratification, and species/varieties that are adapted to drier environments react better to dry stratification.

There are several types of commonly used scarification methods. Mechanical scarification refers to physical damage of the seed coat to allow moisture and air to permeate the seed; this is typically accomplished by rubbing the seed with sandpaper, nicking with a sharp instrument, or processing through a thresher/other mechanical implement (Jensen & Boe, 1991; Kaye & Kuykendall, 2001; Deng et al., 2021). Thermal scarification weakens the seed coat with heat, most often in an oven/hot air chamber or by soaking seed in hot water (Valbuena & Vera, 2002; Ren & Tao, 2004). Chemical scarification damages the seed coat with caustic chemicals such as strong acids or bases. Kindinger (1994) found promising results with *Tripsacum dactyloides* (eastern gamagrass) soaking in 30% hydrogen peroxide for 2 hours. Hydrogen peroxide has also been used to surface sterilize seeds at lower concentrations such as 3-5% (Walker & Erdman, 1926; Landis, 1998). Overall, there is minimal research defining the specific exposure time and concentrations for a given species.

Gibberellic acid stimulation may also be used as a hormonal pretreatment separately from stratification or scarification, from concentrations anywhere from 10 to 1,000 ppm (Chisha-Kasumu, Woodward, & Price, 2007; Su et al., 2011). While the mechanism of gibberellic acid's effect is not well-understood, some have speculated that gibberellic acid treatment may encourage the movement of stored food reserves and advance phytochrome responses to light, thus beginning the early stages of germination (Thomas, 1992; Plummer & Bell, 1995; Loch et al., 2004).

Commercial seed producers often provide generalized germination instructions to consumers, but species-specific protocols could increase germination rates and therefore increase the chance of success at a restoration site. Overall, the goal of this research is to provide insight on germination protocols for species that are native to remnant prairies in Arkansas.

Various prairie species were germinated using the pretreatments discussed to find an optimized germination protocol for each species. The objective of the germination experiments was to determine if scarification or stratification methods could improve germination of native prairie species which often do not have detailed instructions regarding methods to optimize establishment. Twelve commonly used seed germination pretreatments were tested on each of fourteen species. It was hypothesized that pretreatment would have a significant effect on germination rates, and that the most effective pretreatment would differ among species.

Materials and Methods

Fourteen species were included in the germination trials, with seed obtained from a variety of sources in 2021 and early 2022 (Table 1). Multiple seed sources were used for each species to provide diversity in sampling and represent each species more broadly than a single seed source might have provided. Because seed quality can vary widely from various sources and harvest years, we did not consider seed source as a treatment effect in these studies. Written confirmation about the original source material and from where the population was originally found was provided by the seed supplier (Table 1). Upon receipt, the seed was stored in a climate-controlled laboratory until used for germination trials.

All seeds were surface sterilized by soaking in a 30% hydrogen peroxide solution for 30 minutes with occasional stirring (Walker and Erdman, 1926; Landis, 1998). For every 100 mL of solution, 50 µL of Tween was added to ensure proper adhesion to the seed for complete

sterilization (L. Ortega, personal communication, 2019). The solution was poured out over a paper filter to catch seed, then the seed were rinsed three times with distilled water and air dried in a fume hood (Labconco, Kansas City, MO). Based on the possibility that the hydrogen peroxide sterilization treatment not only surface-sterilized the seed but affected germination, a distilled water control of 30 minutes (Treatment 2) was performed for each source. After air drying, seed were stored at room temperature for up to a week. Seed were then divided into approximately 25-seed experimental units and treatments were performed on each 25-seed unit.

Stratification was carried out in a refrigerator held at a constant temperature of 4 °C with no light. For dry stratification, each experimental unit was placed in a separate coin envelope and placed in the refrigerator. For moist stratification, masonry sand (Midwest Block, Springdale, AR) was first oven-heated at 98 °C for at least a week to remove all water before being mixed in a ratio of 100 mL sand to 30 mL distilled water to reliably produce moist sand with a volumetric water content of 30%. Each experimental unit was mixed at a ratio of about 1:2 with the moist sand and placed in a sealed plastic bag in the refrigerator. These treatments were left in the refrigerator for the prescribed amount of time, either 1, 2, or 3 months.

For mechanical scarification, each replication was placed between two sheets of Sandblaster Pro sandpaper (3M, St. Paul, MN) and rubbed vigorously. Duration of the treatment ranged from 15-30 seconds and the grit of the sandpaper was either 80 or 120, depending on preliminary experiments on which combination of time and grit would appropriately affect the seed coat without damaging the endosperm (Table 3).

For thermal scarification, water was boiled, allowed to rest until bubbles were no longer present, and then poured into glass vials containing one experimental unit and allowed to soak for 24 hours. A room temperature (approximately 20 °C) 24-hour soak using distilled water was

used as a control for this treatment to identify any possible effects on germination of the water soak itself. The two chemical treatments were as follows: 0.5 M hydrogen peroxide for 24 hours (Conner, 2018) and 400 ppm gibberellic acid for 24 hours (Su et al., 2011). For every chemical treatment, seeds were rinsed three times with distilled water after soaking.

Germination studies were conducted in a walk-in growth chamber (Model PGW36, Conviron, Winnipeg, Manitoba, Canada) set at 30 °C during an 8-hr light period and 20 °C for a 16-hr night period (Association of Official Seed Analysists, 2016). After pretreatment, the 25 seeds of each experimental unit were placed in a germination pouch (CYG™, Mega International, Roseville, MN) with paper moistened by 40 mL of distilled water (Figure 1). Germination pouches were checked twice a week and water was added to an approximate depth of 2 cm in the bottom of the pouches if they had dried significantly. At three weeks, number of seeds germinated out of 25 was recorded, with germination defined as radicle emergence of at least 2 mm. Seeds that did not germinate within three weeks were tested with tetrazolium chloride (TZ) to determine if they were dormant or dead, following defined protocols (Miller et al., 2010). The final germination rate consisted of the germinated individuals divided by the sum of germinated and dormant individuals. For instance, if a pouch had 10 seeds germinated, 10 seeds dormant, and 5 seeds dead, the final germination rate would be 10 (germinated) divided by 20 (germinated + dormant), or 50%. In the case that all seeds in a pouch were found to be dead, germination was reported as 0% to reflect the possibility that the pretreatment was what caused death of previously viable seeds.

Experimental Design and Statistics

Each experimental run of the germination trial consisted of the 13 seed treatments (Table 2) imposed on each of the 14 grass or forb species (Table 1). Each plant species was also

represented by from two to five seed sources, which were treated and handled separately during each experimental run. As such, the basic experimental unit during each run of the study was the source x seed treatment combination. Each experimental unit contained an excess of 25 seeds to account for seeds slipping out of the germination pouch or becoming moldy. The overall experiment was repeated three times, each approximately two months apart. Treatments were placed in the growth chamber in a completely randomized design.

All statistical analyses and resulting graphs were completed using R statistical software (R Core Team, 2021) and RStudio (RStudio Team, 2022), as well as the packages discussed in the Appendix. Differences in germination rate were compared within species using a one-way analysis of variance (ANOVA) test. In preliminary analysis of the data, type I and type III ANOVA tests both output the same results for every comparison of measurements despite occasions of unbalanced data, and therefore type I was used for all further analyses. A significant difference would support the hypothesis that source did affect restoration efforts. If there was a significant difference, Tukey's honestly significant difference (HSD) test was utilized to determine which sources were different. Assumptions of the ANOVA test, homogeneity of variables and normal distribution, were confirmed by plotting a histogram of residuals and Bartlett's test, respectively. If either assumption was violated, then a nonparametric ANOVA test called the Kruskal-Wallis test by ranks was utilized instead of the standard ANOVA test. Dunn's test for multiple comparisons would then be used to determine pretreatments with significantly different results.

Results and Discussion

Table 4 describes the statistical analysis that was used for germination data from each species, based on whether or not the assumptions of the standard ANOVA test were met. Half of

the species tested, including *A. gerardii*, *P. virgatum*, *S. scoparium*, *S. nutans*, *B. alba*, *B. australis*, *E. yuccifolium*, failed to meet the assumptions and were subsequently analyzed using the Kruskal-Wallis test. Five of the species tested, *S. scoparium*, *B. australis*, *E. pallida*, *L. pycnostachya*, and *S. laciniatum,* showed no significant difference in germination rate based on the seed treatments (Table 4) and will not be discussed further (Figures 2-6).

Within the grasses, *A. gerardii* germination was significantly affected by germination pre-treatments, but no treatment improved germination over the non-sterilized control. The thermal scarification treatment actually reduced germination compared to the non-sterilized control (Figure 7). In *B. curtipendula*, the non-sterilized control and 2-month dry stratification had significantly higher germination rates than thermal scarification and GA pre-treatment, but again none of the treatments improved germination rates over the non-sterilized control (Figure 8). While germination rates of *P. virgatum* were significantly different ($p = 0.024$), Dunn's test could not separate treatments in the pairwise comparisons (Figure 9). Germination of *S. nutans* was significantly improved with a number of the treatments compared to the non-sterilized control (Figure 10). Germination rates in *S. nutans* were significantly improved with all three moist stratifications as well as the 3-month dry stratification. The gibberellic acid and hydrogen peroxide treatments also improved germination compared to the non-sterilized control (Figure 10).

