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The Effects of Gibberellic Acid, Smoke Water, and Cold Stratification on the Germination of Native Perennial Seed

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Abstract

In this study, I evaluated the germination rates of twenty native plant species and their response to cold stratification as well as four chemical solution treatments—water (control), gibberellic acid, smoke water, and gibberellic acid with smoke water. Seeds were evaluated and counted twice a week. Of the twenty evaluated species, sixteen had germination rates over 3%, or 1 seed out of 30. Stratification increased germination by 54% when compared to the control. For chemical treatments, gibberellic acid and gibberellic acid with smoke water were significantly different from the control and increased germination by an average of 47% and 48%, respectively. Six species showed a response to chemical treatment and stratification. Four species showed a response to chemical treatments only, and four species showed a response to stratification only. No growth defects were discovered upon further evaluation after sowing the seed. This study suggested that the combination of stratification with smoke water and/or gibberellic acid has a significant effect on seed germination yield of some native perennials.

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Chapter 1

Introduction

Plant Propagation and Seed Dormancy

Plant propagation is an important process in the production of horticultural crops and the conservation of native plants. While both seed and vegetative propagation are options for growers, propagating plants via seed offers many benefits including genetic variety, hybrid vigor, and decreased risk for disease transmission. Also, some species may only be able to propagate via seed (Sorenson and Garland, 2021).

Seed germination can be affected by many physical and chemical factors and is a process controlled by internal physical regulating factors. Most seeds require moisture, oxygen, and favorable temperature for germination, while some may also have required specific light requirements. Additionally, many native species have some type of seed dormancy. Seed dormancy is believed to be a mechanism that evolved to allow seeds to germinate only under favorable environmental conditions (e.g., moisture, light, temperature). While seed dormancy is an evolutionary advantage for many species, overcoming seed dormancy is a challenge that many growers face when propagating a more diverse plant palette. Without overcoming the dormancy factors, the quality and quantity of the crop can be affected (Jefferson et al., 2014).

Dormancy Classifications

Therefore, understanding seed dormancy classifications and the mechanisms to overcome these types hold importance in improving plant production within the horticulture industry. Baskin and Baskin (2004) classified seed dormancy into five different categories: physiological dormancy, morphological dormancy, morphophysiological dormancy, physical dormancy, and combinational dormancy. Physiological dormancy and morphological dormancy are the most common forms of dormancy (Jefferson et al., 2014).

In physiological dormancy chemical changes need to occur for embryo growth and germination. There are several ways to overcome this type of dormancy. Gibberellic acid, cold or warm stratification, scarification, and dry storage are all treatments that may help overcome physiological dormancy. However, treatment varies species to species and depends on the level of physiological dormancy. There are three levels of physiological dormancy, deep, intermediate, and nondeep (Baskin and Baskin, 2004).

Morphological dormancy occurs when the embryo is underdeveloped. In seeds with this type of dormancy, there is no dormancy breaking pre-treatment required. A period of ripening is needed for the embryo to mature before germination. This process may take up to 30 days (Baskin and Baskin, 2004).

Morphophysiological dormancy occurs when the embryo is underdeveloped, and chemical changes need to occur for embryo growth and germination. Typically, these seeds require some sort of treatment prior to seed germination. The seed germination process for seeds with morphophysiological dormancy is longer than those that experience morphological dormancy (Baskin and Baskin, 2004). In addition, seeds that face morphophysiological dormancy may be put through a treatment called stratification. Cold stratification is the process of chilling a seed in moist conditions for a certain length of time. This process enables the underdeveloped embryo to become fully mature (Luna et al., n.d).

Seeds with physical dormancy have a thicker seed coat that does not easily allow water to penetrate its layers. (Baskin and Baskin, 2004). This seed coat adaptation does not permit the entering of water and oxygen into the seed. Various environmental factors may cause the seed coats to become permeable over time or during a certain time of year. One treatment used to overcome physical dormancy is the process of scarification. Scarification is the breaking of the seed coat to allow water and oxygen into the seed. Scarification may be either a chemical or mechanical process. Chemical scarification may include the use of organic solvents such as alcohol, sulfuric acid, or boiling water. Mechanical scarification may include nicking the seed coat with a knife or using abrasive materials such as sandpaper (Luna et al., n.d).

Combinational dormancy is a combination of physical dormancy and physiological dormancy. Seeds with this type of dormancy have thick seedcoats and require a chemical change to initiate embryo growth and germination. To overcome combinational dormancy, scarification is required first followed by a period of cold stratification (Baskin and Baskin, 2004).

Of the above dormancy types, physiological dormancy and morphophysiological dormancy are interesting because of the role that smoke and gibberellins can play in overcoming dormancy.

Smoke

Fire has been observed for thousands of years to increase the germination of certain plants after a burn. This phenomenon was not officially reported until 1977 when smoke was first scientifically linked to seed germination promotion. Since then, the exact mechanism has been under some debate. When fire sweeps through an ecosystem, the extreme temperatures and chemicals released have effects on the environment. Over time, fire has influenced genetics, plant diversity, and their response to environmental cues. Fire removes vegetation, increases inorganic nutrient availability, decreases microbial populations, and decreases herbivore populations within an environment. Additionally, smoke may also have a positive effect on germination, but in a few cases, it has been reported that smoke may inhibit germination for certain species. Jefferson et al. (2014) describes the use of smoke as one way to overcome physiological and morphophysiological dormancies in specific species. This has been found in many species in a variety of fire prone ecosystems including Australia, South Africa, and even California. Elsadek and Yousef (2019) suggested that smoke contains four active compounds known to stimulate germination. These compounds have been isolated and identified as karrikins, cyanohydrins, butenolides, and hydroquinones. Of these four compounds, karrikins are interesting because of their interaction in promoting the gibberellin pathway (Nelson et al., 2009). Research has shown that smoke can help overcome dormancy of some species and suggested that karrikins, a recently discovered plant hormone that acts similar to strigolactone hormones, which promote germination, may be a contributing factor. In general, seed germination is triggered by internal growth regulators known as gibberellins. The plant hormone, gibberellic acid, is known to be a germination promoter for plant species (Elsadek and Yousef, 2019). Knowing this information can help the horticulture industry better understand and increase the germination rates of specific species of crops.

Smoke Treatments

The use of smoke to improve crops has been practiced for centuries. Current discoveries have led to a deeper understanding of smoke and its effects on certain species. Smoke treatment research has been conducted around the world. In 2012, researchers in Argentina studied the effects of fire on *Fabiana imbricata*, a shrub species, in Patagonian grasslands. Seeds were collected and stored at room temperature. Researchers later tested the effects of scarification. Half of the seeds were scarified using sandpaper abrasion while the other half were left unscarified. Each treatment consisted of 10 replicates containing 100 seed. Researchers then treated the seeds with one of the following factors: no treatment control, smoke, charcoal, heat, and scarification. For smoke treatments, controlled combustion was used to pump smoke into a hermetically sealed box containing seed. Seeds were then exposed to the smoke for 10 minutes. After seeds had been exposed to their treatments, they were then placed in a germination chamber set to imitate the environmental conditions of a natural germination season. In this case, the chamber was set to have 12 hours of light at 19˚C and 12 hours of darkness at 5˚C. Data were collected once a week for 3 months. Results suggested that germination percentages increased significantly when treated with both scarification and smoke. For this research, it was determined that fires were leading to an increase in the *Fabiana imbricata* population and a decrease in biodiversity within Patagonian grasslands (Dudzinsky and Ghermandi, 2013)

In addition, in 2011 the use of smoke water was proven successful in its effects on the germination rate of native medicinal plants in China. Researchers collected seed from *Astragalus mambranaceus*, *Panax notoginseng*, and *Magnolia officinalis*. Seeds were placed in plant derived smoke water and stored under the following six different temperatures: 10, 15, 20, 25, 30, and 35˚C. Four replicates for each treatment contained 25 seeds each. Germination data was collected 20 days after planting in a soil substrate, and seedling growth was further evaluated. While researchers concluded that *Panax notoginseng* exhibited the highest germination rate, the smoke water enhanced germination of all three species significantly. When treated with smoke-water, seedlings of *Astragalus membranaceus* showed a significant increase in shoot length compared to the control seedlings. In addition, smoke-water treatment also showed a significant increase in root growth of each species when compared to the control. At 10˚C with adequate light in combination with high nutrients, maximum germination and growth was achieved for all seedlings. This further suggested that smoke water can be beneficial for seed germination and seedling growth (Zhou et al., 2011).

During March of 2017, a group of researchers also studied the germination response to smoke of different plant species in habitats of Western Asia. This study was conducted in the scrublands of Golestan National Park. Seeds were collected from a soil seed bank during September. The species of seed collected came from the following five groups: legumes, annual forbs, annual grasses, perennial herbs, and perennial grasses. The seeds were then placed under one of the four following treatments: aerosol smoke for 15 minutes, aerosol smoke for 30 minutes, diluted smoke water at a 1:1000 concentration, and diluted smoke water at a 1:500 concentration. Samples were kept moist with tap water and placed under natural lighting within a greenhouse. The smoke water was prepared by mixing distilled water with a smoke extract derived from the burning of plant material. Those samples treated with the smoke water were treated every two weeks with 250 mL of the solution. Samples treated with aerosol smoke were placed in a tent with smoke produced by the burning of plant materials for either 15 or 30 minutes. Germination count data were collected for six months. Germination percentages increased significantly among seeds receiving smoke treatments. Aerosol smoke treatments had a higher germination rate than the smoke water. Furthermore, this study suggested that there is a positive correlation between smoke enhancements and germination rates (Abedi et al., 2017).

Smoke and its effects on germination has also been studied throughout the United States. In the spring of 2004, a series of smoke treatments were performed on thirty-seven midwestern prairie species in the United States. Gaseous smoke was used from a beekeeper’s smoker by pumping smoke through a plastic hose into a smoke chamber that contained the test species. The smoke treatment was repeated four times using 25 seeds per species per treatment. The control was not treated with smoke, whereas the treatments replicates were treated with 1, 10, or 60 minutes of aerosol smoke. After being treated, seeds were sown in a germination substrate covered with vermiculite. Trays were watered on a mist bench and kept in ambient light at 18˚C. Seedling counts were taken twice a week for three weeks. In the end, researchers reported that 13 of the 37 species responded positively to all the smoke treatments; however, those treated with 60 minutes of aerosol smoke had a much higher response than those that were treated for a shorter time interval. The most significant increase in germination percentage was shown in 5 out of 6 *Echinacea* species tested. To contrast, the article stated that the smoke treatments had a negative effect on three of the species tested: *Silene regia, Monarda fistulosa, and Epilobium glandulosum*. In their research, the authors indicated that smoke does in fact play a major role in promoting seed germination and may aid in the conservation of endangered species (Jefferson et al., 2008).

Wada and Reed (2011) tested the effects of several treatments on the germination rates of 17 *Rubus* species. Treatments consisted of deionized water, gibberellic acid with potassium nitrate, gibberellic acid, and a smoke gas solution. All seeds were scarified using sodium hypochlorite and concentrated sulfuric acid then placed on germination blotters soaked in deionized water. Then, seeds were soaked in one of the four previously mentioned treatments. All seeds were then stratified for one month at 18˚C with a 16-hour photoperiod. The smoke gas solution was obtained by soaking smoke infused paper in deionized water at 4˚C for 24 hours. Data counts were performed one month after stratification and then once a month for four to twelve months. In the end, results suggested that the treatments enhanced the germination rates of six of the seventeen tested species (Wada and Reed, 2011).

In spring of 2015, smoke water treatments were tested on 10 *Penstemon* species native to western North America. *Penstemon* seed was collected from 9 habitats in Montana, Colorado, New Mexico, and Wyoming. Seeds were air dried and stored at 22˚C until ready for later use. Treatments included smoke with stratification or smoke with no stratification. Seeds that were stratified were chilled for 10 weeks. The selected smoke method used for this experiment was a smoke water solution using a smoke water concentrate. Seeds receiving smoke water treatments were soaked for 12 hours in 15 mL of solution. Seeds were then drained and placed in Petri dishes. Stratified seeds were placed in an incubator for 10 weeks at 5˚C, while seeds not stratified were placed into an incubator at 22˚C with a 12-hour photoperiod. Once the stratification period was over, stratified seeds were placed in an incubator with the same conditions as the non-stratified seed. Germination counts for both stratified and non-stratified seeds were taken every day for one week then every two days for 23 days. Researchers concluded that percent germination for 3 of the 10 *Penstemon* species tested was enhanced by smoke treatments while one species was inhibited. Furthermore, percent germination for 6 species increased when stratified. These findings led to a better understanding of smoke's role in *Penstemon* germination (Fornwalt, 2015).

