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Identifying Optimum Germination Temperatures and Analysis Weights in Seed Testing for Stiff Goldenrod (*Solidago rigida*) and River Bulrush (*Bolboschoenus fluviatilis*)

Rachel Geary

IDENTIFYING OPTIMUM GERMINATION TEMPERATURES AND ANALYSIS WEIGHTS IN SEED TESTING FOR STIFF GOLDENROD (*Solidago rigida*) AND RIVER BULRUSH (*Bolboschoenus fluviatilis*)

BY

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THESIS ACCEPTANCE PAGE

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

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ABBREVIATIONS

AOSA	Association of Seed Analysts
CV	Coefficient of Variance
TZ	tetrazolium
KNO_3	potassium nitrate
ANOVA	Analysis of Variance
ABA	abscisic acid
GA_3	gibberellic acid

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ABSTRACT

IDENTIFYING OPTIMUM GERMINATION TEMPERATURES AND ANALYSIS WEIGHTS IN SEED TESTING FOR STIFF GOLDENROD (*SOLIDAGO RIGIDA*) AND RIVER BULRUSH (*BOLBOSCHOENUS FLUVIATILIS*)

RACHEL GEARY

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Federal and state seed laws require seed lots offered for sale to be tested and labeled with the appropriate information before they can be sold. Those tests are conducted in seed testing labs all around the world. In the United States, accredited seed testing labs follow the Association of Official Seed Analysts (AOSA) Rules for Testing Seeds to provide information for labeling. The rules lack instructions for weights and germination for many native species. Stiff goldenrod (*Solidago rigida*) and river bulrush (*Bolboschoenus fluviatilis*) are two species that lack any information required for testing. When seed testing labs use correct weights and optimum germination temperatures, they can provide customers with accurate results in a timely manner. This research evaluates the weights needed for purity and noxious weed seed testing and the optimum temperature(s) needed for germination.

Multiple seed lots of each species were gathered to determine the weights required for a purity and noxious analysis. Following the steps laid out in the AOSA Rules to calculate weights, eight replications of 100 seeds were counted out from each sample and weighed. The average weights of the replications, minimum weight for a purity and noxious analysis, and the number of seeds per gram were determined for each species. The average weight for 100 stiff goldenrod pure seed units was 0.0548 grams.

The weight required for a purity and noxious analysis were 1.4 and 14 grams, which provides approximately 2500 pure seed units for a purity and 25,000 seeds for a noxious exam. The number of seeds per gram was 1824.7391. River bulrush had an average weight of 0.6516 grams per 100 pure seed units. The weight needed for a purity and noxious exam were 16 and 160 grams. River bulrush contained 153.4794 seeds per gram.

A thermogradient table was used to evaluate the optimum temperature for germination of the selected species. The thermogradient table allowed multiple temperatures to be tested at once. The temperatures created with the thermogradient table were 13°C, 17°C, 19°C, 22°C, 23°C, 24°C, 26°C, 27°C, 28°C, 30°C, 31°C, and 32°C. In addition, six germinators set to temperatures typically used for species in the AOSA Rules were used. These temperatures included three constant temperatures (15°C, 20°C, 25°C) and three alternating temperatures (15-25°C, 15-30°C, 20-30°C). A total of 18 temperatures were assessed. Four lots were chosen for each species. Four replications of 50 seeds from each lot were planted at every temperature evaluated. Stiff goldenrod exhibited the highest germination at a constant 25°C with an average germination 63.8%. River bulrush exhibited the highest germination of 2.5% at the alternating temperature 20-30°C.