In forbs, *A. tuberosa* had a significantly lower germination rate from thermal scarification than the non-sterilized control (Figure 11). *B. alba* had no treatments that yielded significantly higher germination rates than the non-sterilized control, but the 3-month stratification had significantly higher germination rates than the 1-month dry stratification (Figure 12). While *E. yuccifolium* was shown to have significant differences, Dunn's test could not separate the

treatments in the pairwise comparisons (Figure 13). Thermal scarification had significantly lower germination rates than the non-sterilized control in *R. columnifera* (Figure 14). In *R. hirta*, germination was reduced by several pretreatments in comparison to the non-sterilized control, including 1-month and 2-month dry stratification, all scarification treatments, and the gibberellic acid hormone treatment (Figure 15).

The seed from each source, as obtained, was verified to be at least partially viable based on the non-sterilized control for each species (Figures 2-15). Germination rates of the non-sterilized treatments ranged from less than 10% for *B. alba* (Figure 12) and *B. australis* (Figure 3) to over 90% for *A. gerardii* (Figure 7), *P. virgatum* (Figure 9), *S. scoparium* (Figure 2), *A. tuberosa* (Figure 11), *R. columnifera* (Figure 14), and *R. hirta* (Figure 15). Statistically, the non-sterilized seeds had the highest germination rates for all of the species except *S. nutans* (Figure 10). As hypothesized, pretreatment of seeds did affect germination in a number of species, and the most effective treatments varied by species. Thermal scarification significantly reduced germination rate from the non-sterilized control for five different species (*A. gerardii*, *B. curtipendula*, *A. tuberosa*, *R. columnifera*, and *R. hirta*). However, thermal scarification causing lower germination in seeds is not uncommon, especially in heat-sensitive and thin-coated species (Khasa, 1992). Unfortunately, there were no consistent pre-treatments that improved germination across the entire range of forb or grass species, which would allow more broad applications of the results to be made.

There is a general lack of rigorous seed pretreatment studies in the literature, especially regarding prairie natives, and therefore it is difficult to compare these findings to others. While further research should be conducted on the species included in this germination study, along

with other species not included, a few conclusions may be made regarding native prairie seed germination as a whole.

Boiling water scarification should not be used unless outlined by the seed supplier or other species-specific protocols, as this treatment consistently reduced overall germination of most of the species tested (even if not statistically significant). Similarly, surface-sterilization also had some negative effects and should be avoided unless specifically required (e.g. mold consistently killing seed before they can germinate). Even in the case of mold problems, a method other than hydrogen peroxide should be investigated.

The broadest, safest conclusions that may be drawn from the study are to first attempt to sow seed without pretreatments, or to dry stratify seed for as long as possible before planting. Moist stratification occasionally reduced germination rate in the study, but the main concern would be growth of mold during the stratification period. If low germination rates occur even after dry stratification, the next suggestion would be then to try moist stratifying the seed, especially for forbs. Germination rates in relation to scarification pretreatments were often inconsistent when compared across species, though a hydrogen peroxide overnight soak appeared to be slightly more effective than mechanical scarification.

These three methods of pretreatment—dry stratification, moist stratification, and hydrogen peroxide soaks—are all cost effective, as bags, sand, watertight containers, and hydrogen peroxide can be easily purchased in bulk. In the event of poor germination, those seeking to restore or establish a tallgrass prairie will be able to utilize these pretreatments at a relatively low cost in materials and labor.

A couple of limitations were noted over the course of the germination experiments. The first was the inability to distinguish loss of seed viability during the pretreatment and nonviable

seed prior to the pretreatment. If any of the three, 25-seed replicates in a pretreatment contained no viable seed, then the overall germination rate would be scored as 0, regardless of the treatment. However, if a pretreatment was the cause of loss of viability, there would be no functional difference between loss of viability and failure to break dormancy, as both cases would result in the pretreatment receiving the same germination rate.

There was also an issue during the majority of the second repetition where the tetrazolium chloride (TZ) solution stopped dying seed red. A possible reason for the issue is that the supplier had sent poorly stored TZ, which had expired, though this was never confirmed. A new supplier was chosen, and while the TZ was being shipped, the protocol was edited slightly. After germination counts, germination pouches were placed back in the growth chamber until the seed inside could be tested. Any seed that germinated during this period (after the usual three weeks) was still considered dormant.

Conclusion

The overall objectives of the research were fulfilled: for each species in the study, it was determined if 1) pretreatment had a significant effect on germination rate, and 2) if applicable, what pretreatment yielded the highest and lowest germination rate. The hypothesis that pretreatment would have a significant effect on germination rates, and that the most effective pretreatment would differ among species was somewhat supported, in that each species reacted differently to pretreatments, despite there being a non-significant difference in germination rate in some species. While there were no pretreatments that clearly proved to be effective across many species, it was concluded that boiling water scarification could lower germination rates.

Tables and Figures

Table 1. Summary of individuals used in the 2022 germination studies. While production source denotes where the seed was purchased/received from, source material origin refers to the location where the plant material was found before human interference.

Species	Production Source	Cultivar	Source Material Origin
Andropogon gerardii Vitman Big bluestem	Bismarck, ND Plant Materials Center	'Bison'	Oliver County, ND
	Bismarck, ND Plant Materials Center	'Bonilla'	Beadle County, SD
	Roundstone Seed		LaRue County, KY
	Wildflower Farm		Coldwater, Ontario, Canada
Bouteloua curtipendula (Michx.) Torr. Sideoats grama	Bismarck, ND Plant Materials Center	'Pierre'	Stanley County, SD
	Minnesota Native Landscapes		Douglas County, MN
	Roundstone Seed	'El Reno'	Canadian County, OK
	Wildflower Farm		Coldwater, Ontario, Canada
Panicum virgatum L. Switchgrass	Bismarck, ND Plant Materials Center	'Dacotah'	Morton County, ND
	Bismarck, ND Plant Materials Center	'Forestburg'	Sanborn County, ND
	Minnesota Native Landscapes		Houston County, MN
	Roundstone Seed	'Cave-in-Rock'	Hardin County, IL
	Wildflower Farm		Coldwater, Ontario, Canada
Schizachyrium scoparium (Michx.) Nash Little bluestem	Bismarck, ND Plant Materials Center	'Badlands Ecotype'	Badlands, ND & SD
	Bismarck, ND Plant Materials Center	'Itasca'	ND, SD, & MN
	Roundstone Seed	'Aldous'	Hart County, KY
	Wildflower Farm		Coldwater, Ontario, Canada
Sorghastrum nutans (L.) Nash Indiangrass	Bismarck, ND Plant Materials Center	'Tomahawk'	Dickey County, ND; Marshall County, SD; & Brown County, SD
	Minnesota Native Landscapes		Benton, Sherburne, Mille Lacs, & McLeod County, MN
	Roundstone Seed		Hart County, KY
	Wildflower Farm		Coldwater, Ontario, Canada

Table 1. (cont.)

Species	Production Source	Source Material Origin
Asclepias tuberosa L. Butterflyweed	Minnesota Native Landscapes	Kossuth, Madison, Lucas, & Union County, IA
	Roundstone Seed	Colorado
	Wildflower Farm	Coldwater, Ontario, Canada
Baptisia alba (L.) Vent. White wild indigo	Roundstone Seed	Missouri
	Wildflower Farm	Pennsylvania*
Baptisia australis (L.) R. Br. Blue wild indigo	Roundstone Seed	Todd County, KY
	Wildflower Farm	Pennsylvania*
Echinacea pallida (Nutt.) Nutt. Pale purple coneflower	Minnesota Native Landscapes	Madison County, IA
	Wildflower Farm	Coldwater, Ontario, Canada
Eryngium yuccifolium Michx. Rattlesnake master	Minnesota Native Landscapes	Dakota County, MN
	Roundstone Seed	Priceville, KY
	Searles Prairie	Benton County, AR
	Wildflower Farm	Pennsylvania*
Liatris pycnostachya Michx. Prairie blazingstar	Grand Prairie	Arkansas, Prairie, Lonoke, & White County, AR
	Minnesota Native Landscapes	McLeod County, MN
	Searles Prairie	Benton County, AR
	Wildflower Farm	Coldwater, Ontario, Canada
Ratibida columnifera (Nutt.) Woot. & Standl. Prairie coneflower	Minnesota Native Landscapes	Houston County, MN
	Roundstone Seed	Texas
	Wildflower Farm	Coldwater, Ontario, Canada
Rudbeckia hirta L. Black-eyed Susan	Cherokee National Forest Ecotype	Cherokee National Forest, TN
	Minnesota Native Landscapes	Dakota County, MN
	Wildflower Farm	Coldwater, Ontario, Canada
Silphium laciniatum L. Compassplant	Grand Prairie	Arkansas, Prairie, Lonoke, & White County, AR
	Minnesota Native Landscapes	Kossuth & Greene County, IA
	Searles Prairie	Benton County, AR

*Source material from Pennsylvania, collected by Wildflower Farm 30 years ago to be grown in Coldwater, Ontario, Canada since then.