Gibberellins

Gibberellins are hormones that stimulate plant growth and development. Over 100 forms of gibberellins have been discovered, but the most common naturally occurring ones are GA_1, GA_4, and GA_7. The gibberellic acid sold commercially, GA_3, is produced by fungi. Gibberellins are known to trigger seed germination, stem elongation, and flowering while also determining sex expression and grain development. Gibberellic acid works to increase embryo growth potential and induces hydrolytic enzymes to aid in seed germination. However, the exact mechanism of GAs in plant growth and seed germination is not entirely understood (Gupta and Chakrabarty, 2013).

Studies have shown that GA increases germination in a variety of species and genera. Research was done on GA and its effects on seed germination in *Penstemon digitalis.* Seeds were soaked for 24 hours in 50 mL of six different concentrations of GA treatments. The GA concentrations were 0, 10, 50, 100, 200 and 500 mg/L^{-1}. Once seeds were soaked, they were then filtered, rinsed under distilled water for two minutes, placed into filter paper and then placed into a Petri dish. There were two seed germination experiments placed in dark growth chambers at 21.3˚C and a third seed germination experiment was potted and placed in a greenhouse at 32˚C during the day and 24˚C during the night. There was an observed increase in germination for each concentration of GA per experiment on average starting with the control at 9%, 21%, 18%, 26%, 33%, and 54%, respectively. For each experiment, not only was seed germination increased but rate of germination was increased as well. The higher the concentration of GA, the less days the seeds took to germinate when compared to the control (Mello et al., 2009).

A similar study examined temperature and gibberellic acid effects on *Eranthis hyemalis* seed. Initially, seeds were sterilized by washing them in distilled water, soaked in 96% alcohol for 3 minutes, soaked in 30% sodium hypochloride for 20 minutes, and washing in distilled water. Seeds were then soaked in solutions of distilled water with gibberellic acid concentrations of either 0.1, 5 or 10 mM for 24 hours. Seeds were then placed in germination dishes and left in a growth chamber at 23˚C and in a refrigerator at 4˚C. Germination percentages were evaluated after 69, 75, 90, 105, and 120 days. There was no observed germination for the control and treatment groups placed in the growth chamber. However, for those placed in the refrigerator there was an observed increase for those placed in GA when compared to the control. On average, starting with the control, germination percentages were 76%, 87%, 84%, and 81%, for the 0.1, 5.0, and 10 mM treatments, respectively. In this case, those treatments with higher GA concentrations had lower germination percentages than treatments with lower GA concentrations. Results revealed that gibberellic acid along with cooler temperature caused a significant increase in germination of *Eranthis hyemalis* seed (Tipirdamaz and Gomurgen, 2000).

According to previous research, it is obvious that smoke enhances the germination counts of specific species; however, these studies have demonstrated that smoke treatments can cause a variety of responses in different species. There are not many resources that have researched the effects of smoke water and gibberellins alone and in combination on southern native plant species in the United States with stratification treatments. Thus, the purpose of this study is to evaluate and compare the effects of temperature in combination with gibberellic acid and smoke water on 20 native plant species. With the knowledge gained from this research, the hope is to help horticulturists and native plant conversationists better propagate a more diverse palette of plants.

Chapter 2

Materials and Methods

Seeds

Seeds were sown from twenty different species for this experiment at Stephen F. Austin State University in Nacogdoches, TX in 2020 (Table 1). Eleven species, *Agastache foeniculum, Amorpha canescens, Aquilegia canadensis, Arnoglossum plantagineum, Callirhoe digitata, Callirhoe involucrata, Liatris ligulistylis, Oenothera macrocarpa*, *Penstemon cobaea*, *Penstemon grandiflorus,* and *Phlox glaberrima subsp. interior* were purchased from Prairie Moon Nursery (Winona, MN), one species, *Muhlenbergia dumosa*, was donated by Hoffman Nursery (Rougemont, NC), and eight species, *Dalea villosa, Delphinium carolinianum, Ipomopsis rubra, Liatris aspera, Penstemon murrayanus, Sarracenia alata*, *Stenanthium gramineum,* and *Verbesina virginica* were collected from locations in East Texas. Seeds were stored at room temperature before applying treatments.

Species Description

Asteraceae

Arnoglossum plantagineum- Commonly known as Indian plantain or prairie plantain, is a perennial of the aster family. It can be found in prairies, fields, and wetland habitats throughout east, southeast, and central Texas up to the Edwards Plateau. In Texas, *A. plantagineum* blooms during the warmer months of June, July, and August. However, it is known for its thick, rubbery foliage more than its white blooms (Kartesz, 2021; NPIN, 2021). *A. plantagineum* seed germinates best after a two-month cold, moist stratification period (Prairie Moon Nursery, 2021).

Liatris aspera- Commonly known as tall blazing star or tall gay feather, is a perennial member of the aster family. It can be found in thin woods, dry plains, and prairies throughout Texas up through the Midwest into Canada and stretching to the eastern United States. *L. aspera* is known for its pink or lavender spike rounded flower heads and four-foot-tall erect fuzzy stems. In Texas, *L. aspera* is known to bloom during the months of August and September. *L. aspera* may be found in pollinator gardens as it is a natural attractor of hummingbirds and butterflies. This species germinates best after scarification and a three-month cold stratification period (Kartesz, 2021; NPIN, 2021).

Liatris ligulistylis- Commonly known as rocky mountain blazing star or meadow blazing star, is a perennial member of the aster family. This species can be found in rocky woods, rocky slopes, along streams, and prairies in New Mexico east to Wisconsin and Missouri and to Canada. *L. ligulistylis* is known for its deep purple fluffy feathery flowerheads which attracts butterflies, specifically Monarchs. This plant is said to bloom from July to September (Kartesz, 2021; NPIN, 2021). *L. ligulistylis* seed germinates best after a two-month cold, moist stratification period (Prairie Moon Nursery, 2021).

Verbesina virginica- Commonly known as frostweed or ice plant, is a perennial or sometimes biennial member of the aster family. It can be found at the edge of woodlands and along streambanks throughout Texas up to central Iowa stretching to the eastern United States from Florida to Maryland. *V. virginica* is known for its white flower heads and dark green leaves. During the winter, its roots are known to divide and grow frost flowers, an interesting ice phenomenon. In Texas, *V. virginica* is known to bloom from August to September. This species is best used as a transitional plant and attracts butterflies (Kartesz, 2021; NPIN, 2021).

Fabaceae

Amorpha canescens- Commonly known as leadplant, is a legume in the pea family native to habitats such as open woodlands, rocky bluffs, and prairies. *A. canescens* can be found throughout Texas up throughout the midwestern United States and into Canada. This species contains small purple or blue single petal flowers that are grouped together in the form of spikes. Its leaves are covered in fine grey hairs. *A. canescens* blooms during the months of June and July. Due to its sweet nectar, it attracts beneficial insects such as bees. Scarification and stratification are often required for seed germination in the species (Kartesz, 2021; NPIN, 2021). *A. canescens* seed were provided by Prairie Moon Nursery and were scarified prior to receiving them (Prairie Moon Nursery, 2021) suggested that *A. canescens* seed germinates best after a 10-day cold, moist stratification period.

Dalea villosa- Commonly known as silky prairie clover, is a deciduous shrub in the pea family that is native to habitats such as sandy meadows, prairies, and fields. Like *A. canescens, D. villosa* grows in clumps and contains single petal flowers resembling spikes with leaves and stems covered in fine hairs. Though similar in appearance, *D. villosa* is much smaller in height and leaf span than *A. canescens*. *D. villosa* is in bloom from July to August and can be found throughout Texas up throughout the midwestern United States and into Canada. This species is listed as a threatened species throughout its range and important to beneficial insects such as honey and bumble bees (Kartesz, 2021; NPIN, 2021). Prairie Moon Nursery (2021) suggested that *D. villosa* seeds germinate best in warm environments after scarification and no stratification period is needed.

Lamiaceae

Agastache foeniculum- Commonly known as anise hyssop, is a perennial member of the mint family. It can be found in open and dry habitats such as prairies and roadsides. During July and August when it blooms, *A. foeniculum* can be distinguished by its small tubular blue or purple flower cluster. This species is known to attract butterflies and hummingbirds with its sweet nectar and is important to native bees. *A. foeniculum* can be found in Alabama, upper midwestern and eastern states, and Canada (Kartesz, 2021; NPIN, 2021). *A. foeniculum* seed germinates best after a one-month cold, moist stratification period and requires light to germinate due to the small size of the seed (Prairie Moon Nursery, 2021).

Melanthiaceae

Stenanthium gramineum- Commonly known as eastern feather bells is a perennial member of the lily family that blooms from June to September. This species can be found in open areas, rocky woods, and sand bogs throughout Texas to the east coast. *S. gramineum* can be identified by its small white nodding flowers in the shape of a pyramidal panicle (Kartesz, 2021; NPIN, 2021).

Malvaceae

Callirhoe digitata- Commonly known as winecup or poppy mallow, is a perennial member of the mallow family. It can be found in native habitats such as open woods, dry prairies, and grassy slopes. *C. digitata* is distributed in the following seven states: Georgia, Louisiana, Arkansas, Oklahoma, Kansas, Missouri, and Illinois. *C. digitata* may be identified by its magenta colored five petaled cup shaped flowers, that bloom from April to August, which attract butterflies (Kartesz, 2021; NPIN, 2021). Prairie Moon Nursery (2021) suggested that *C. digitata* seed be scarified by soaking the seed in a hot water treatment for 24 hours prior to sowing. After scarification treatment, *C. digitata* seed germinates best after a one-month cold, moist stratification period (Prairie Moon Nursery, 2021).

Callirhoe involucrata- Commonly known as purple poppy mallow, is a perennial member of the mallow family. It can be found in native habitats such as open woods and on rocky hills in shrublands and thickets. *C. involucrate* is distributed in Oregon, Florida, Pennsylvania and through central and midwestern United States. When in bloom from March to June, *C. involucrate* may be identified by its magenta colored five petaled cup shaped flowers which attract bees and butterflies. Scarification is important for germination in this species (Kartesz, 2021; NPIN, 2021). *C. involucrate* seed should be scarified by soaking the seed in a hot water treatment for 24 hours prior to sowing. After scarification treatment, *C. involucrate* seed germinates best after a one-month cold, moist stratification period (Prairie Moon Nursery, 2021).

Poaceae

Muhlenbergia dumosa- Commonly known as bamboo muhly, is a drought tolerant perennial member of the grass family. It can be identified by its fine textured foliage. *M. dumosa* is natively distributed in Arizona (Hoffman Nursery, 2021; NPIN, 2021).

Polemoniaceae

Ipomopsis rubra- Commonly known as red Texas star or Texas plume, is a biennial member of the phlox family. It can be found in rocky fields and open woods throughout Texas, eastern United States, and Canada. *I. rubra* attracts hummingbird with its nectar and bright red or orange tubular flowers. This species blooms from May to July (Kartesz, 2021; NPIN, 2021).

Phlox glaberrima subsp. interior- Commonly known as marsh phlox, is a perennial member of the phlox family found in wet woods and thickets. This species can be found throughout Texas and the eastern United States. When in bloom from June to September, *P. glaberrima* ssp. *interior* can be identified by its pink and purple partially joined five petaled flowers. During the spring, this species is important to butterflies and hummingbirds (Kartesz, 2021; NPIN, 2021). Prairie Moon Nursery (2021) suggested that *P. glaberrima* ssp. *interior* seeds be stored at 3˚C prior to sowing or pretreatments and will germinate best after a two-month cold, moist stratification period.

Ranunculaceae

Aquilegia canadensis- A perennial member of the buttercup family, is commonly known as eastern red columbine. This beautiful wildflower can be identified through its drooping bell like red and yellow flowers. *A. canadensis* can be found throughout Texas and the Midwest all the way to the eastern coast of the United States, Louisiana excluded and up through Canada. This species can be found in partly shaded wooded habitats blooming from February to July. *A. canadensis* is beneficial to wildlife such as hummingbirds, bees, butterflies, and hawkmoths (Kartesz, 2021; NPIN, 2021). This species germinates best after a two-month cold, moist stratification period (Prairie Moon Nursery, 2021).

Delphinium carolinianum- A perennial member of the buttercup family and is commonly known as Carolina larkspur or blue larkspur. When in bloom from April to July, this species can be identified by blue, lavender, and white, spurred flowers in a narrow cluster. *D. carolinianum* can be found in open woods, sandy hills, and brushlands through Arizona to the southeastern United States and north to Canada (Kartesz, 2021; NPIN, 2021).

Sarraceniaceae

Sarracenia alata- Commonly known as pale pitcher plant, is a perennial member of the pitcher-plant family and is a carnivorous plant. It has hollow leaves with an open partially covered top. During the blooming months of March and April, its yellow flowers face downward hanging from the tip of the stem. *S. alata* can be found in native habitats such as marshes and bogs in Alabama, Mississippi, Louisiana, and Texas (Kartesz, 2021; NPIN, 2021).