I. INTRODUCTION

The market for native seed is growing due to the interest of habitat restoration projects due to fires and floods, USDA programs to address at-risk acreages from water and wind erosion, and restoration of degraded lands (soils). With the increase in interest, seed testing laboratories are seeing more species that do not have standardized testing procedures published in the Association of Official Seed Analysts (AOSA) Rules for Testing Seeds. Seed companies and producers rely on seed testing laboratories to provide test results in a timely manner and provide accurate results. Customers need accurate and timely results so they can label their seed lot with the appropriate information and ship to markets in a timely manner. Seed lots sold in the United States must be labeled according to state and federal seed laws, which require a purity analysis providing the percentage by weight of pure seed, weed seed, other crop seed, and inert matter, the percentage of total viable seed (% germination + % dormant + % hard seed), and the kind and rate of any noxious weed seed found in the noxious exam.

Currently, there are hundreds of species with standardized testing procedures in the AOSA Rules, and even more that do not. The increased interest in native species for habitat restoration or other purposes means seed testing laboratories are receiving more and more species without standardized testing procedures. Without standardized testing procedures for many native species, seed testing laboratories are providing customers with results that vary from one seed testing lab to another, causing an uneven playing field for companies selling those seeds. Standardized testing procedures ensure that all labs are using the same methods, providing less variation from lab to lab, while currently many different methods are being used with these species, and the industry tends to use

labs that provide higher results. Purity and noxious analysis sample sizes and optimum germination temperature and testing methods are not standardized or studied for many of these native species. Purity and noxious analyses require a minimum weight of seed to be examined within the test, a minimum of 2500 pure seed units in a purity analysis, and a minimum of 25,000 pure seed units in the noxious exam (which includes the purity analysis sample size).

Reporting the germination for many of these species is difficult due to the lack of knowledge on how to test them and at which temperature optimum germination occurs. There is still a lack of understanding of the requirements needed for germinating a tremendous number of native species. Native species can have very different levels and types of dormancy that need breaking before germination can take place. Many seed testing laboratories do not have the experience or the equipment to evaluate the wide variety of native species being used in various projects. Creating standard and optimum germination procedures for species not in the AOSA Rules can improve the results reported to producers and consumers.

Stiff goldenrod and river bulrush are two among the hundreds of species without standardized testing procedures. The intention of this experiment is to determine the weights required to conduct an official purity and noxious analysis on stiff goldenrod and river bulrush. The dormancy and germination requirements of these two species are not well understood. The goal of this research is to identify an optimum germination method, and appropriate weights for testing of these two species, which can be used in developing a standardized testing procedure.

II. LITERATURE REVIEW

Purity and Noxious Analyses

Seed testing labs in the United States follow the AOSA Rules for Testing Seeds to conduct analyses of crops for labeling information. To accurately test crops, it is important to understand how many seeds are in a gram on average. This is used to determine the weight required for purity and noxious analyses. A purity analysis is based off the approximate weight of 2500 pure seed units and a noxious analysis based off a minimum of 25,000 pure seed units, with a maximum of 500 grams required for a noxious weed seed exam (AOSA, 2021). A purity analysis provides the percent pure seed; the kind, number, and percent of other crop seeds; the kind, number, and percent of weed seed; and percent inert matter. To determine what is pure seed, the seed analysts must understand the many different definitions of pure seed units found in the AOSA Rules for Testing Seeds. A pure seed unit for stiff goldenrod is considered an intact achene with or without pappus or seed coat and contains some extent of embryo development that is detectable (AOSA, 2021). A pure seed unit for river bulrush is an intact achene that does or does not have a seed present. River bulrush can be missing its seed coat. It can have these structures present, or be missing the beak, bristle, hairs, pappus, or wing (AOSA, 2021). For both species, a broken seed is considered pure seed as long as it is larger than one-half the original size. A noxious analysis provides the customer with information of the kind and number of noxious weed seeds found in the representative portion of the seed lot analyzed. The analyses are crucial and provide required information for labeling purposes.

Germination Examination

When conducting a germination exam, it is useful to know the optimum temperature needed for a crop to reach optimum germination within the shortest time possible. The requirements needed for many native seeds to germinate are poorly understood. However, the idea of using seed for revegetation is a more economical option than using vegetative propagules (Marty and Kettenring, 2017). Since there are not any standardized testing procedures for stiff goldenrod and river bulrush in the AOSA Rules for Testing Seeds, it would be valuable to research and create standards for the benefit of companies and consumers who are working on restoration projects, as well as seed testing laboratories who are performing analyses on the two species.