Table 2. A summary of all pretreatments used in the germination study. All seeds but the non-sterilized control were first surface-sterilized in a solution of 30% hydrogen peroxide (Walker and Erdman, 1926; Landis, 1998) and 0.005% Tween before being dried up to a week in a fume hood. All controls and pretreatments were then performed on the sterilized, dried seed.

#	Controls	Pretreatment Description
1	Non-sterilized	None—30-minute soak in water
2	Sterilized	None—30-minute soak in sterilization solution
#	**Stratification Pretreatments***	**Pretreatment Description**
3	1-month moist	1 month under moist conditions
4	2-month moist	2 months under moist conditions
5	3-month moist	3 months under moist conditions
6	1-month dry	1 month under dry conditions
7	2-month dry	2 months under dry conditions
8	3-month dry	3 months under dry conditions
#	**Scarification Pretreatments**	**Pretreatment Description**
9	Mechanical	Rubbed between two sheets of sandpaper**
10	Thermal control	24-hour soak in room temperature water
11	Thermal	24-hour soak in water that was just boiled
12	Hydrogen peroxide	24-hour soak in 0.5 M hydrogen peroxide
#	**Hormonal Pretreatment**	**Pretreatment Description**
13	Gibberellic acid	24-hour soak in 400 ppm gibberellic acid

*Stratification pretreatments were carried out in a refrigerator at 4 ºC. Moist stratification refers to seed being mixed with sand at a 30% volumetric water content and sealed in a plastic envelope for the entire duration of the stratification, while dry stratification refers to seed being placed in a sealed paper envelope for the entire duration of the stratification.

**Protocols of mechanical scarification for each species are detailed in Table 3.

Table 3. A list of mechanical scarification methods used for each species in the germination studies, determined by preliminary experiments on which combination of time and sandpaper grit would appropriately affect the seed coat without damaging the endosperm.

Species	Common Name	Sandpaper (grit)	Rubbing time (s)
Andropogon gerardii	Big bluestem	120	10
Bouteloua curtipendula	Sideoats grama	120	10
Panicum virgatum	Switchgrass	120	10
Schizachyrium scoparium	Little bluestem	120	5
Sorghastrum nutans	Indiangrass	120	10
Echinacea pallida	Pale purple coneflower	120	5
Eryngium yuccifolium	Rattlesnake master	120	5
Liatris pycnostachya	Prairie blazingstar	120	5
Ratibida columnifera	Prairie coneflower	120	5
Rudbeckia hirta	Black-eyed Susan	120	5
Silphium laciniatum	Compassplant	120	10
Asclepias tuberosa	Butterflyweed	60	5
Baptisia alba	Blue wild indigo	60	15
Baptisia australis	White wild indigo	60	15

Table 4. For the germination study, assumptions of the standard ANOVA test were confirmed by plotting a histogram of residuals and Bartlett's test. If the histogram did not show approximately normal distribution or Bartlett's test showed that the assumption of equal variances was violated, then a nonparametric Kruskal-Wallis test by ranks was utilized instead of the standard ANOVA test. For each test, p-values are reported.

Species	Standard	Kruskal-Wallis
	---------- p-value ----------	
Andropogon gerardii		<0.001
Bouteloua curtipendula	<0.001	
Panicum virgatum		0.024
Schizachyrium scoparium		0.189
Sorghastrum nutans		<0.001
Asclepias tuberosa	0.010	
Baptisia alba		0.022
Baptisia australis		0.619
Echinacea pallida	0.138	
Eryngium yuccifolium		0.047
Liatris pycnostachya	0.353	
Ratibida columnifera	<0.001	
Rudbeckia hirta	0.001	
Silphium laciniatum	0.356	

Figure 1. A germination pouch with paper moistened by 40 mL of distilled water (CYG™, Mega International, Roseville, MN). Seeds are placed in the top pocket of the paper within the pouch while distilled water is poured into the bottom of the bag. Germination occurs with the pouches standing upright.

Figure 2. *Schizachyrium scoparium* (little bluestem) germination rates, separated by pretreatment type. Reported rates are averages of all utilized seed sources and all replications. The letter at the top of each bar represents pairwise comparisons of least-squared means using Dunn's test with p = 0.05. Bars sharing the same letter are not significantly different.

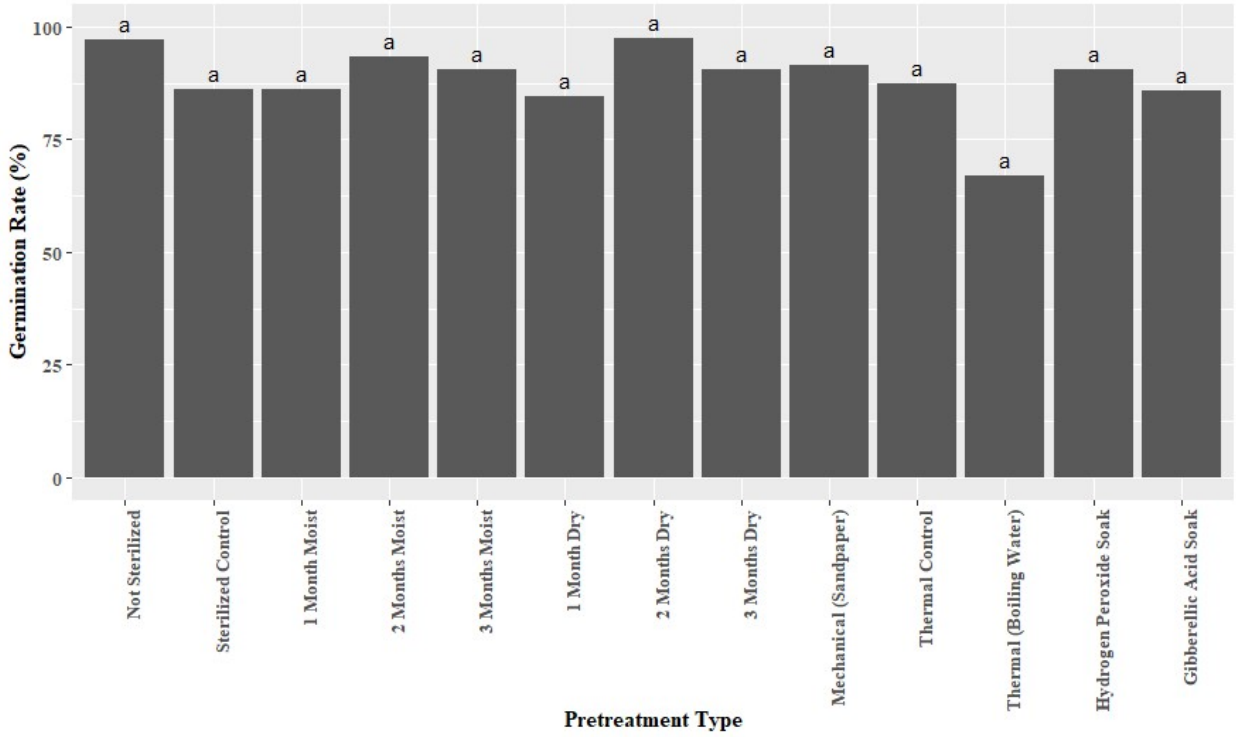


Figure 3. *Baptisia australis* (blue wild indigo) germination rates, separated by pretreatment type. Reported rates are averages of all utilized seed sources and all replications. The letter at the top of each bar represents pairwise comparisons of least-squared means using Dunn's test with p = 0.05. Bars sharing the same letter are not significantly different.