Plantaginaceae

Penstemon cobaea- Commonly known as prairie beardtongue or wild foxglove, is a perennial member of the figwort family. When in bloom during April and May, *P. cobaea* can be identified by its white or pink flowers with dark purple lines inside the floral tube. This species is known to attract moths, butterflies, and hummingbirds. *P. cobaea* can be found in open hillsides, plains, and prairies in Arkansas, Arizona, Colorado, Iowa, Illinois, Kansas, Missouri, Nevada, New Mexico, Ohio, Oklahoma, and Texas (Kartesz, 2021; NPIN, 2021). *P. cobaea* seed germinates best after a one-month cold, moist stratification period (Prairie Moon Nursery, 2021).

Penstemon grandiflorus- Commonly known as large beardtongue, is a perennial member of the figwort family. This species is native to dry prairies throughout Texas up through the Midwest and Connecticut. This beautiful plant blooms during May and June and holds special importance to bumble bees. *P. grandiflorus* can be identified by its large, downward hanging lavender, tubular flowers.(Kartesz, 2021; NPIN, 2021). A one-month cold, moist stratification period is normally required to achieve the best germination for this species (Prairie Moon Nursery, 2021).

Penstemon murrayanus- Commonly known as scarlet beardtongue, is a perennial member of the figwort family. Like *P. cobaea* and *P. grandiflorus*, *P. murrayanus* has downward hanging tubular shaped flowers. From April to June, the showy scarlet flowers attract hummingbirds with its sweet nectar. This species can be found in habitats such as prairies, plains, and meadows in Arkansas, Louisiana, Oklahoma, and Texas. Generally, cold stratification is required for seed germination in *P. murrayanus* (Kartesz, 2021; NPIN, 2021).

Onagraceae

Oenothera macrocarpa- Commonly known as big fruit evening primrose, is a perennial member of the evening primrose family. When in bloom from April to August, it can be identified by its 4-inch-wide yellow flowers. This species is native to habitats such as roadsides, bluffs, hillsides and rocky prairies in Arkansas, Illinois, Kansas, Missouri, Nevada, Oklahoma, Tennessee, and Texas. *O. macrocarpa* is important to hawk moths and hummingbirds (Kartesz, 2021; NPIN, 2021). *O. macrocarpa* seed germinates best after a two-month cold, moist stratification period (Prairie Moon Nursery, 2021).

Preparation

To test for germination, the technique was utilized as described in *Seed Germination Theory and Practice* by Dr. Norman C. Deno (1993). Baggies®, a polyethylene bag, and paper towels supplied by Stephen F. Austin State University were used to incubate seeds for germination. For each replicate, a paper towel was folded into a 10.16 × 10.16 cm square and moistened. Cut from polyethylene bags, a 7.62 × 7.62 cm square that housed a 5.08 × 5.08 cm square cut from paper towel was placed into the larger 10.16 × 10.16 cm moistened paper towel square. The larger 10.16 × 10.16 cm square helped to moderate humidity, and the plastic 7.62 × 7.62 cm square held the smaller 5.08 × 5.08 cm square that was treated with 1 mL of one of four chemical treatments: control (deionized H_2O), gibberellic acid (GA), smoke water (SW), and gibberellic acid with smoke water (GA+SW). The gibberellic acid mixture was composed of 0.05 g of solid gibberellic acid dissolved in 100 mL of deionized water to achieve a 0.00050 g/mL aqueous concentration (Deno, 1993).

Wright's® Hickory Flavored Liquid Smoke was used for this experiment. It is composed of water and natural hickory smoke concentrate made from smoke of hickory wood. To achieve an 1:50 ratio aqueous smoke solution, 2.0 mL of Wright's® Hickory Flavored Liquid Smoke was used diluted in 100 mL of deionized water. For each species, the control and GA treatments were administered before the smoke water treatments to minimize the effect from the hormones in the smoke water.

Once the chemical treatments had been administered, the 10.16 × 10.16 cm square that held the plastic 7.62 × 7.62 cm square and smaller 5.08 × 5.08 cm square was folded in half lengthwise so that the seeds were in good contact with the 5.08 × 5.08 cm square that held the treatment. This folded stack was then placed into a non-cut Baggie®. Each Baggie® contained 30 seeds in each 5.08 × 5.08 cm paper towel square resulting in 720 seeds being used per species. Six replicates were prepared per species of seed for each treatment. These replicates were then divided into two groups of three to expose them to different air temperature treatments, unstratified at 22°C and stratified at 3°C.

Data Collection and Statistical Analysis

Seeds in the unstratified treatment were kept in Agriculture room 118 at 22°C, and number germinated was counted twice a week for four weeks. Seeds in the stratified treatment were stored in a cooler for 3 months at 3°C. After 3 months, stratified seeds were removed from the cooler then number germinated was counted twice a week for two weeks. Seeds were considered to be germinated once the radicle had emerged. Germinated seed were then potted up and evaluated for changes in growth and physiology. SAS version 9 from Stephen F. Austin State University to statistically analyze the data. The PROC GLM procedure was used to determine the statistical significance of temperature and chemical interactions for the entire data set and then used to break down data for each individual species. The alpha value was 0.05. Least squares means was used to separate the treatment groups within each species.

Table 1. Seven hundred and twenty seeds derived from various locations were used for each prairie forb species. Seeds were sown on different days.

Family	Species	Source	Date Sown
Asteraceae	*Arnoglossum plantagineum*	Prairie Moon Nursery	6/24/2020
	Liatris aspera	East Texas	5/27/2020
	Liatris ligulistylis	Prairie Moon Nursery	7/8/2020
	Verbesina virginica	East Texas	5/27/2020
Fabaceae	*Amorpha canescens*	Prairie Moon Nursery	7/8/2020
	Dalea villosa	East Texas	9/16/2020
Lamiaceae	*Agastache foeniculum*	Prairie Moon Nursery	6/30/2020
Melanthiaceae	*Stenanthium gramineum*	East Texas	9/16/2020
Malvaceae	*Callirhoe digitata*	Prairie Moon Nursery	6/24/2020
	Callirhoe involucrata	Prairie Moon Nursery	6/24/2020
Poaceae	*Muhlenbergia dumosa*	Hoffman Nursery	6/24/2020
Polemoniaceae	*Ipomopsis rubra*	East Texas	9/16/2020
	Phlox glaberrima ssp. interior	Prairie Moon Nursery	7/8/2020
Ranunculaceae	*Aquilegia canadensis*	Prairie Moon Nursery	7/8/2020
	Delphinium carolinianum	East Texas	9/16/2020
Sarraceniaceae	*Sarracenia alata*	East Texas	5/27/2020
Plantaginaceae	*Penstemon cobaea*	Prairie Moon Nursery	6/30/2020
	Penstemon grandiflorus	Prairie Moon Nursery	6/30/2020
	Penstemon murrayanus	East Texas	9/16/2020
Onagraceae	*Oenothera macrocarpa*	Prairie Moon Nursery	6/30/2020

RESULTS AND DISCUSSION

Overall Species Effects

Of the twenty species evaluated, 16 species had an average of at least 3.3% seed germination (1/30) seeds germinated (Table 2). Two of the 16 species (*Liatris aspera, Callirhoe involucrata*) had no statistically significant chemical and temperature effect. The remaining 4 species had less than 3.3% percent germination for chemical and temperature treatments and were excluded from the statistical analysis. These species were *Sarracenia alata, Muhlenbergia dumosa, Phlox glaberrima* ssp. *interior,* and *Dalea villosa.* There is not enough evidence to suggest why these species either did not respond or had no statistically significant interaction with the treatment. There could be several reasons such as age of seed, location of seed source, dormancy state, natural environment, and response to light that could have caused variations within each species (Jefferson et al., 2014).

After running all 16 species together, GA and GA+SW were statistically significant with a p-value <0.0001. Both GA and GA+SW increased germination by an average of 47% and 48%, respectively. Smoke water was not significantly different from the control. Therefore, the data was broken down by individual species. Stratified treatment was statistically significant from the unstratified control as well and increased germination percentage by an average of 54%, respectively. After statistical analysis, the species were divided into three groups: chemical + temperature effects, chemical effects, and temperature effects.

Table 2. Treatment that exhibited best germination, increase in germination, decrease in germination, and no change in germination from the control in each species for chemical treatments of water, gibberellic acid (GA), smoke water (SW), and gibberellic acid and smoke water (GA+SW) and unstratified or stratified temperature treatments.

Family	Species	Unstratified				Stratified			
		H_2O	GA	SW	GA+SW	H_2O	GA	SW	GA+SW
Asteraceae	*Arnoglossum plantagineum*	-	INC	NO	INC	BEST	BEST	BEST	BEST
	Liatris aspera	-	NO	NO	NO	NO	NO	NO	NO
	Liatris ligulistylis	-	INC	NO	INC	BEST	BEST	INC	BEST
	Verbesina virginica	-	NO	NO	NO	BEST	BEST	BEST	BEST
Fabaceae	*Amorpha canescens*	-	BEST	BEST	BEST	DEC	DEC	DEC	DEC
	Dalea villosa	-	-	-	-	-	-	-	-
Lamiaceae	*Agastache foeniculum*	-	BEST	BEST	BEST	DEC	INC	DEC	BEST
Melanthiaceae	*Stenanthium gramineum*	-	NO	NO	NO	INC	BEST	INC	BEST
Malvaceae	*Callirhoe digitata*	-	INC	BEST	INC	INC	INC	NO	INC
	Callirhoe involucrata	-	NO	NO	NO	NO	NO	NO	NO
Poaceae	*Muhlenbergia dumosa*	-	-	-	-	-	-	-	-
Polemoniaceae	*Ipomopsis rubra*	-	BEST	BEST	BEST	NO	BEST	BEST	BEST
	Phlox glaberrima ssp. interior	-	-	-	-	-	-	-	-
Ranunculaceae	*Aquilegia canadensis*	-	BEST	NO	INC	NO	BEST	NO	INC
	Delphinium carolinianum	-	NO	NO	NO	INC	BEST	BEST	BEST
Sarraceniaceae	*Sarracenia alata*	-	-	-	-	-	-	-	-
Plantaginaceae	*Penstemon cobaea*	-	BEST	NO	INC	INC	INC	INC	BEST
	Penstemon grandiflorus	-	BEST	INC	BEST	INC	INC	INC	INC
	Penstemon murrayanus	-	INC	NO	INC	NO	INC	BEST	BEST
Onagraceae	*Oenothera macrocarpa*	-	NO	NO	NO	INC	BEST	INC	BEST

Total Changes in Germination								
Best germination	0	6	4	4	3	8	5	10
Not best, but germination increased	0	4	1	6	6	5	5	3
Best + increase in germination	0	10	5	10	9	13	10	13
Decrease in germination	0	0	0	0	2	1	2	1

The overall effects of GA, SW, or a combination of the two along with non-stratified or stratified seed were evaluated on the 20 species by totaling the occurrences of best germination, an increase in germination over the control but not the best, a decrease in germination from the control, and no change from the control (Table 2). Overall, GA+SW stratified (10 species) had the most occurrences for best germination treatments. GA+SW stratified and GA GA+SW stratified both caused increases for 13 species, while GA unstratified, GA+SW unstratified, and SW stratified caused increases for 10 species. GA stratified and GA+SW stratified caused a decrease in 1 species, while SW stratified caused a decrease in 2 species. In many cases, unstratified SW showed no change in germination percentage from the control; however, unstratified SW did cause an increase in germination percentages for 5 species compared to unstratified water. On the other hand, stratified SW showed an increase in germination percentages for 10 species compared to the unstratified control but was no different from stratified water.

Chemical + Temperature effects

Six species (*Arnoglossum plantagineum, Liatris ligulistylis, Agastache foeniculum, Oenothera macrocarpa, Penstemon cobaea,* and *Penstemon murrayanus*) had a statistically significant chemical and temperature interaction (Table 3).

Table 3. Germination percentages of species that exhibited significant interaction for chemical treatments of water, gibberellic acid (GA), smoke water (SW), and gibberellic acid and smoke water (GA+SW) and unstratified or stratified temperature treatments.