Seed testing laboratories use various methods to germinate species. They often use multiple germination chambers set at different temperature regimes to accommodate most of the species they receive for testing. The germination chambers can operate at a constant or alternating temperature regimes. The most common temperatures used in the SDSU Seed Testing Laboratory are 15°C, 20°C, 25°C, 15-25°C, 15-30°C, and 20-30°C. Alternating temperature chambers are set at the lower temperature for 16 hours, followed by the higher temperature for 8 hours, which simulates daytime/nighttime temperature fluctuations in nature. During the high temperature, lights can be set to come on during the high temperature period, or longer, simulating the darkness/light period of a 24-hour period. The chambers can be easily changed to accommodate species with differing temperature requirements. A pre-chill chamber, essentially a refrigerator at 5-10°C, aids with breaking dormancy. A pre-chill can be necessary for newly harvested crops or required because of the standardized testing methods. The chambers have either LED or fluorescent lights set to be on for eight hours and off for sixteen. The AOSA Rules for

Testing Seeds states the minimum required lumens is between 807 and 1346 (AOSA, 2021). The lights can also be set to any desired or required time period. This helps signify day and night, and sometimes is needed to aid in breaking dormancy. Some species need to be germinated in the dark, as light will delay or inhibit germination.

A variety of wetting agents can be used on the planting media to induce germination, or aid in breaking easily broken dormancy. The two most commonly used wetting agents are potassium nitrate (KNO_3) and water. Water used in seed testing laboratories should be purified if needed as tap water can vary from lab to lab, which can cause results to vary. The water pH should be between 6-8 as anything higher or lower may inhibit germination of some species. Growth chemicals such as gibberellic acid (GA_3) and ethylene gas are occasionally required to break dormancy.

The current germination method currently used in the SDSU Seed Testing Laboratory for stiff goldenrod is planting the seeds on top of two blotters moistened with deionized water and placing the seeds in the 20-30°C chamber. A 7-day first count is performed and a 14-day final count. The lab observes some germination with this method, but many of the seeds do not germinate. After the final count, the ungerminated seeds are subjected to a tetrazolium (TZ) test to determine the percent dormancy of ungerminated seed.

The methods for river bulrush being used at the SDSU Seed Testing Laboratory is planting the seeds on top of two blotters moistened with water and placing the seeds in the 15-30°C germination chamber after a 7-day pre-chill at 5°C. After the 7-day pre-chill, a first count is conducted 7-days after being placed in the germinator, and a 14-day final count. The test takes 21 days in total, and a TZ test is used after the completion of the

germination exam to determine the percent dormancy of ungerminated seed. The lab observes very little germination but high dormancy rates with the current method.

Tetrazolium Testing

TZ testing is primarily utilized in seed testing for various reasons. A TZ test conducted first on 200 seeds prior to germination allows seed testing laboratories to quickly evaluate whether a seed sample is viable or not, and it provides an estimated potential germination capability. TZ testing is the best method available for determination of dormant seeds in a germination test after the completion of the germination period as the ungerminated seeds are subjected to a TZ test as recommended by the AOSA Rules for Testing Seeds. Essential seed structures (cotyledon(s), roots, shoots, etc.) in non-germinated seed that stain red/pinkish are considered viable after germination and reported as dormant seed.

To determine if seeds are viable but dormant, they are cut or nicked with a razor and placed in tetrazolium solution. This colorless TZ solution contains 2, 3, 5-triphenyl tetrazolium chloride (TTC) and water, which is measured to form a 1% TZ solution. The reduction of TTC creates formazan, which is carmine red in color. This reaction stains the actively respiring tissues a reddish-pink color suggesting the seed is viable (Tetrazolium Testing Handbook, 2010). The staining of seed is dependent on the species and their tissues. The embryo will stain if the seed is viable. However, the endosperm (non-living tissue) will not stain in all grass species. Outside the grass family, some species have living endosperm and others have non-living.