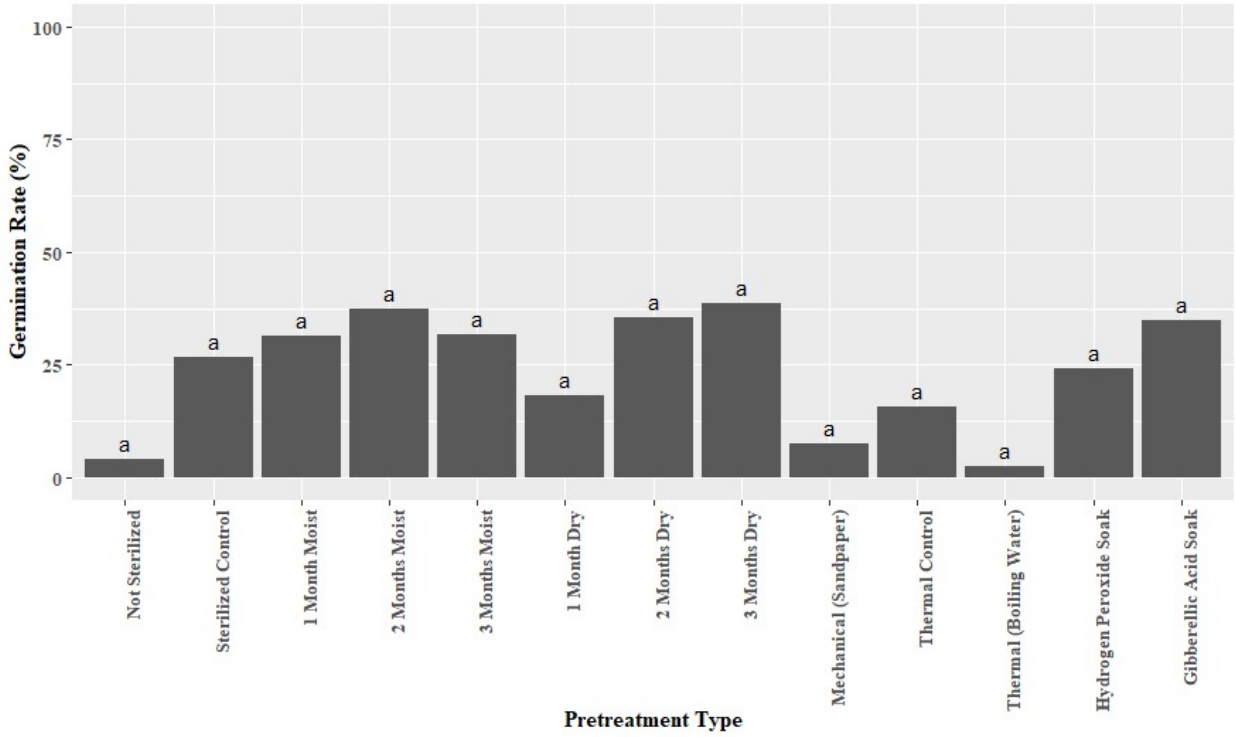


Figure 4. *Echinacea pallida* (pale purple coneflower) germination rates, separated by pretreatment type. Reported rates are averages of all utilized seed sources and all replications. The letter at the top of each bar represents pairwise comparisons of least-squared means using Dunn's test with p = 0.05. Bars sharing the same letter are not significantly different.

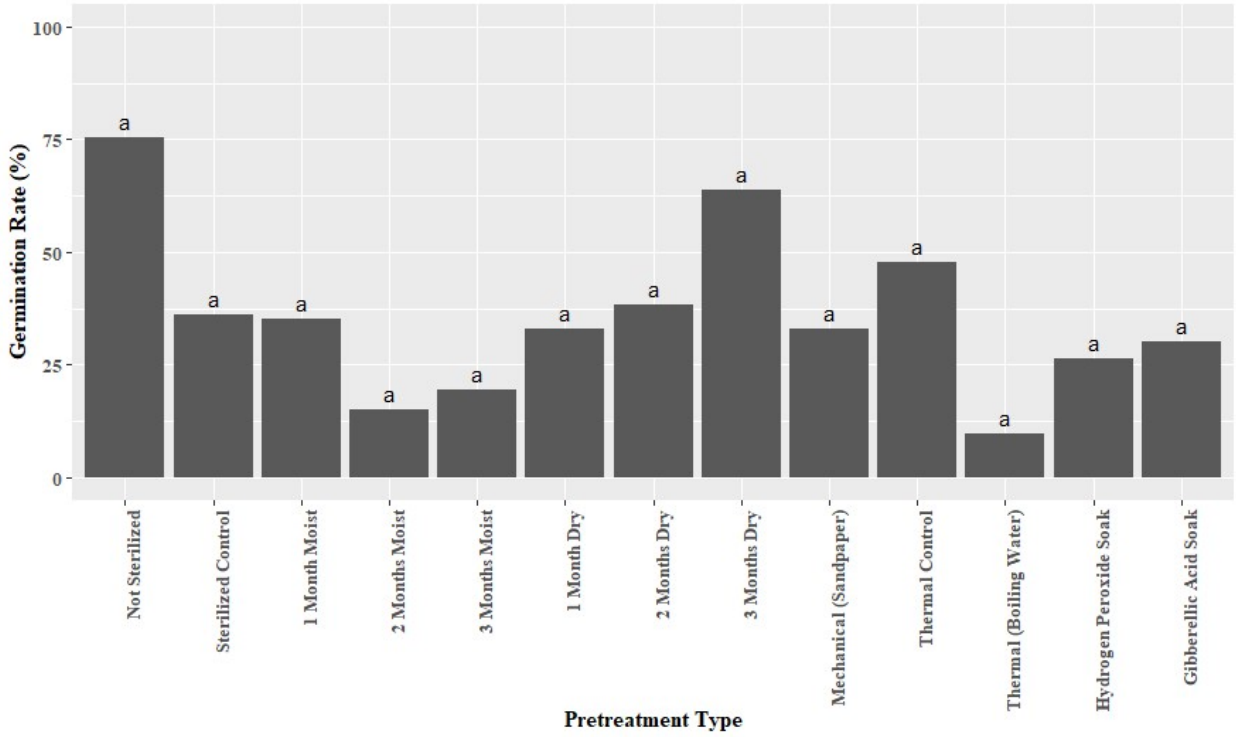


Figure 5. *Liatris pycnostachya* (prairie blazingstar) germination rates, separated by pretreatment type. Reported rates are averages of all utilized seed sources and all replications. The letter at the top of each bar represents pairwise comparisons of least-squared means using Tukey's HSD test with p = 0.05. Bars sharing the same letter are not significantly different.

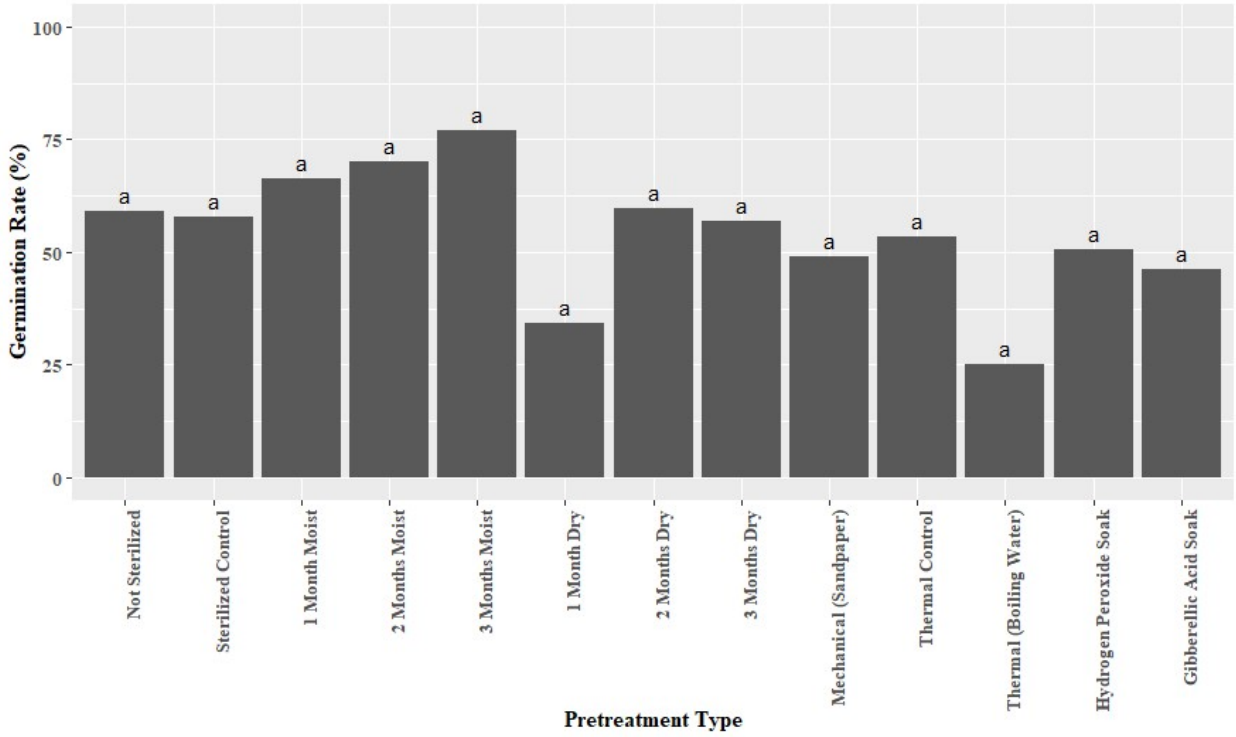


Figure 6. *Silphium laciniatum* (compassplant) germination rates, separated by pretreatment type. Reported rates are averages of all utilized seed sources and all replications. The letter at the top of each bar represents pairwise comparisons of least-squared means using Tukey's HSD test with p = 0.05. Bars sharing the same letter are not significantly different.