Species	**p-value**	**Unstratified**				**Stratified**			
		Water	GA	SW	GA+SW	Water	GA	SW	GA+SW
Arnoglossum plantagineum	0.0003	9%	33%	21%	38%	20%	39%	46%	54%
Liatris ligulistylis	<0.0001	4%	9%	7%	3%	22%	40%	33%	44%
Agastache foeniculum	<0.0001	2%	78%	8%	59%	47%	31%	43%	66%
Penstemon cobaea	<0.0001	0%	40%	4%	39%	57%	78%	61%	56%
Penstemon murrayanus	0.0001	20%	56%	17%	57%	71%	79%	58%	77%
Oenothera macrocarpa	<0.0001	40%	83%	69%	70%	2%	64%	13%	81%

Asteraceae

Arnoglossum plantagineum

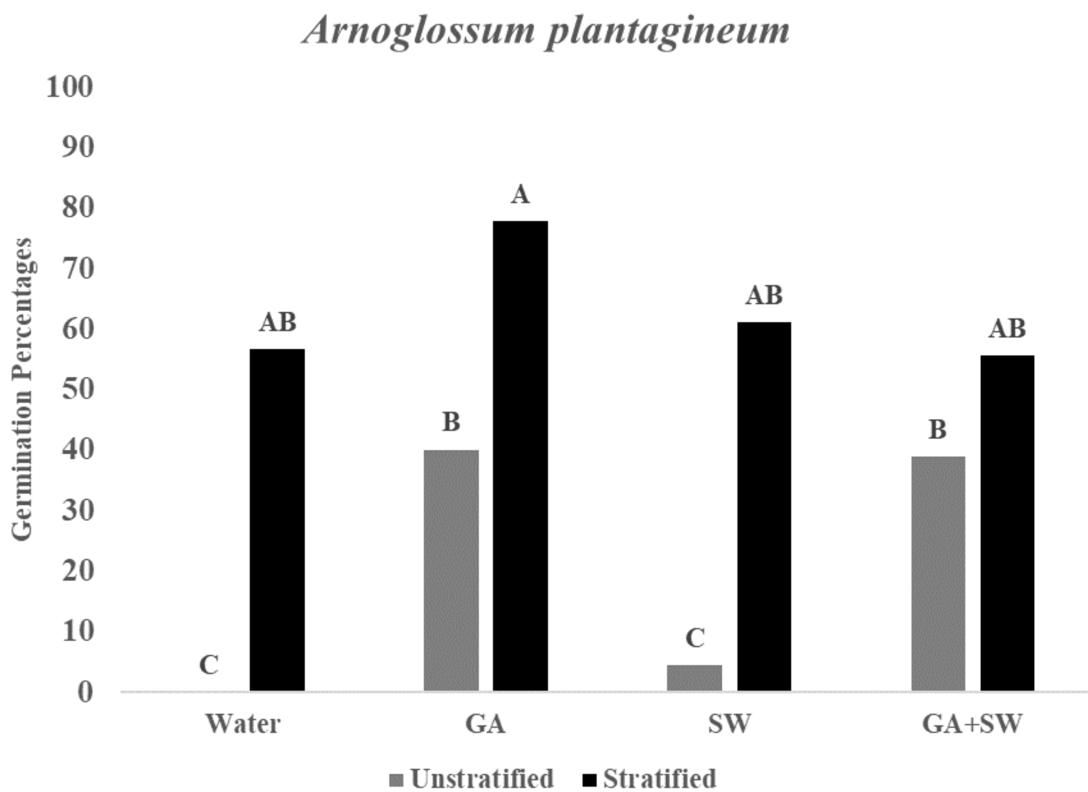

Figure 1. *Arnoglossum plantagineum* germination percentages for chemical treatments of water, gibberellic acid (GA), smoke water (SW), and gibberellic acid and smoke water (GA+SW) and unstratified or stratified temperature treatments

For *Arnoglossum plantagineum*, GA stratified (78%), SW stratified (61%), GA+SW stratified (56%), and water stratified (57%, Fig. 1) had the highest germination percentages, respectively compared to the unstratified control. *Arnoglossum plantagineum* can be found in tall prairies throughout the Midwest and into Canada. These are areas that usually have harder and longer winters, and prairies are known fire prone areas. The results seen here could be a result of provenance. (Winter et al., 2015).

This could explain why the higher germination percentages are amongst the stratified treatments. Prairie Moon Nursery (2021) suggested that *Arnoglossum plantagineum* seed germinates best after a two-month cold, moist stratification period.

Liatris ligulistylis

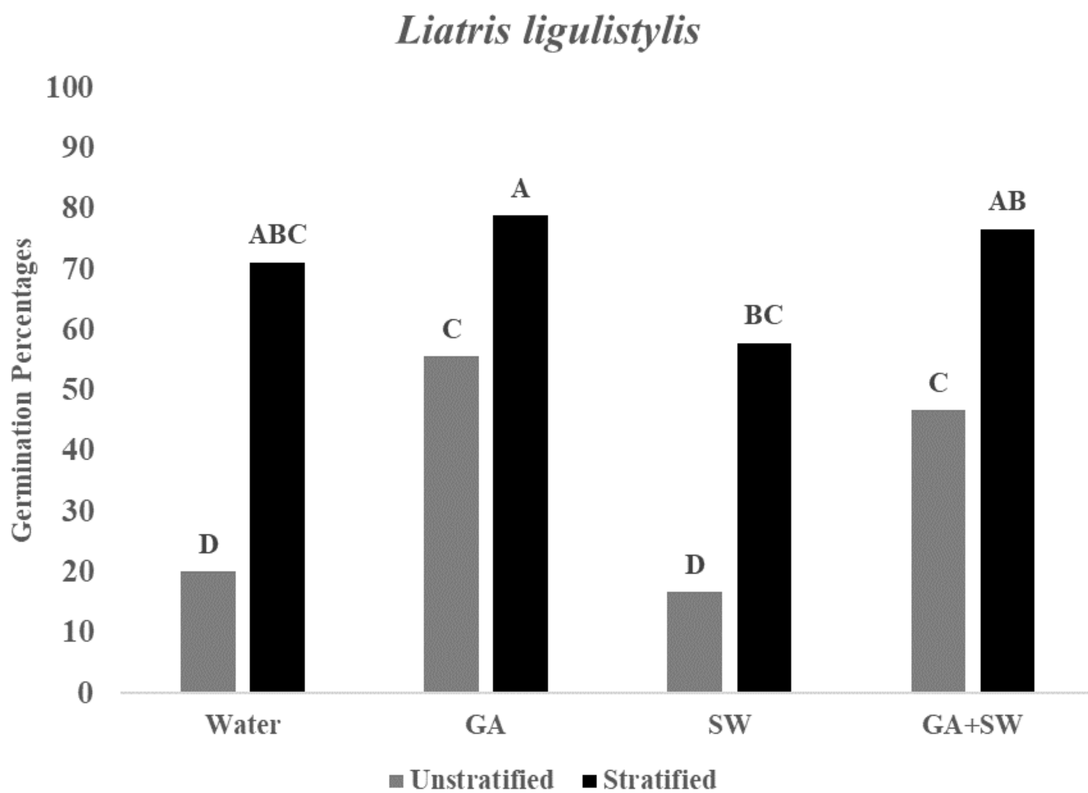

Figure 2. *Liatris ligulistylis* germination percentages for chemical treatments of water, gibberellic acid (GA), smoke water (SW), and gibberellic acid and smoke water (GA+SW) and unstratified or stratified temperature treatments

For *Liatris ligulistylis*, GA stratified (79%), GA+SW stratified (77%), and water stratified (71%) gave the best results respectively, compared to the unstratified control at 20% (Fig. 2). Interestingly, SW unstratified showed no change in germination percentage from the control. Those treatments that were stratified showed an increase in germination percentages. Prairie Moon Nursery (2021) suggested that *Liatris ligulistylis* seed germinates after 60 days of cold, moist stratification. In, *Germination Studies of*

Wisconsin Prairie Plants, germination percentages for *Liatris ligulistylis* seed unstratified and stratified for three months were 26% and 42%, respectively (Greene and Curtis, 1950).

Lamiaceae

Agastache foeniculum

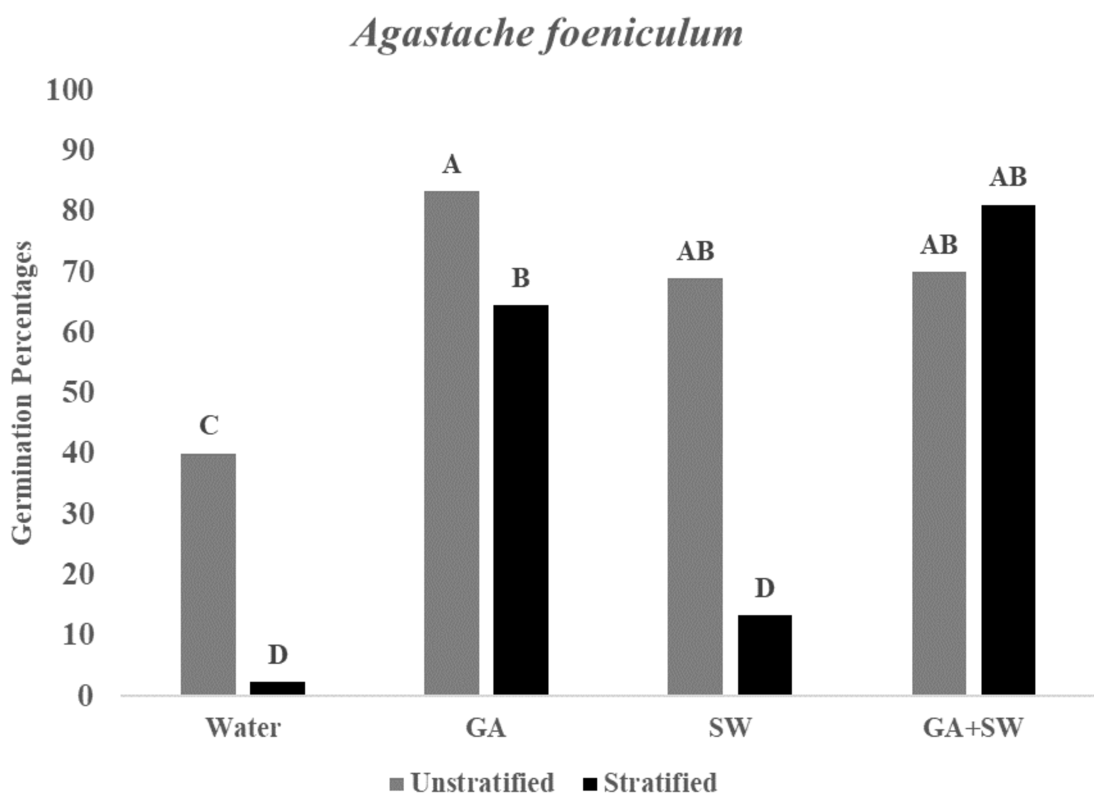

Figure 3. ***Agastache foeniculum*** **germination percentages for chemical treatments of water, gibberellic acid (GA), smoke water (SW), and gibberellic acid and smoke water (GA+SW) and unstratified or stratified temperature treatments**

For *Agastache foeniculum*, GA unstratified (83%), GA+SW stratified (81%), GA+SW unstratified (70%), and SW unstratified (69%) had higher germination percentages compared to the unstratified water control (40%, Fig.3). Interestingly, stratification caused a decrease in germination percentages in all chemical treatments except for GA+SW. Prairie Moon Nursery suggested that *Agastache foeniculum* seed should undergo cold, moist stratification for 30 days and that this species needs light to germinate. There may be a decrease in seed germination for those treatments stratified due to them being stored in a dark cooler since this species requires light to germinate. The reason there is a higher germination percentage for unstratified SW compared to stratified SW may be because smoke treatments have been shown to be effective on many species that have a light requirement for germination (Van Staden et al., 2008). Also, the length of the stratification period may have affected seed germination for this species (Prairie Moon Nursery, 2021).