Seed Dormancy

One obstacle seed testing laboratories experience when working with various native seeds is seed dormancy. The AOSA defines seed dormancy as “the physical or physiological condition of a viable seed that prevents germination from occurring even in the presence of otherwise favorable germination conditions” (Tetrazolium Testing Handbook, 2010). Dormancy is very common in native species because dormancy allows these species to maintain a presence in the habitat over time. Dormant seeds can remain in the soil for years before the right conditions exist, allowing it to germinate.

Seeds can exhibit different types of dormancies. Many seeds can be impacted by exogenous or endogenous dormancy. The AOSA explains that a seed experiences exogenous dormancy when the germination requirements (water, oxygen, temperature, etc.) are not met or when expansion and growth are constrained due to an impermeable or thick seed coat. Endogenous dormancy occurs more frequently in native species and is caused by physiological properties inhibiting it from germinating, such as an imbalance of abscisic acid (ABA) and GA_3. Seeds can also exhibit a combination of different dormancy mechanisms.

Thermogradient table

A thermogradient table may be used help to determine the optimal temperature(s) for germination. The thermogradient table allows for germination testing across multiple temperatures to be tested at the same time along with multiple lots. The table can provide a broad range of temperatures at once, allowing an evaluation of multiple germination regimes in a shorter period of time. The table has insulated sides, bottom, and lids to help maintain the temperature inside. The table works by having two circulating baths, one at each end of the table. One bath is refrigerated and the other heated (see Figure 1 in the

Appendix). The baths can be filled with different liquids depending on the temperature range needed. Typically, an equal mix of water and antifreeze is used, which enhances the exchange of heat and reduces the chance of the water lines freezing (Welbaum et al., 2016). Each bath has lines that run under the special aluminum alloy plate surface, causing a temperature gradient to form across the table from the cold end to the warm end (see Figure 2 in the Appendix). Once the water baths are prepared, the table should be given 24 hours to equilibrate and reach the chosen temperatures. To reduce condensation within the germination boxes on the warm side of the table, it is recommended to use smaller temperature ranges and perform multiple experiments to cover a wider range of temperatures overall. It should be noted that the warmer side of the table will need to be monitored and watered more often than the cooler side (Welbaum et al., 2016).

Stiff Goldenrod (*Solidago rigida*)

Stiff goldenrod is a native perennial plant in the Asteraceae family. It can commonly be found in prairies and woods across the United States (USDA Planting Guide). Most stiff goldenrod samples received by the SDSU Seed Testing Laboratory are received from Minnesota. Stiff goldenrod can be used in a variety of locations including ditches, prairie restoration projects, gardens, or in wildlife habitats (USDA Planting Guide). Stiff goldenrod has been observed to attract many insects including butterflies, bees, ladybirds, lacewings, and hoverflies (Brown, 2002). It has been noted that different types of birds and small rodents feed on stiff goldenrod seeds (Brown, 2002). Figure 3 shows the details of stiff goldenrod seed under the microscope.

It has been suggested that there is a relationship between seed traits and germination. Barak et al. (2018) states that rounded seeds typically germinate slower than

elongated seeds. From this statement, the assumption that stiff goldenrod seeds should germinate rapidly compared to other seeds can be concluded. However, elongated seeds do not persist in the soil as long as rounded seeds (Barak et al., 2018). This suggests stiff goldenrod has a harder time maintaining a presence in its native habitat. It emphasizes the need for finding the optimum germination temperature for stiff goldenrod so that the restoration efforts are successful. It is important to mention that seed mass can also impact seed germination (Barak et al., 2018). Seed mass influences seed dispersal, and likely influences the germination potential, as larger seeds would have more cotyledonary reserves. Lighter seeds are capable of traveling a farther distance than heavier seeds, which can impact the competition found in an environment. As long as there are established populations of stiff goldenrod, there is an advantage for spread and expansion of the population.