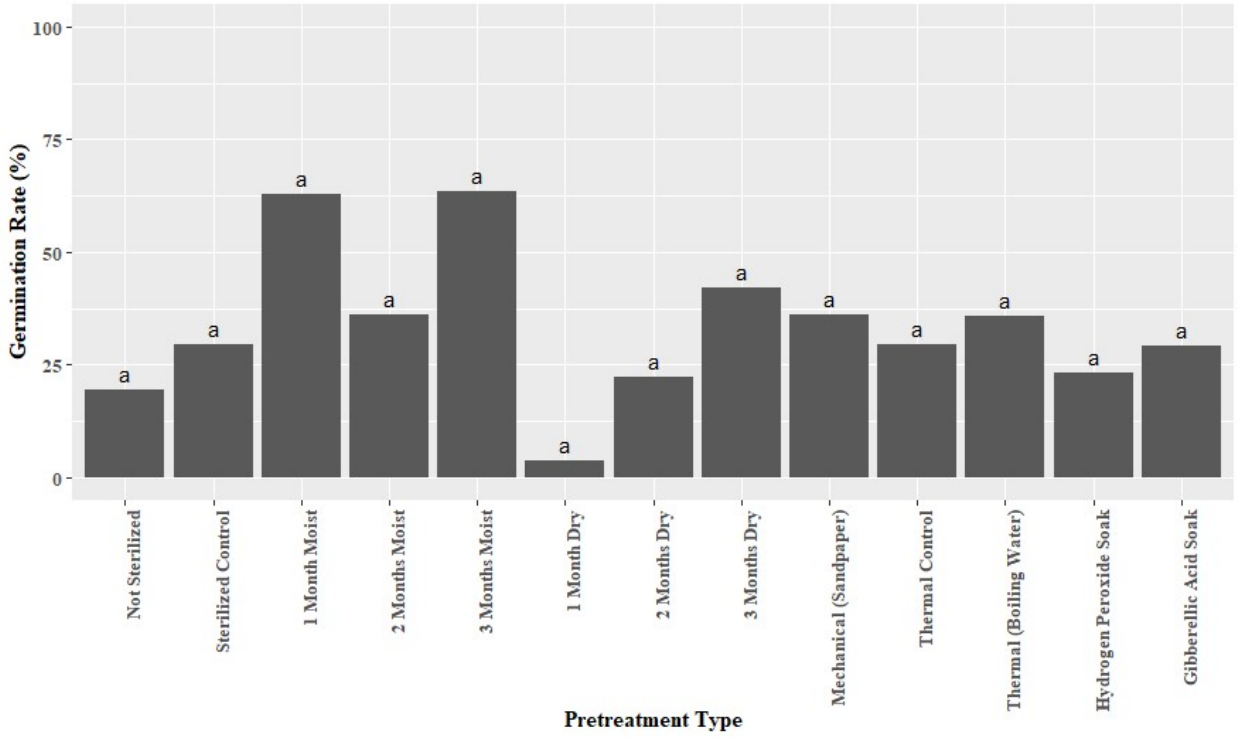


Figure 7. *Andropogon gerardii* (big bluestem) germination rates, separated by pretreatment type. Reported rates are averages of all utilized seed sources and all replications. The letter at the top of each bar represents pairwise comparisons of least-squared means using Dunn's test with p = 0.05. Bars sharing the same letter are not significantly different.

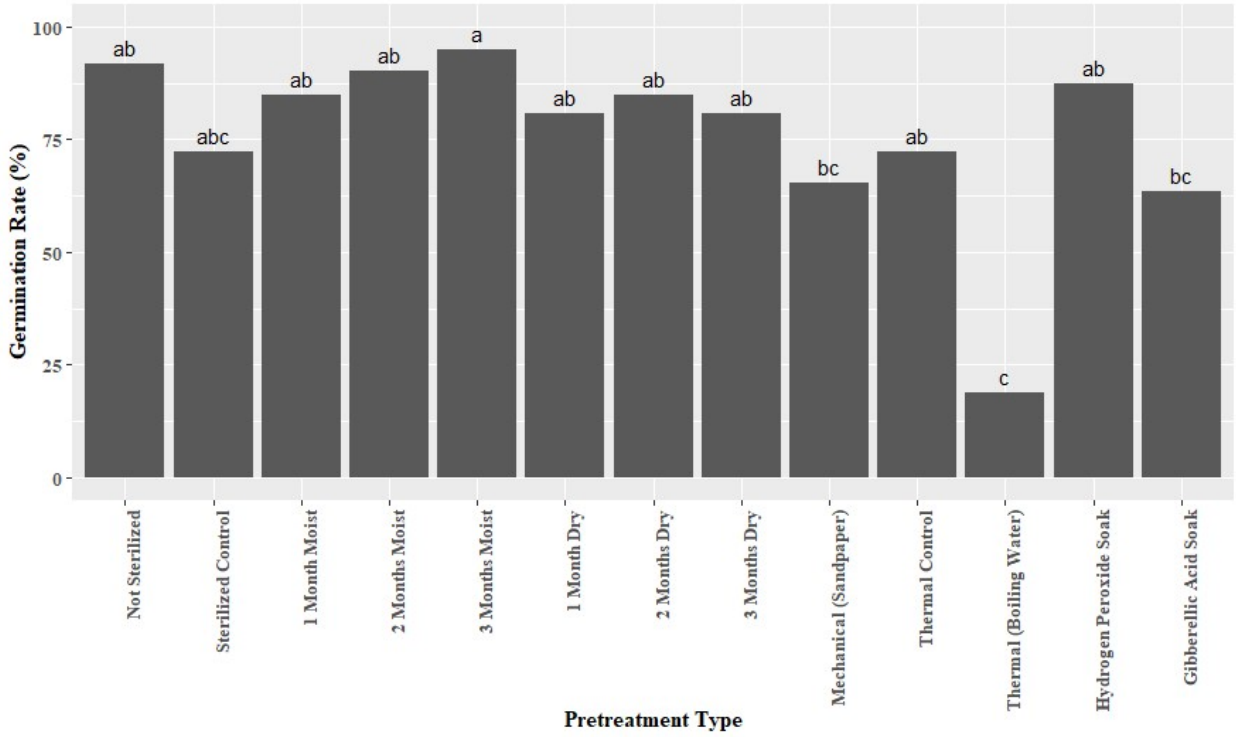


Figure 8. *Bouteloua curtipendula* (sideoats grama) germination rates, separated by pretreatment type. Reported rates are averages of all utilized seed sources and all replications. The letter at the top of each bar represents pairwise comparisons of least-squared means using Tukey's HSD test with p = 0.05. Bars sharing the same letter are not significantly different.

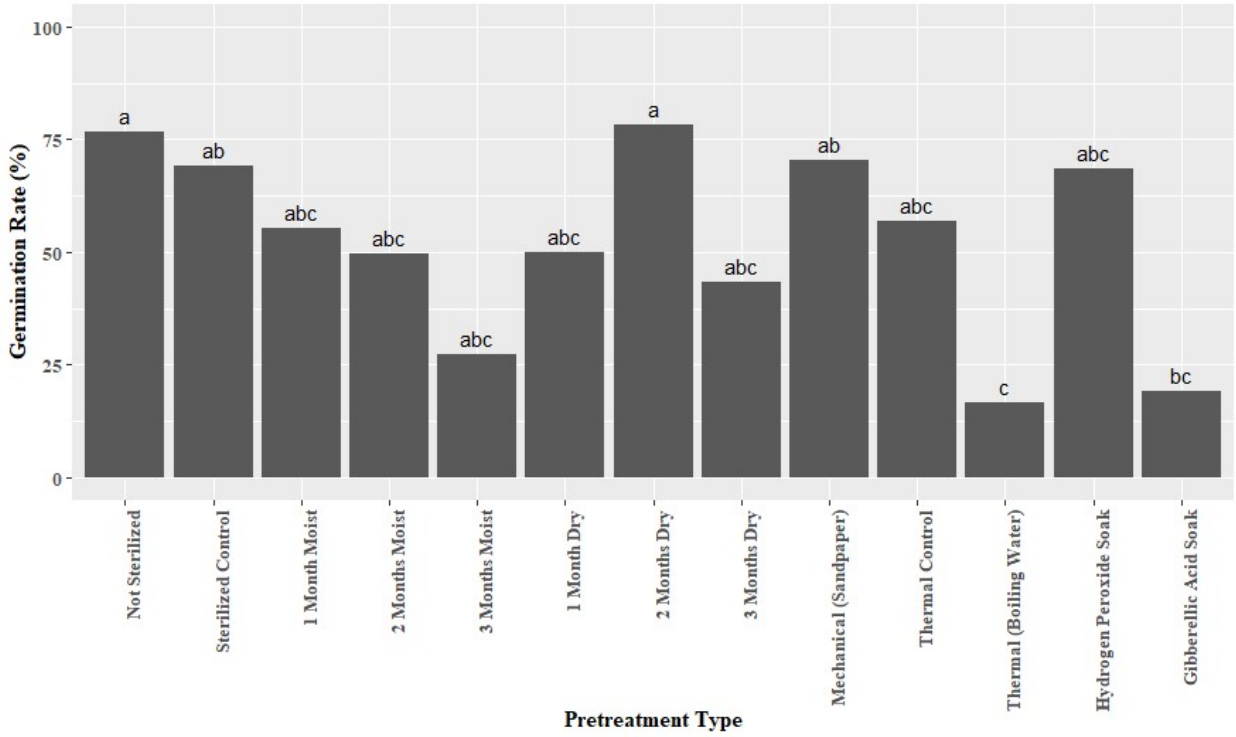


Figure 9. *Panicum virgatum* (switchgrass) germination rates, separated by pretreatment type. Reported rates are averages of all utilized seed sources and all replications. The letter at the top of each bar represents pairwise comparisons of least-squared means using Dunn's test with p = 0.05. Bars sharing the same letter are not significantly different.