Plantaginaceae

Penstemon cobaea

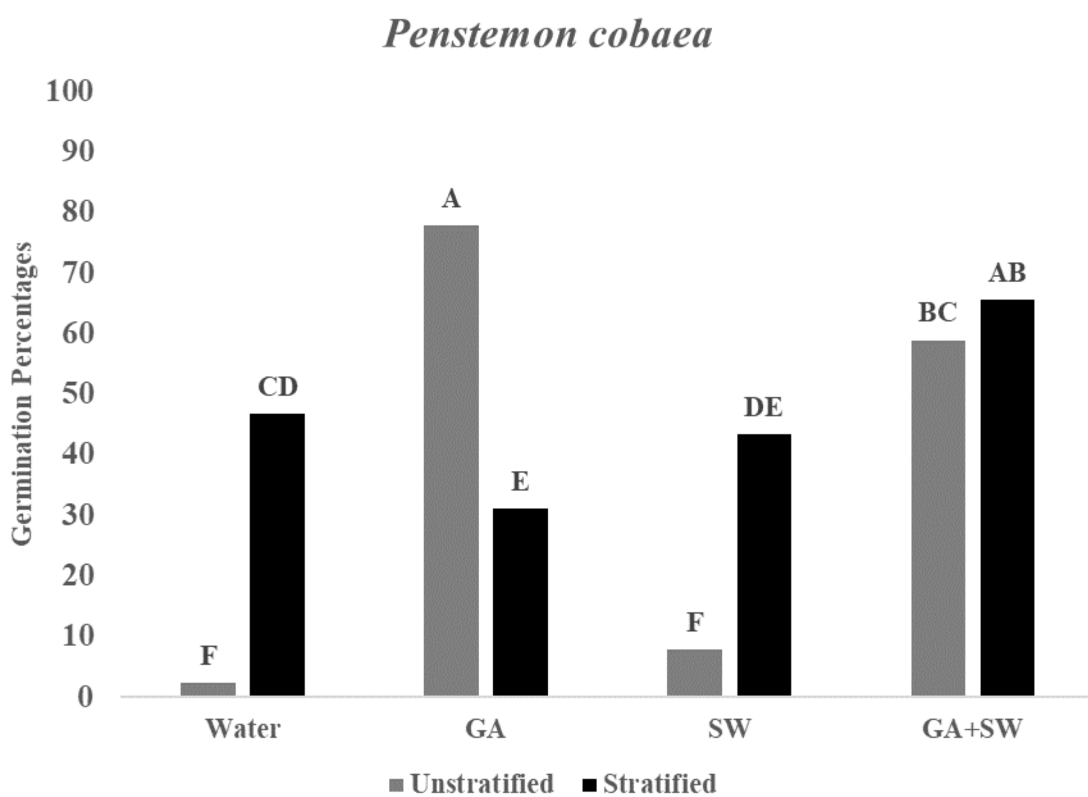

Figure 4. *Penstemon cobaea* germination percentages for chemical treatments of water, gibberellic acid (GA), smoke water (SW), and gibberellic acid and smoke water (GA+SW) and unstratified or stratified temperature treatments

For *Penstemon cobaea*, GA unstratified (78%) and GA+SW stratified (66%) gave the best results, respectively, compared to the unstratified control at 2% (Fig. 4). In each chemical treatment except for GA, there was an observed increase in germination when stratified. As mentioned in the *Penstemon murrayanus* section, cold, moist stratification is usually required for seed germination in *Penstemon* species. According to Jefferson et al. (2014) in a study conducted on *Penstemon cobaea* testing the effects of aerosol smoke and stratification, germination percentages were higher for seeds treated with cold, moist stratification and aerosol smoke than those treated and stored in a dry environment. This matches the results given here from the smoke water stratified treatments. Based on what is known of the *Penstemon cobaea* native habitats and their native distribution, there should be increases in seed germination for smoke treatment and stratification treatment. This could be due to them having come from fire prone areas such as prairies in the Midwestern United States. (Kartesz, 2021; NPIN, 2021). In a study conducted on the effects of gibberellic acid on *Penstemon digitalis*, those seeds treated with 500 GA_3 mg · L^{-1} and stored at a higher temperature reached 100% germination, while those stored at a lower temperature reached only 56%, respectively. *Penstemon digitalis* and *Penstemon cobaea* come from the same family and can be found in similar habitats (Mello, 2009). High concentrations of GA_3 can be toxic for some species. Stratification can cause an increase in GA_3 concentrations. The additional GA_3 application along with stratification may have become toxic for this species therefore resulting in a germination decrease as seen in the previously mention *Eranthis hyemalis* study (Tipirdamaz and Gomurgen, 2000).

Penstemon murrayanus

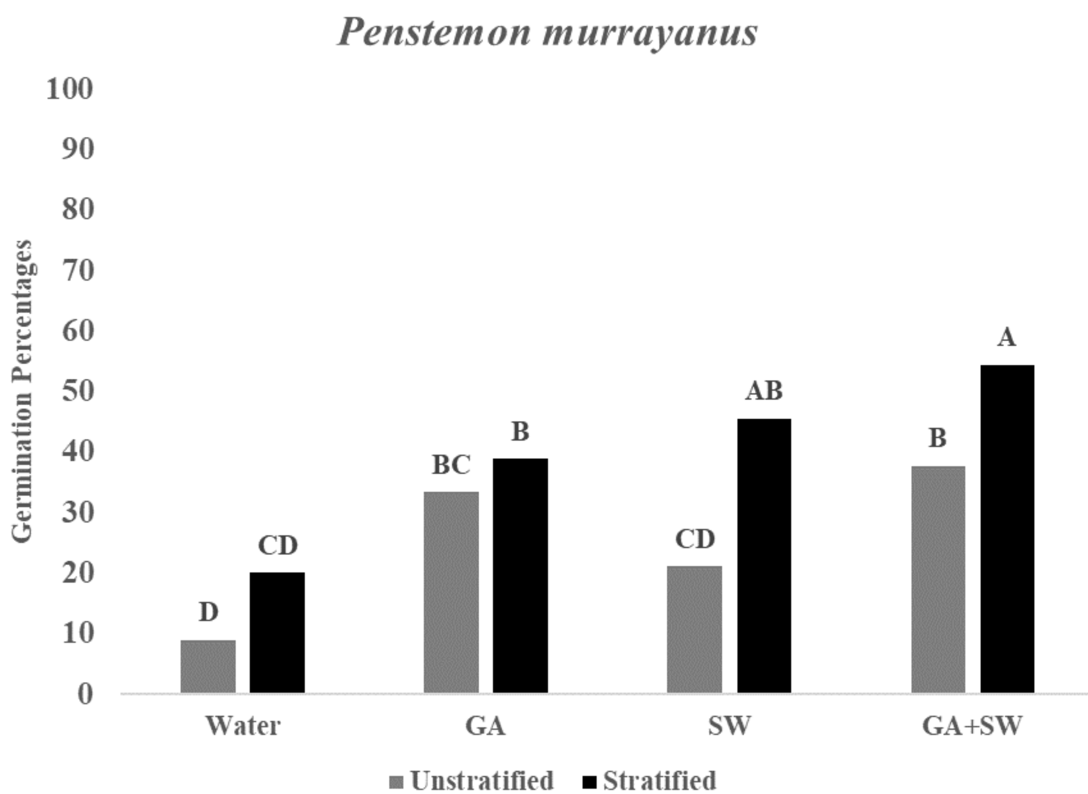

Figure 5. *Penstemon murrayanus* germination percentages for chemical treatments of water, gibberellic acid (GA), smoke water (SW), and gibberellic acid and smoke water (GA+SW) and unstratified or stratified temperature treatments

For *Penstemon murrayanus,* stratified GA+SW and stratified SW had the highest germination percentage at 54% and 46%, respectively compared to the unstratified control at 9% (Fig. 5). According to Mello et al. (2009), *Penstemon* species are known for their low germination percentages and inconsistency; however, germination requirements vary species to species. Many *Penstemon* seed are dormant when dispersed and require a moist chilling period (stratification) for germination which could be why there was an increase in germination for the stratified seed. In a smoke study conducted on ten interior western *Penstemon* species, three species exposed to smoke did show an increase in germination while six species responded to stratification. *Penstemon murrayanus* being native to fire prone habitats such as east Texas prairies could explain why there is an observed increase in germination for stratified SW and stratified GA+SW (Fornwalt, 2015).

Onagraceae

Oenothera macrocarpa

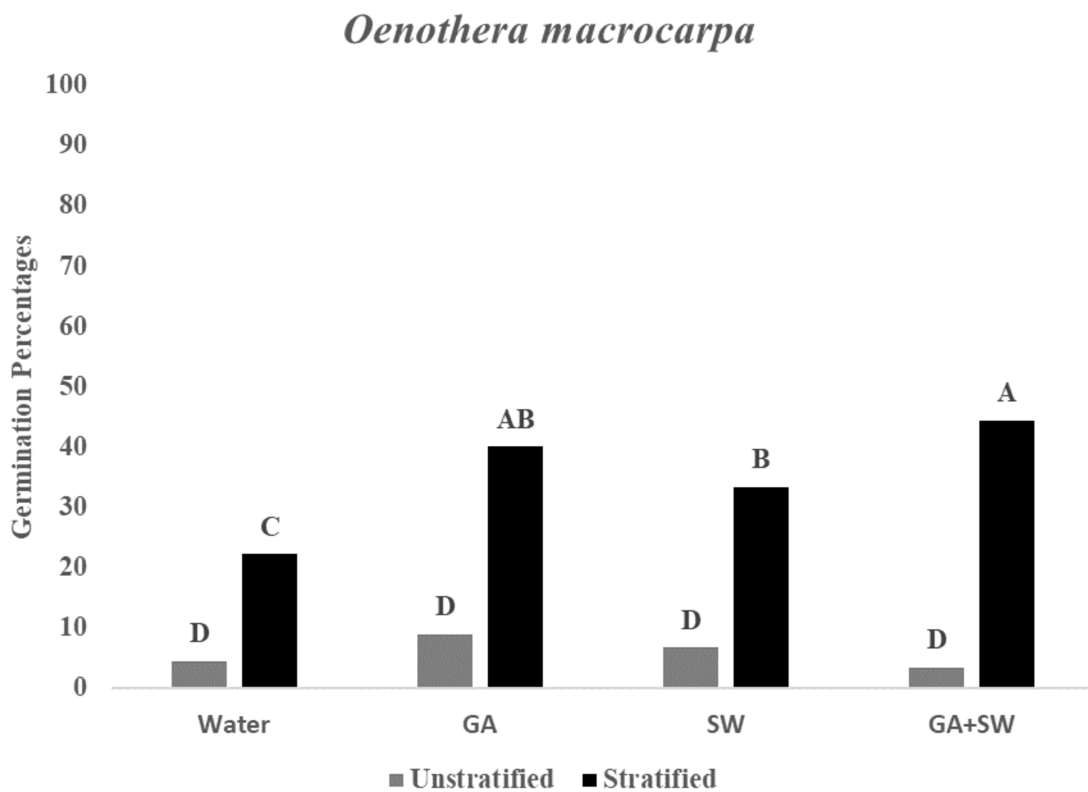

Figure 6. *Oenothera macrocarpa* germination percentages for chemical treatments of water, gibberellic acid (GA), smoke water (SW), and gibberellic acid and smoke water (GA+SW) and unstratified or stratified temperature treatments

For *Oenothera macrocarpa*, stratified GA+SW (44%), and GA stratified (40%) had higher germination percentages respectively compared to the unstratified control at 4% (Fig. 6). This species seems to show more of a chemical effect. Prairie Moon Nursery (2021) suggested that this species germinates best when cold, moist stratified for two months. *Oenothera macrocarpa* comes from fire prone environments with long hard winters (Riveiro et al., 2019). It makes sense to see increase in germination amongst the stratified GA+SW and GA treatments, but to also see an increase with stratified SW (33%) compared to both the unstratified (4%) and stratified control (22%, Fig.6) based on *Oenothera macrocarpa's* environmental background.

Chemical Effects

Four species (*Callirhoe digitata*, *Penstemon grandiflorus*, *Ipomopsis rubra*, and *Aquilegia canadensis*,) had a statistically significant chemical effect (Table 4).

Table 4. Germination percentages of species that exhibited significant effects for chemical treatments of water, gibberellic acid (GA), smoke water (SW), and gibberellic acid and smoke water (GA+SW) and unstratified or stratified temperature treatments.

Species	p-value	Unstratified				Stratified			
		Water	GA	SW	GA+SW	Water	GA	SW	GA+SW
Callirhoe digitata	0.0022	2%	9%	17%	10%	8%	9%	4%	8%
Penstemon grandiflorus	<0.0001	7%	86%	33%	90%	46%	57%	47%	60%
Ipomopsis rubra	0.0011	40%	87%	84%	90%	58%	79%	97%	88%
Aquilegia canadensis	<0.0001	11%	42%	8%	24%	0%	38%	4%	22%

Malvaceae

Callirhoe digitata

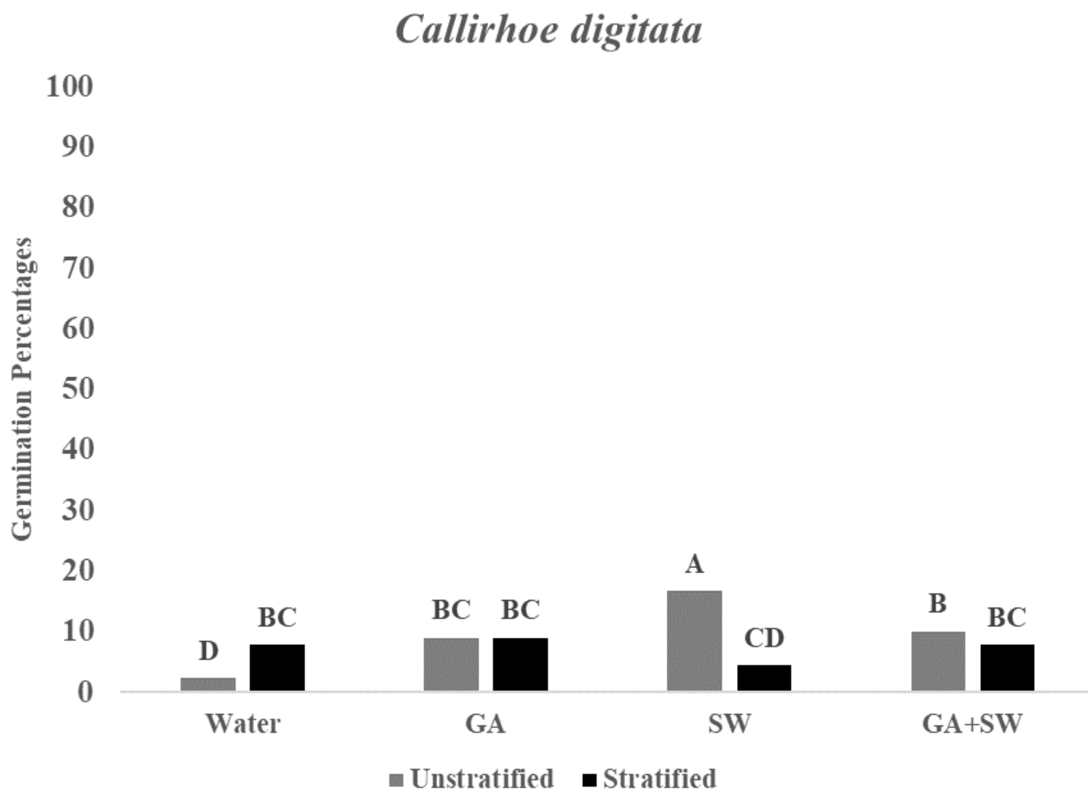

Figure 7. ***Callirhoe digitata*** **germination percentages for chemical treatments of water, gibberellic acid (GA), smoke water (SW), and gibberellic acid and smoke water (GA+SW) and unstratified or stratified temperature treatments**

For *Callirhoe digitata*, SW unstratified had the highest germination percentage (17%) respectively, compared to the unstratified control at 2% (Fig. 7). *Callirhoe digitata* is native to prairies and open woods (NPIN, 2021) in states such as Oklahoma, Kansas, and Missouri (Kartesz, 2021). Many *Callirhoe* species require scarification and stratification to overcome seed dormancy. This may be the case for this species as well. If seeds were scarified, in this case there may have been a larger increase in germination percentage among the treatments (Alberts and Mandel, 2004).