One can find very few previous studies on the germination of stiff goldenrod, and of the few studies conducted, many were performed in greenhouse or field settings. According to Nuzzo (1976), stiff goldenrod takes about a week to observe germination, but only takes approximately two weeks to reach peak germination. In the study conducted by Hillhouse and Zelder (2011), stiff goldenrod experienced peak germination later than many other native species in the study. It did reach peak germination by 16 days. Nuzzo (1976) subjected the stiff goldenrod to greenhouse daytime temperatures of 21° to 32°C and nighttime temperatures 4° to 21°C. The seeds were subjected to a ten-week dry stratification process, and then planted in a 50/50 mix of potting soil and silica sand (Nuzzo, 1976). The stiff goldenrod seedlings performed well in the field after being transplanted and flowered within two years of being transferred; however, stiff goldenrod

is unlikely to perform well in the field if directly seeded (Nuzzo, 1976). Stiff goldenrod experienced only a 0.3% emergence rate in the field even though it had a germination rate of 55.5% (Hillhouse and Zelder, 2011). A study conducted by the New England Wildflower Society compared directly planting stiff goldenrod outside compared to cold stratification before planting. They planted 100 seeds three months after harvest outside, while an additional 100 seeds were placed in a refrigerator for 12 weeks. After 12 weeks, the seeds were planted outside. The seeds planted right away after harvest only experienced 15% germination. The seeds that underwent cold stratification before they were sown reached 50 to 68% germination (Brown, 2002).

River Bulrush (*Bolboschoenus fluviatilis*)

River bulrush is a native grasslike perennial plant found on the banks of many bodies of water across the United States. It is in the Cyperaceae family. River bulrush seeds and roots are consumed by waterfowl, and it provides a spawning habitat for some fish species (Illinois Planting Guide, 2004). River bulrush can spread through seeds or rhizomes (Kettenring et al., 2019). It is used in restoration projects of shorelines to reduce erosion (Illinois Planting Guide, 2004). The majority of river bulrush samples received by the SDSU Seed Testing Laboratory are from producers in Minnesota. Figure 4 in the Appendix displays seed characteristics of river bulrush seed under the microscope.

There is limited existing literature on the germination and growing of river bulrush. However, other related species have been studied. *Bolboschoenus maritimus*, *Schoenoplectus acutus*, and *Schoenoplectus americanus* are grown in similar environments as river bulrush. The ability to break seed dormancy and germination has been studied for these species. Marty and Kettenring (2017) performed two experiments.

The first experiment evaluated cold stratification followed by germination. In addition to cold stratification, they performed a germination study with the seeds subjected to a saturated and flooded environment. *B. maritimus* was significantly impacted by the stratification, source site, and moisture, and *S. acutus* was only impacted by the stratification and source site (Marty and Kettenring, 2017). The germination of *S. americanus* was not different between the various treatments. The study found that the stratification treatment of 180-days produced the highest germination (Marty and Kettenring, 2017). River bulrush can be established with seed and needs to experience cold and moist stratification to germinate (Illinois Planting Guide, 2004). Barak et al. (2018) suggests that cold stratification can increase germination because it can help break dormancy in native species.

Another method applied to increase germination in these species was scarification. Marty and Kettenring (2017) performed a second experiment where they evaluated using sulfuric acid, bleach, and water soak to break dormancy. The chemical scarification with bleach produced higher germination for two of the three species. Wagner and Oplinger (2016) performed mechanical and chemical scarification on four different species. Of the four species studied, two are related to river bulrush, *Schoenoplectus americanus* and *S. acutus*. Mechanical scarification was achieved by rubbing the seeds over sandpaper for three minutes. The chemical scarification involved the seeds soaking in bleach for one day or five days (Wagner and Oplinger, 2016). There was some improvement in germination with the five-day bleach treatments for both *Schoenoplectus spp.* used in the study, but mechanical scarification provided little to no difference in germination.