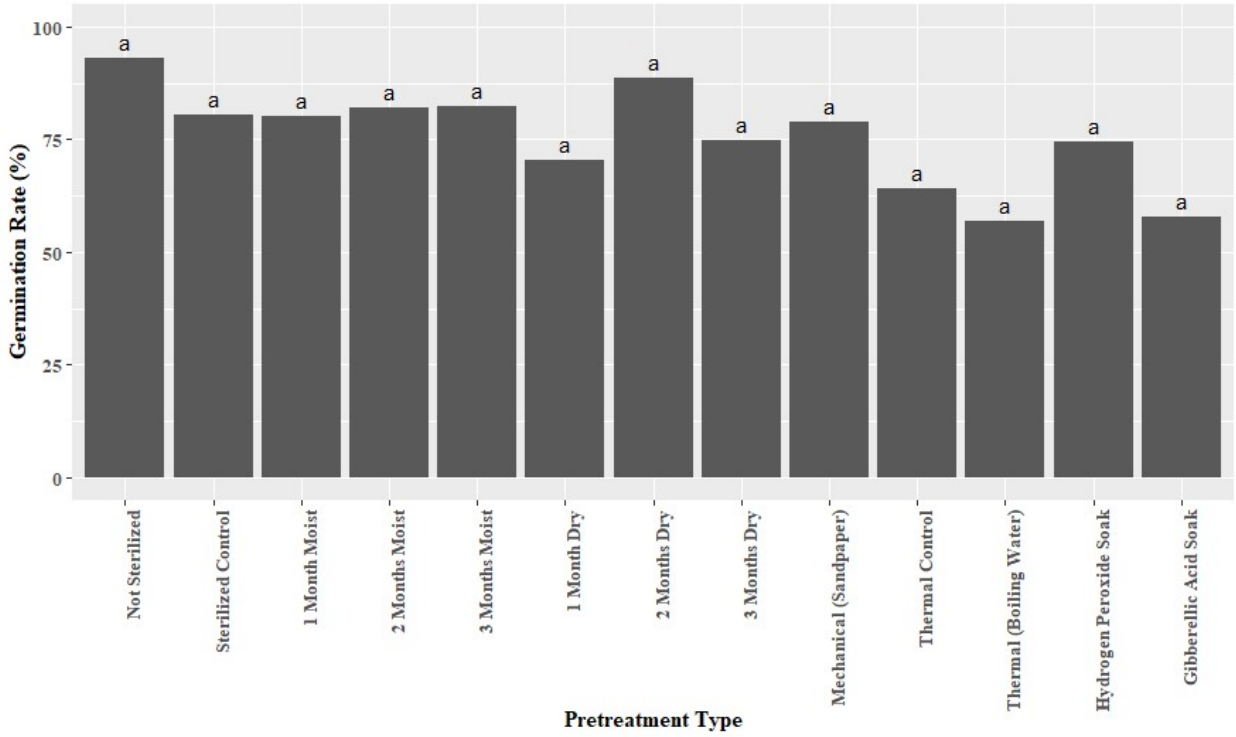


Figure 10. *Sorghastrum nutans* (Indiangrass) germination rates, separated by pretreatment type. Reported rates are averages of all utilized seed sources and all replications. The letter at the top of each bar represents pairwise comparisons of least-squared means using Dunn's test with p = 0.05. Bars sharing the same letter are not significantly different.

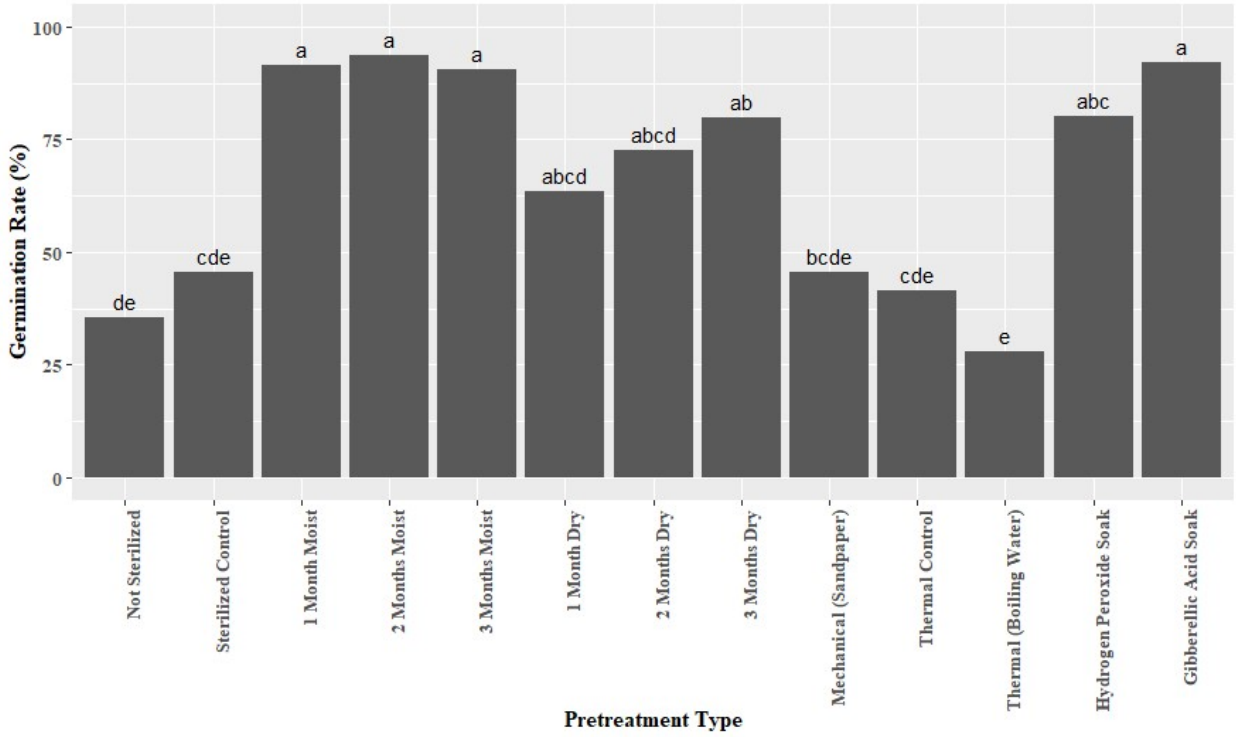


Figure 11. *Asclepias tuberosa* (butterflyweed) germination rates, separated by pretreatment type. Reported rates are averages of all utilized seed sources and all replications. The letter at the top of each bar represents pairwise comparisons of least-squared means using Tukey's HSD test with p = 0.05. Bars sharing the same letter are not significantly different.

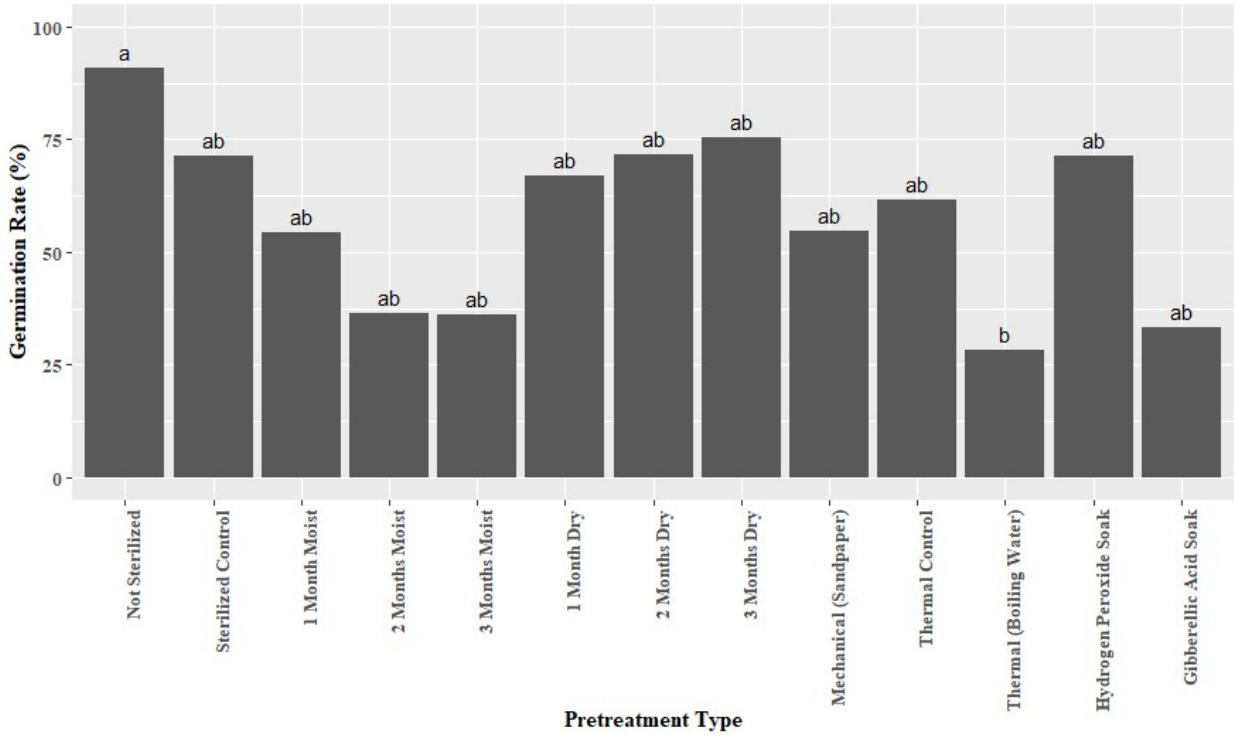


Figure 12. *Baptisia alba* (white wild indigo) germination rates, separated by pretreatment type. Reported rates are averages of all utilized seed sources and all replications. The letter at the top of each bar represents pairwise comparisons of least-squared means using Dunn's test with p = 0.05. Bars sharing the same letter are not significantly different.