Plantaginaceae

Penstemon grandiflorus

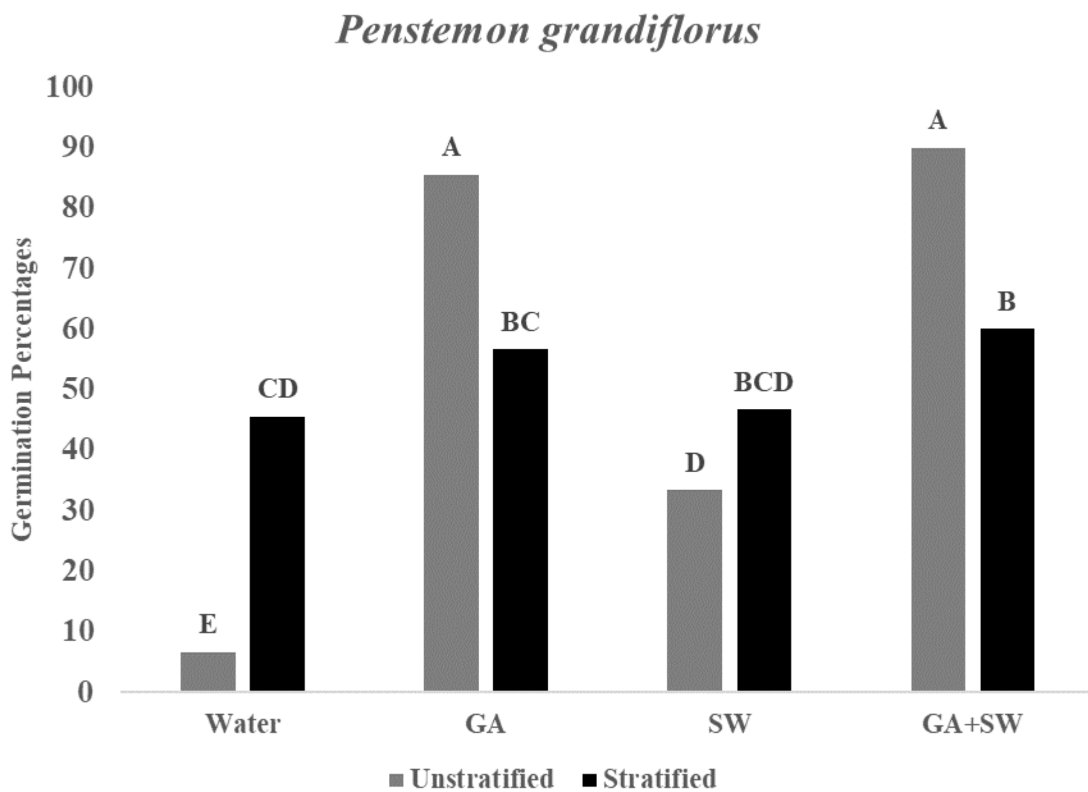

Figure 8. *Penstemon grandiflorus* germination percentages for chemical treatments of water, gibberellic acid (GA), smoke water (SW), and gibberellic acid and smoke water (GA+SW) and unstratified or stratified temperature treatments

According to the data for *Penstemon grandiflorus*, GA+ SW unstratified (90%) and GA unstratified (86%) germinated better when compared to the unstratified control at 7%, respectively (Fig. 8). Both SW stratified and SW unstratified show no change in germination percentage from the control. The genus *Penstemon* is composed of 250 species, many who have undergone adaptive radiation to fit various habitats. These species have genetically changed and spread to adjust to new environments. Many southern *Penstemon* species produce nondormant seed that may respond negatively cold stratification (Meyers et al., 1995). Prairie Moon Nursery (2021) suggested that a one-month cold, moist stratification period is normally required to achieve the best germination for this species. There is an observed decreased from GA unstratified (86%) to GA stratified (57%) and from GA+SW unstratified (90%) to GA+SW stratified (60%, Fig. 8). High concentrations of GA_3 can be toxic for some species. Stratification can cause an increase in GA_3 concentrations. The additional GA_3 application along with stratification may have become toxic for this species therefore resulting in a germination decrease as seen in the previously mention *Eranthis hyemalis* (Tipirdamaz and Gomurgen, 2000) and *Penstemon digitalis* study (Mello, 2009).

Polemoniaceae

Ipomopsis rubra

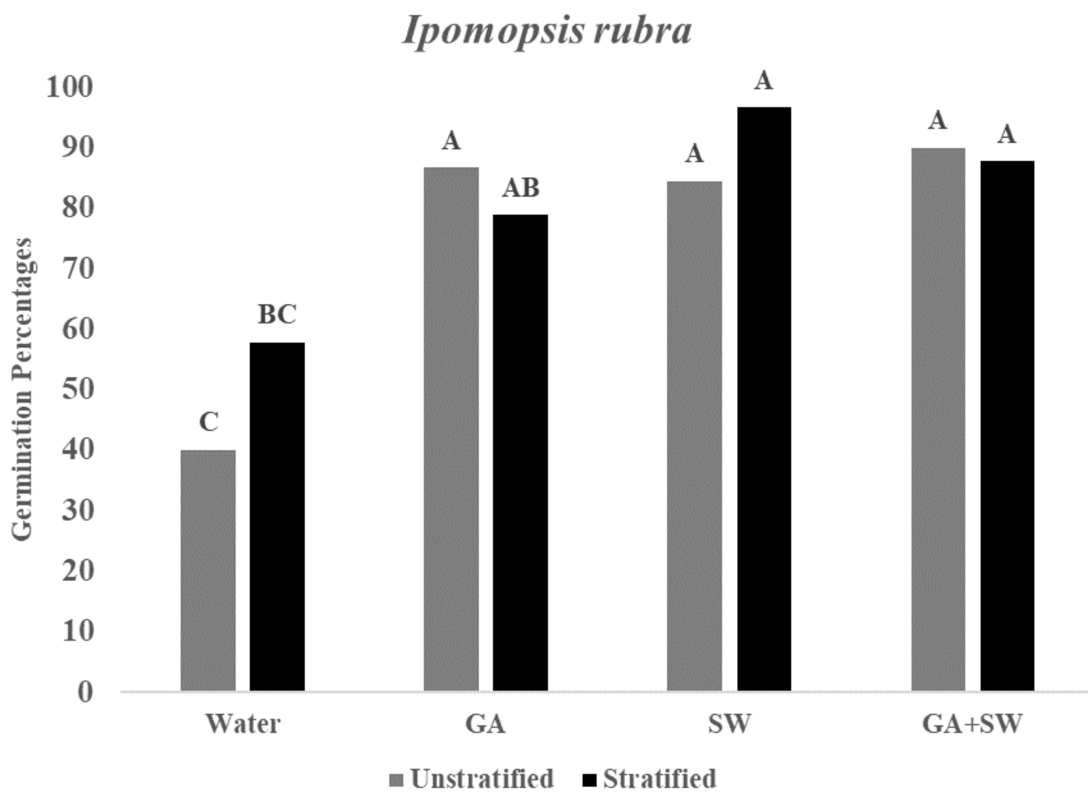

Figure 9. *Ipomopsis rubra* germination percentages for chemical treatments of water, gibberellic acid (GA), smoke water (SW), and gibberellic acid and smoke water (GA+SW) and unstratified or stratified temperature treatments

For *Ipomopsis rubra*, SW stratified (97%), GA+SW stratified (88%), GA stratified (79%), SW unstratified (84%), GA+SW unstratified (90%), and GA unstratified (87%) gave the best results, respectively when compared to the unstratified water control at 40% (Fig. 9). *Ipomopsis rubra* can be found in rocky fields, along roadsides, and open woods (NPIN, 2021) throughout Texas, eastern United States, and Canada (Kartesz, 2021). *Ipomopsis rubra* is also a biennial whose goal is to reproduce as quickly as possible in areas where temperature and winter length vary which is why this species may have responded more to treatment than temperature as it adapts to a variety of habitats. (Sorrie et al., 2018).

Ranunculaceae

Aquilegia canadensis

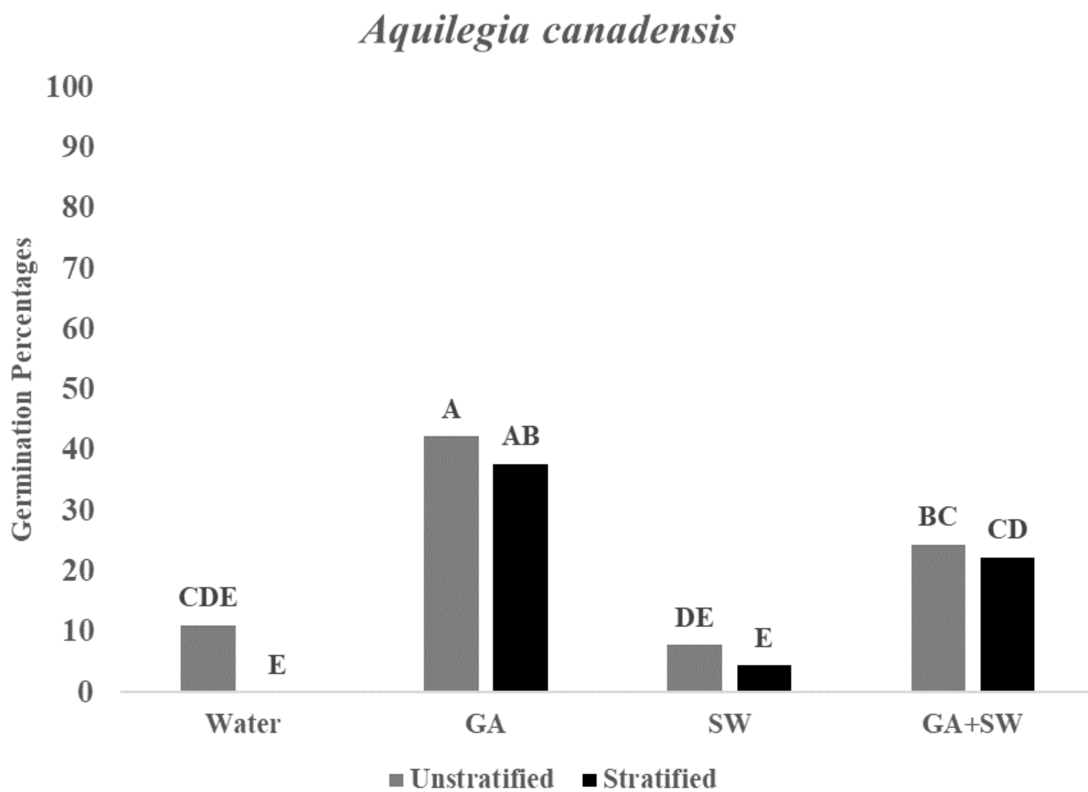

Figure 10. *Aquilegia canadensis* germination percentages for chemical treatments of water, gibberellic acid (GA), smoke water (SW), and gibberellic acid and smoke water (GA+SW) and unstratified or stratified temperature treatments

For *Aquilegia canadensis*, GA stratified and GA unstratified had higher germination percentages at 42% and 38%, respectively, compared to the unstratified control at 11% (Fig. 10). As previously mentioned, from Kartesz (2021), *Aquilegia canadensis* can be found throughout Texas and the Midwest all the way to the eastern coast of the United States, Louisiana excluded and up through Canada. It can be found in wooded areas (NPIN, 2021). Prairie Moon Nursery (2021) suggested that *Aquilegia canadensis* germinates best when cold, moist stratified for two months. For each chemical treatment, there is no observed difference in germination percentages for unstratified versus stratified. Both unstratified and stratified SW show no observed change in germination percentages from the unstratified and stratified control. Some species have been known to respond negatively to smoke. In this case, smoke toxicity may be the reason there is no observed change in germination percentages for seeds treated with SW from the control (Van Staden, 2008).

Temperature effects

Four species (*Verbesina virginica, Amorpha canescens, Delphinium carolinianum,* and *Stenanthium gramineum*) had a statistically significant temperature effect (Table 5).

Table 5. Germination percentages of species that exhibited significant temperature effects. for chemical treatments of water, gibberellic acid (GA), smoke water (SW), and gibberellic acid and smoke water (GA+SW) and unstratified or stratified temperature treatments.

Species	p-value	Unstratified				Stratified			
		Water	GA	SW	GA+SW	Water	GA	SW	GA+SW
Verbesina virginica	<0.0001	1%	10%	1%	1%	92%	93%	90%	90%
Amorpha canescens	0.0002	95%	97%	93%	93%	78%	80%	59%	63%
Stenanthium gramineum	<0.0001	0%	0%	0%	0%	28%	38%	14%	41%
Delphinium carolinianum	<0.0001	1%	0%	0%	0%	68%	72%	91%	93%

Asteraceae

Verbesina virginica

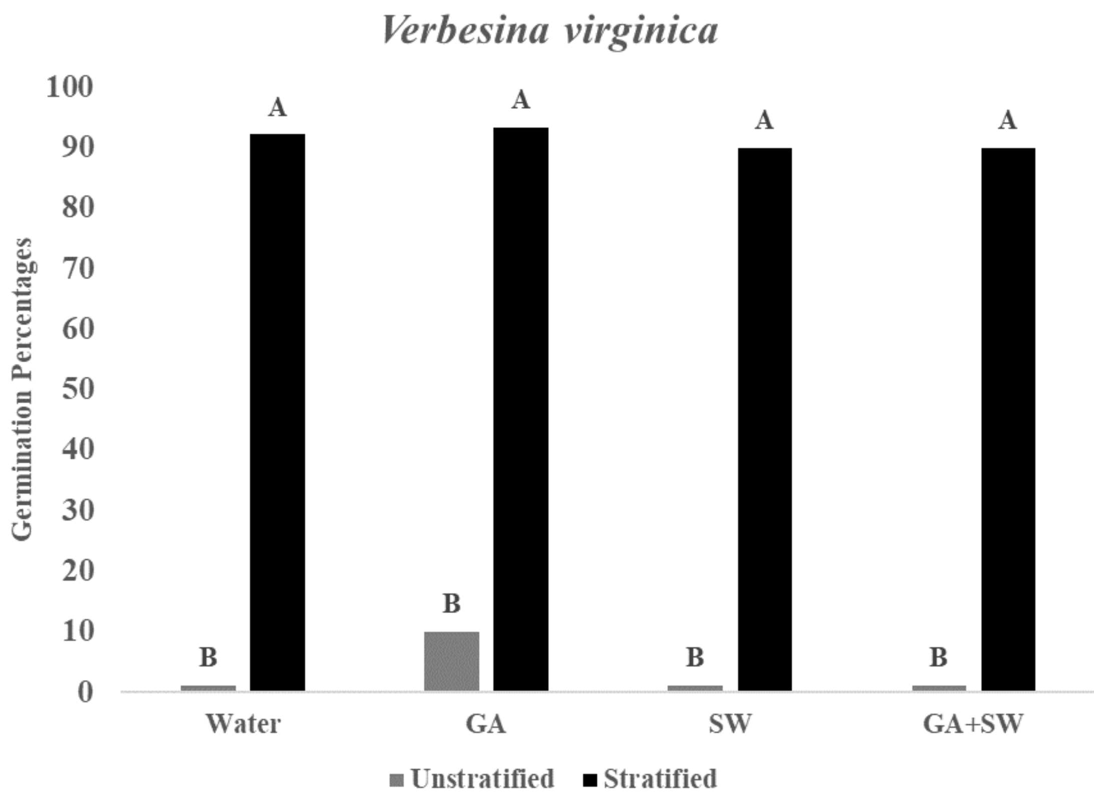

Figure 11. *Verbesina virginica* germination percentages for chemical treatments of water, gibberellic acid (GA), smoke water (SW), and gibberellic acid and smoke water (GA+SW) and unstratified or stratified temperature treatments

For *Verbesina virginica*, GA stratified (93%), control stratified (92%), SW stratified (90%), and GA+SW stratified (90%) germinated better, respectively, when compared to unstratified water at 1% (Fig. 11). Stratification played a key role in the germination of this species as chemical treatment did not have a significant effect. GA may be involved in germination mechanism; however, based on the results shown GA unstratified shows no change in germination percentage compared to the other unstratified treatments.

Verbesina virginica is native to habitats such as the edge of woodlands and along streambanks (NPIN, 2021) throughout Texas to Iowa stretching to the eastern United States from Florida to Maryland (Kartesz, 2021). Based on their environments, it could be that *Verbesina virginica* seeds require a ripening period to allow the embryo to mature while undergoing stratification (Baskin et al., 1993)

Fabaceae

Amorpha canescens

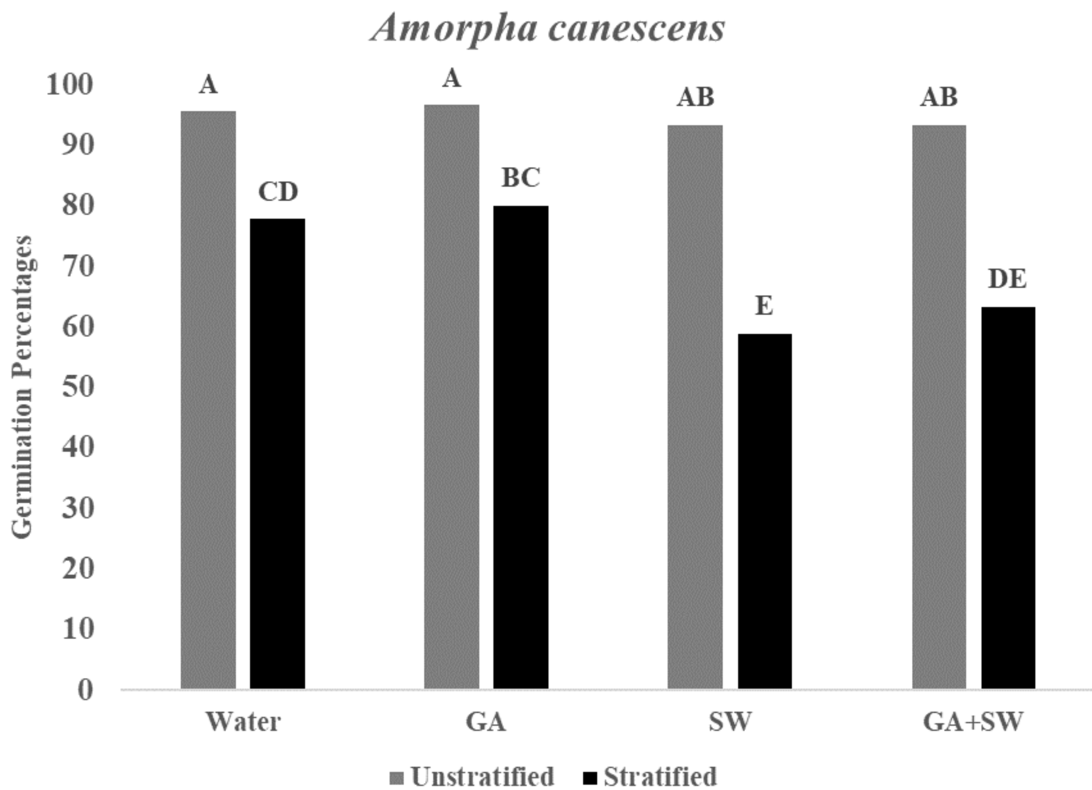

Figure 12. ***Amorpha canescens*** **germination percentages for chemical treatments of water, gibberellic acid (GA), smoke water (SW), and gibberellic acid and smoke water (GA+SW) and unstratified or stratified temperature treatments**.

For *Amorpha canescens*, GA unstratified (97%), control unstratified (96%), GA unstratified (93%), and GA+SW unstratified (93%) gave the best results (Fig. 12), respectively. For this species, there is an observed decrease in germination percentages among the stratified treatments. Prairie Moon Nursery (2021) suggested that *Amorpha canescens* germinates best when cold, moist stratified for ten days. *Amorpha canescens* may show higher germination percentages for unstratified treatments than stratified treatments due to the length of stratification in this experiment which was 3 months. The longer than normal stratification period may have caused a GA toxicity for *Amorpha canescens*, therefore resulting in lower germination percentages for stratified treatments (Tipirdamaz and Gomurgen, 2000). According to Jefferson et al. (2008), in a study conducted on the effects of smoke on seed germination for *Amorpha canescens*, there was a zero percent germination response to aerosol smoke treatments. However, for this experiment, there was an observed positive germination response to those seeds treated with smoke water and no stratification. Stratified SW (59%) and stratified GA+SW (63%), respectively, had the lowest germination percentages (Fig.12).

Melanthiaceae

Stenanthium gramineum

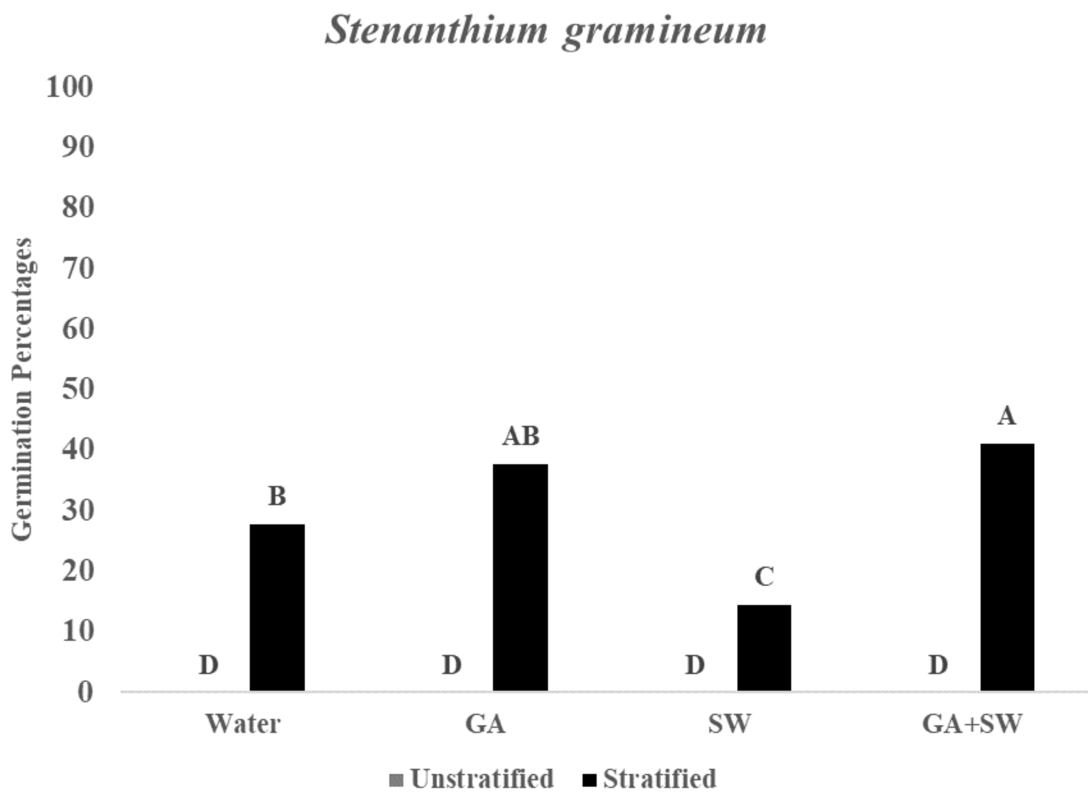

Figure 13. *Stenanthium gramineum* germination percentages for chemical treatments of water, gibberellic acid (GA), smoke water (SW), and gibberellic acid and smoke water (GA+SW) and unstratified or stratified temperature treatments.