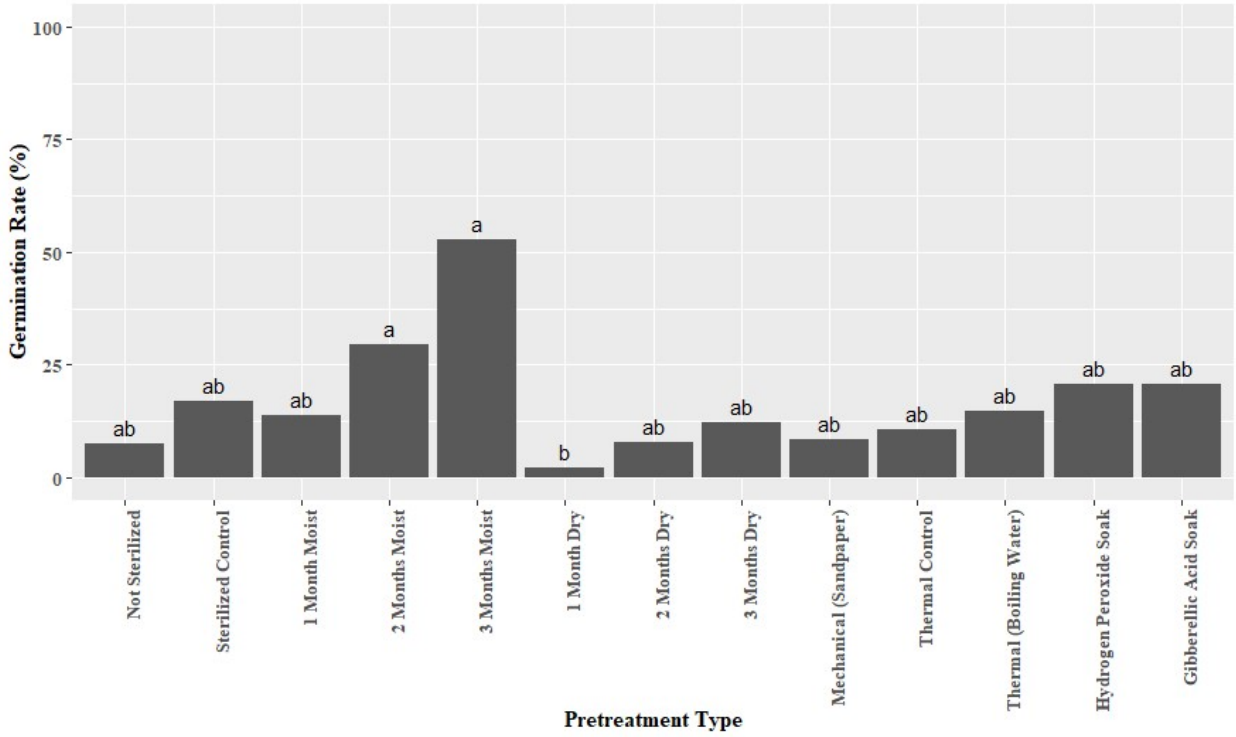


Figure 13. *Eryngium yuccifolium* (rattlesnake master) germination rates, separated by pretreatment type. Reported rates are averages of all utilized seed sources and all replications. The letter at the top of each bar represents pairwise comparisons of least-squared means using Dunn's test with p = 0.05. Bars sharing the same letter are not significantly different.

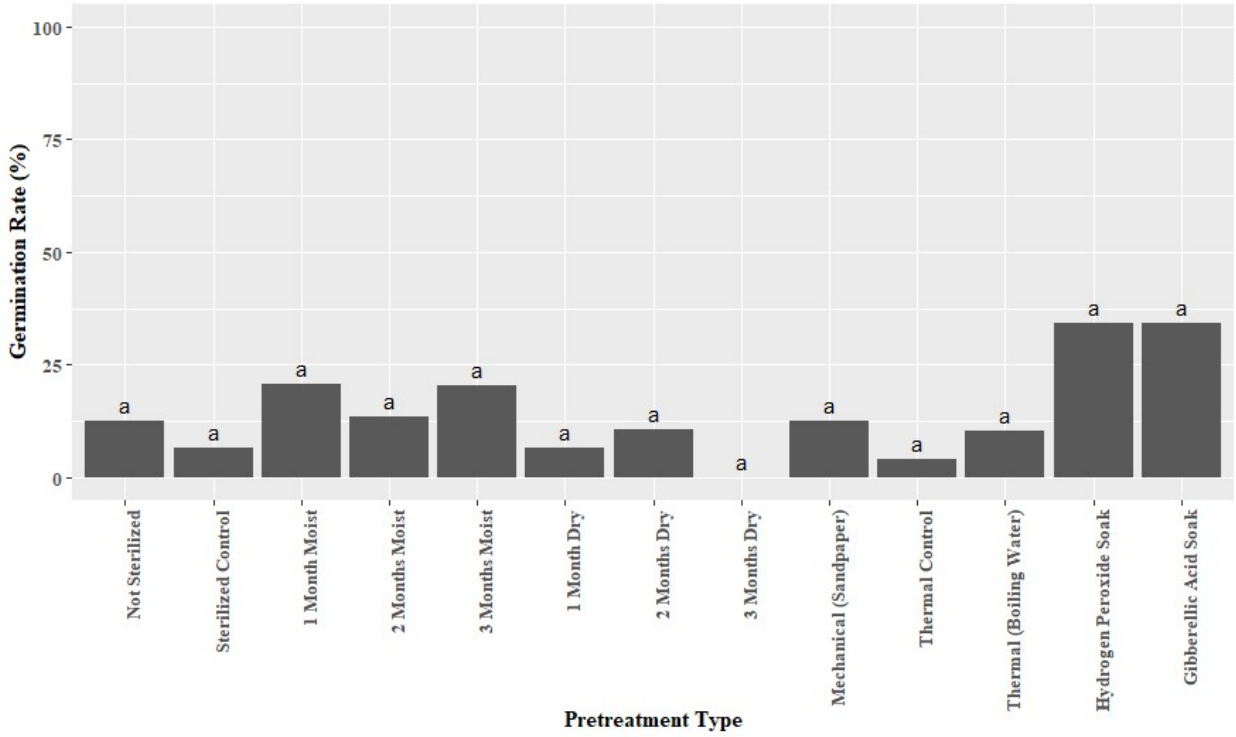


Figure 14. *Ratibida columnifera* (prairie coneflower) germination rates, separated by pretreatment type. Reported rates are averages of all utilized seed sources and all replications. The letter at the top of each bar represents pairwise comparisons of least-squared means using Tukey's HSD test with p = 0.05. Bars sharing the same letter are not significantly different.