Based on the data for *Stenanthium gramineum*, GA+SW stratified (41%) and GA stratified (38%) gave the best results respectively, when compared to the unstratified control at 0% (Fig. 13). *Stenanthium gramineum* is natively distributed throughout eastern and southeastern United States (Kartesz, 2021). This species can be found in open rocky areas and sandy bogs (NPIN, 2021). Based on their environments, it could be that *Stenanthium gramineum* seeds require a ripening period to allow the embryo to fully mature while undergoing stratification (Edgin, 2004) Though unstratified SW showed no change from unstratified water, stratified SW (14%) showed a decrease in germination percentages compared to stratified water (28%, Fig. 13). In some cases, smoke has inhibited germination for some species. This may be the case for *Stenanthium gramineum.* Smoke toxicity may have contributed to the decrease in germination percentages for stratified SW compared to stratified water (Van Staden, 2008).

Ranunculaceae

Delphinium carolinianum

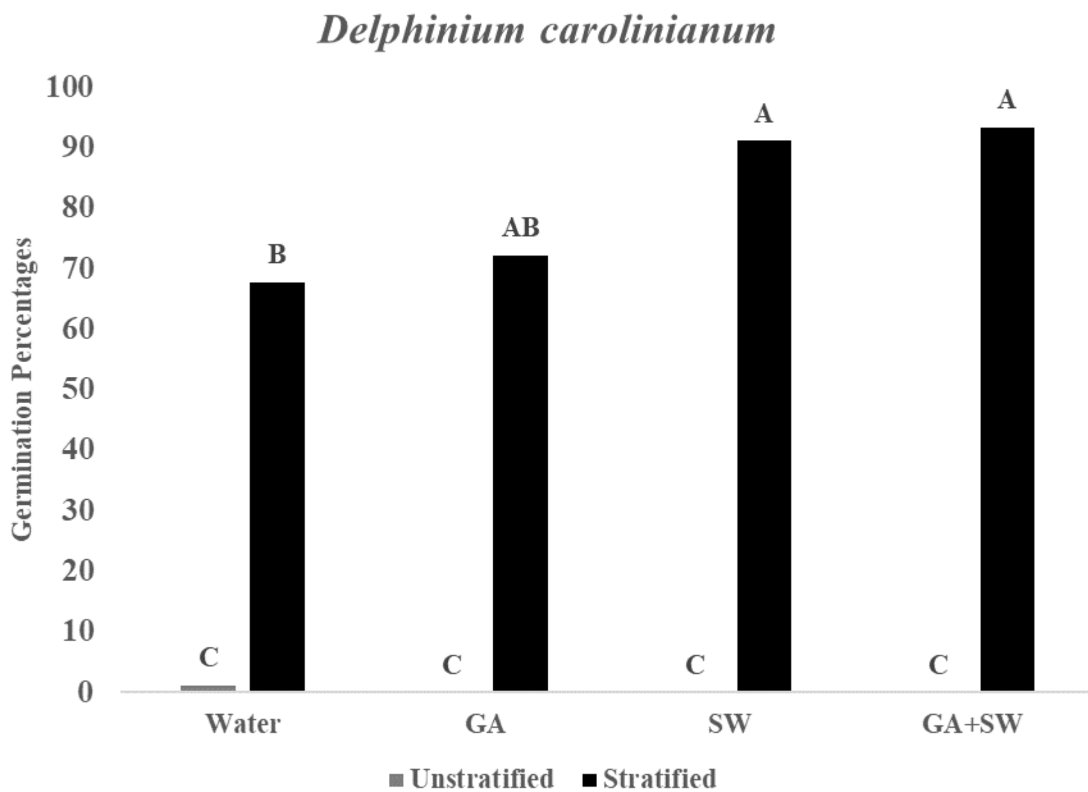

Figure 14. *Delphinium carolinianum* germination percentages for chemical treatments of water, gibberellic acid (GA), smoke water (SW), and gibberellic acid and smoke water (GA+SW) and unstratified or stratified temperature treatments.

For *Delphinium carolinianum,* GA+SW stratified (93%), SW stratified (91%), and GA stratified (72%), gave the best results, respectively when compared to the unstratified control (1%, Fig. 14). Stratification significantly increased germination rates for this species. *Delphinium carolinianum* can be found in open woods, sandy hills, and brushlands (NPIN, 2021) through Arizona to the southeastern United States and as far north as North Dakota (Kartesz, 2021). Based on their environments, it could be that

Delphinium carolinianum seeds require time to allow the embryo to mature, ripening period, while undergoing stratification (Warnock, 2021).

No effect

Two species (*Liatris aspera* and *Callirhoe involucrata*) had no statistically significant chemical and temperature effect (Table 6).

Table 6. Germination percentages of species that did not exhibit significant temperature or chemical interaction for chemical treatments of water, gibberellic acid (GA), smoke water (SW), and gibberellic acid and smoke water (GA+SW) and unstratified or stratified temperature treatments.

Species	p-value	Unstratified				Stratified			
		Water	GA	SW	GA+SW	Water	GA	SW	GA+SW
Liatris aspera	0.7316	2%	9%	17%	10%	8%	9%	4%	8%
Callirhoe involucrata	0.4037	7%	86%	33%	90%	46%	57%	47%	60%

Asteraceae

Liatris aspera

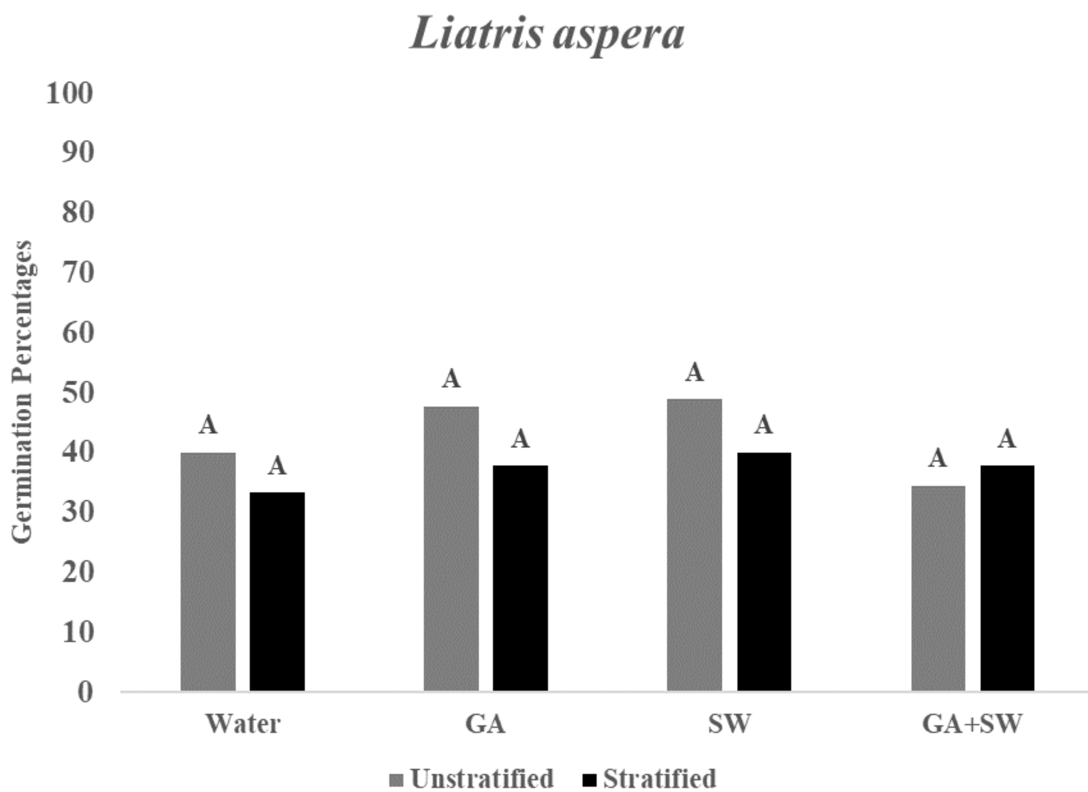

Figure 15. ***Liatris aspera*** **germination percentages for chemical treatments of water, gibberellic acid (GA), smoke water (SW), and gibberellic acid and smoke water (GA+SW) and unstratified or stratified temperature treatments.**

For *Liatris aspera,* chemical treatment and temperature had no significant effect on seed germination percentage. In a study conducted on the effects of smoke on Midwestern plant species, *Liatris aspera* also showed no response to smoke treatment and was recorded as not significant. However, there is not enough evidence to suggest why this species shows no significant response to chemical and temperature treatment (Jefferson et al., 2008). Scarification and three-month stratification are commonly used when propagating this species (NPIN, 2021). For this experiment, the seeds were not scarified. There may have been more of a significant effect with chemical treatments if seeds were scarified prior to treatments.

Malvaceae

Callirhoe involucrata

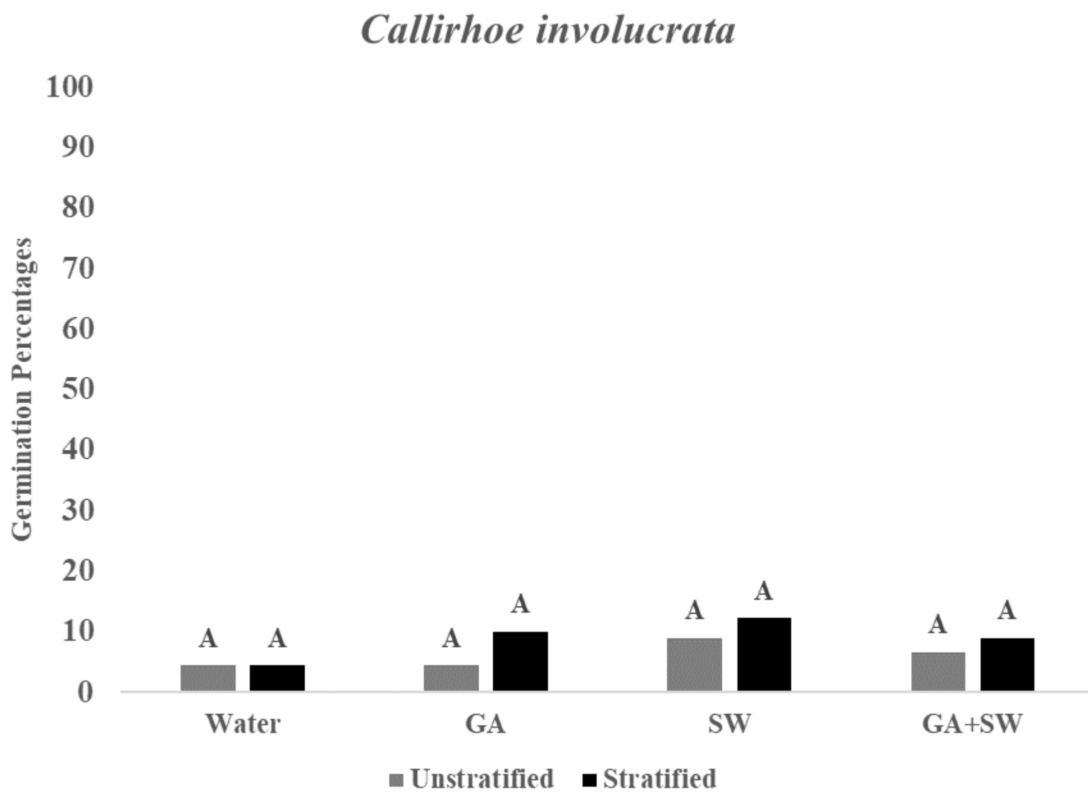

Figure 16. *Callirhoe involucrata* germination percentages for chemical treatments of water, gibberellic acid (GA), smoke water (SW), and gibberellic acid and smoke water (GA+SW) and unstratified or stratified temperature treatments.

For *Callirhoe involucrata,* chemical treatment and temperature had no significant effect on seed germination percentage. Other species in the *Callirhoe* genus have been known to have a more positive response to chemical and/or temperature treatments. Scarification is important for germination in this species. Prairie Moon Nursery (2021) suggested that *Callirhoe involucrata* seed should be scarified by soaking the seed in a hot water treatment for 24 hours prior to sowing. Then, seeds should be cold, moist stratified for one-month. If the seeds were scarified, they may have had more of a response to chemical treatment. *Callirhoe involucrata* seeds were stratified for longer than the recommended stratification period which may have led to this species not showing a significant response in germination percentages for temperature treatments compared to the control. However, there is not enough evidence to suggest why this species showed no significant response to either chemical or temperature treatment (Alberts and Mandel, 2004).

Conclusion

Plant propagation gives growers access to a variety of plants and species. Overcoming seed dormancy is often a problem faced within the consistently growing horticulture industry (Jefferson et al., 2014). This research expands on ways to overcome seed dormancy and increase seed germination in certain species.

To conclude, as germination increases for individual species, this is beneficial for horticulturists and conservationists. This research matters because the response to GA and SW is very species specific. Gibberellic acid is easily accessible but costly while, smoke water is inexpensive and easy to apply. Through simple solutions such as smoke water, growers can increase germination without having to spend a ton of money, and gibberellic acid, smoke water, or a combination of the two along with stratification may aid in the survival of these native perennials.

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Vita

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