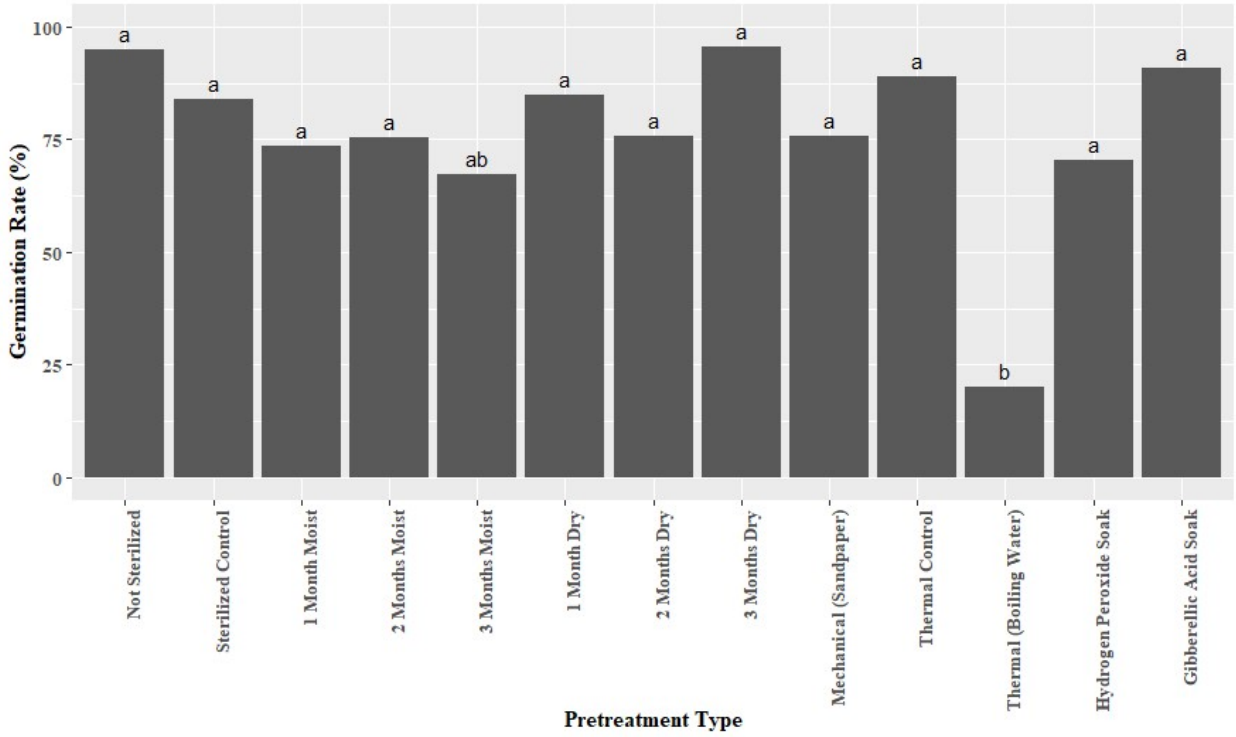
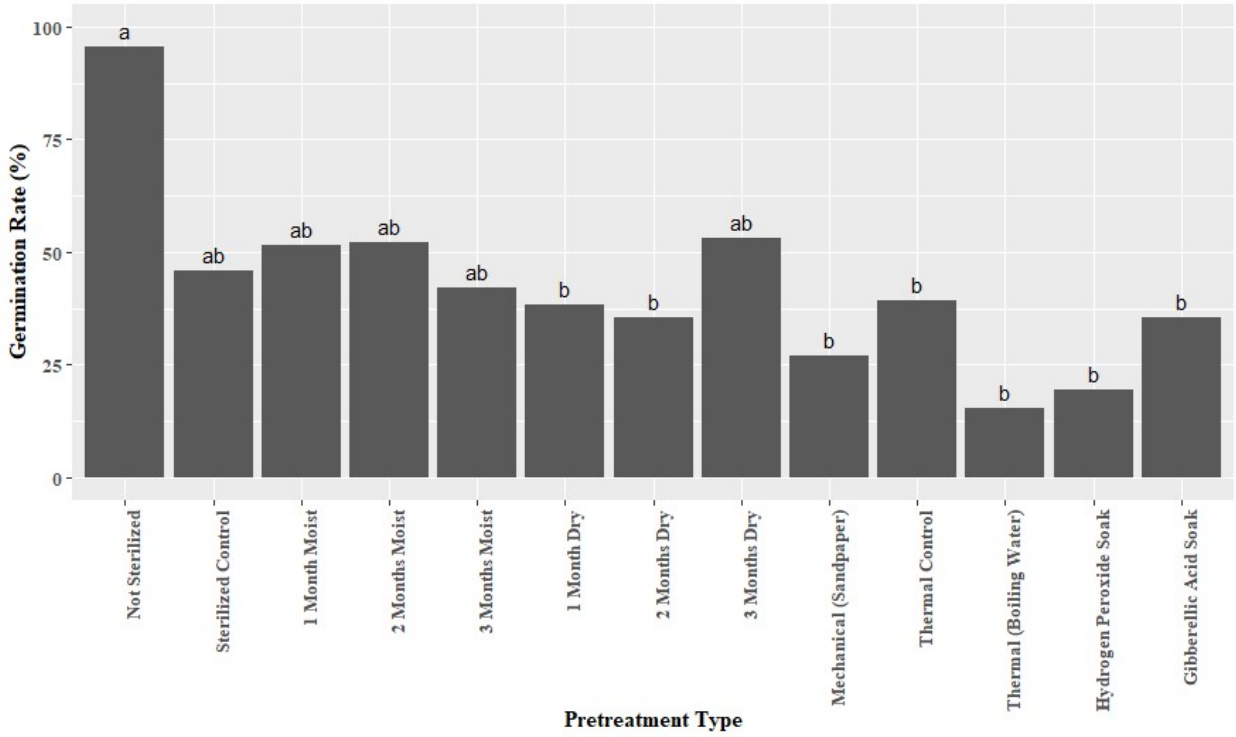


Figure 15. *Rudbeckia hirta* (black-eyed Susan) germination rates, separated by pretreatment type. Reported rates are averages of all utilized seed sources and all replications. The letter at the top of each bar represents pairwise comparisons of least-squared means using Tukey's HSD test with p = 0.05. Bars sharing the same letter are not significantly different.



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Conclusions

The end goal of this research was to provide anyone working with native seed with information on how to increase the chance of a successful prairie restoration, both on informed decisions regarding seed sourcing and how to obtain higher germination rates.

While the preliminary conclusions from the seed sourcing experiment were that source did not significantly affect individual success of native plants in restoration or establishment sites, another year at least of data must be collected before stronger conclusions are drawn. Further common garden research in different locations, with different species, and various sources would also be beneficial.

The germination study suggested that, as hypothesized, each species reacts differently to various pretreatments. General conclusions are that pretreatments should only be applied if non-treated seed has low germination to begin with. Additional conclusions are that boiling water scarification should be avoided if possible. As with the seed sourcing experiment, more replications and additional species in future studies would allow for more broad generalizations, even if those generalizations are on what pretreatments to avoid.

Appendix

R Packages Used in Statistical Analysis

There were several R packages utilized in the final statistical analysis in both chapters of this thesis. readxl: Read Excel Files (Wickham & Bryan, 2022) reads the Excel files of raw data and imports that raw data into R for analysis. car: *An {R} Companion to Applied Regression* (Fox & Weisberg, 2019) enables R to run analysis of variables (ANOVA) tests. FSA: Fisheries Stock Analysis (Ogle et al., 2022) enables R to run Dunn's test. multcompView: Visualizations of Paired Comparisons (Graves et al., 2019) is used to form the compact letter display in relation to Tukey's HSD test in graphs. rcompanion: Functions to Support Extension Education Program Evaluation (Mangiafico, 2022) is used to form the compact letter display in relation to Dunn's test in graphs. dplyr: A Grammar of Data Manipulation (Wickham et al., 2022) was used to organize and manipulate data in tables required for the creation of graphs. ggplot2: Elegant Graphics for Data Analysis (Wickham, 2016) allowed for the creation of complex graphs which supported the various compact letter displays for pairwise comparisons. ggpubr: 'ggplot2' Based Publication Read Plots (Kassambara, 2020) was a support package to ggplot2 which included the ability to make box plots, change font, and make other edits to the original graphs.

Tetrazolium Chloride (TZ) Testing Protocols

The following protocols were adapted from the AOSA Tetrazolium Testing Handbook (Miller, 2010). Most of the species in the experiment were not included in the handbook, and so generalized protocols for families were edited according to species-specific characteristics and assimilation into the rest of the experiment.

All protocols begin with seed in the germination pouch after three weeks in the growth chamber and utilize an overnight soak in an incubator (Shel Lab, Cornelius, OR) at 29-30 ºC. As recommended by Miller (2010), if seed was unable to be evaluated directly after an overnight soak, it was stored in the refrigerator at 4 ºC until evaluation.

Apiaceae—*Eryngium yuccifolium*

Each seed was sliced longitudinally from distal end just into the endosperm with a razor blade before being soaked overnight in 1.0% TZ solution. Seed was evaluated as viable if, once completely sliced longitudinally, the endosperm and embryo were completely or mostly stained. In the case that the three weeks of germination softened the seed so much that it was unable to be sliced cleanly, the seed would first be squeezed gently with forceps until a small amount of endosperm showed, soaked overnight, and then torn apart to evaluate.

Apocynaceae—*Asclepias tuberosa*

Each seed was squeezed gently with forceps until a small amount of endosperm showed and soaked overnight in 0.1% TZ solution. Seed was evaluated as viable if, when torn apart, it was completely or mostly dyed.

Asteraceae—*Echinacea pallida*, *Liatris pycnostachya*, *Ratibida columnifera*, *Rudbeckia hirta*,

Each seed was squeezed gently with forceps until a small amount of endosperm showed and soaked overnight in 0.1% TZ solution for *Ratibida columnifera* and *Rudbeckia hirta*, 1.0% TZ solution for *E. pallida* and *L. pycnostachya*. Seed was evaluated as viable if, when torn apart, it was completely or mostly dyed.

Asteraceae—*Silphium laciniatum*

Each seed was sliced longitudinally from distal end just into the endosperm with a razor blade before being soaked overnight in 1.0% TZ solution. Seed was evaluated as viable if, once completely sliced longitudinally, the endosperm and embryo were completely or mostly stained.

Fabaceae—*Baptisia alba*, *Baptisia australis*

Each seed was clipped with a razor blade to expose the endosperm before soaking overnight in 1.0% TZ solution. Seed was evaluated as viable if, once completely sliced longitudinally, the endosperm and embryo were completely or mostly stained. In the case that the three weeks of germination softened the seed so much that it was unable to be sliced cleanly, the seed would first be squeezed gently with forceps until a small amount of endosperm showed, soaked overnight, and then torn apart to evaluate.

Poaceae—*Andropogon gerardii*, *Bouteloua curtipendula*, *Panicum virgatum*, *Schizachyrium scoparium*, *Sorghastrum nutans*

Each seed was pierced in the endosperm with a needle, excluding *P. virgatum*, before being soaked overnight in 1.0% TZ solution. Seed was evaluated as viable if, once sliced longitudinally, the embryo was completely or mostly stained. In the case that the three weeks of germination softened the seed so much that it was unable to be sliced cleanly, the seed would first be squeezed with forceps to puncture the endosperm, soaked overnight, and then torn apart to evaluate. The exception is *B. curtipendula*, which had clusters of papery, difficult to dissect seed. The stained embryos, however, were visible due to the translucent seed coat and thus able to be counted without dissection.