Since there is evidence of waterfowl feeding on river bulrush, a study evaluated the germination of three related species collected from the gizzards of waterfowl. The three species evaluated in this study were *B. maritimus*, *S. acutus*, and *S. americanus*. The bulrush seeds were collected from mallard gizzards. Kettenring et al. (2019) found that the seeds collected from the gizzards experienced high germination. The seeds were germinated under a constant daytime temperature of 35°C and a constant nighttime temperature of 28°C. *B. maritimus* reached 33.0±7.8% germination, and since *S. acutus*, and *S. americanus* could not be visually separated, they reached a combined germination of 59.3±16.7% (Kettenring et al., 2019). As waterfowl consume bulrush seeds, they are helping with the spread and germination of these species. It may be necessary to use processes such as chemical or physical scarification that imitate the seeds passing through the gizzards of waterfowl to increase germination.

Various temperature regimes have been used in these studies. Marty and Kettenring (2017) used a growth chamber set to 35°C for 12 hours and 18°C for 12 hours. These temperatures were chosen based off of the average summer temperatures found in the Great Salt Lake wetland area. Wagner and Oplinger (2016) experienced higher germination with *S. acutus* when using fluctuating temperatures. The highest germination experienced was 50-54% with the fluctuating temperatures 32°C and 38°C. In order to see river bulrush germinate, it may be necessary that a combination of cold stratification, chemical scarification, and high temperatures be utilized.

III. MATERIAL AND METHODS

Determining the weights

Samples used for determining the weights were collected from the South Dakota State University Seed Testing Laboratory and Agrecol Native Nursery. A total of 29 stiff goldenrod samples were used. River bulrush had a total of 18 samples. Following the procedure set forth in Section 13 of the AOSA Rules for Testing Seeds Volume 1 (2021), the weights required for stiff goldenrod and river bulrush were determined. Eight replications of 100 pure seeds were counted from a working sample. Each replication of 100 seeds was weighed to four decimal places. The mean, variance, standard deviation, and coefficient of variation (CV) were calculated from the eight replications. The CV cannot exceed 4.0 for non-chaffy seeds and 6.0 for chaffy seeds. According to the AOSA Rules for Testing Seeds (2021), a chaffy seed consists of "seed units that adhere to other seed units or other seed units or other surfaces because of their structure or texture, making it difficult to sample a seed lot or mixture and divide a representative working sample." If the CV is exceeded, an additional eight replications must be counted and weighed. A new standard deviation is determined from the 16 replicates. If a replicate diverges from the mean by more than twice the standard deviation, it must be removed. A new mean is calculated from the remaining replicates. The weight for a purity and noxious analysis is determined by the following equations:

$$weight\ for\ purity\ analysis\ =\ mean\ weight\ of\ replications\ x\ 25$$

$$weight\ for\ noxious\ analysis\ =\ purity\ analysis\ weight\ x\ 10$$

Germination

Four different seed lots of stiff goldenrod were used in this study to identify an optimum temperature for germination. The lots used in this study came from customers in Minnesota of the SDSU Seed Testing Laboratory. Lot 10954 and 11466 originated in MN. The age of the lots is unknown, but the SDSU Seed Testing Lab received Lot 10954 in June 2020 and Lot 11466 in May 2018. Lot 5955 and 9183 originated in Sherburne CO., MN and received in July 2021 and January 2020 respectively. Three seed lots of river bulrush received by the SDSU Seed Testing Laboratory for testing, and one which was donated from Agrecol Native Nursery were used in the study. Lots 3028, 8259, and 9377 originated in Sherburne CO., MN. The lab received the lots in January 2019, December 2019, and February 2020 respectively. The lot donated by Agrecol Native Nursery was produced in MN, and according to the label provided, it was tested in Mach 2021. For both species, the exact age of the lots is unknown. Only the date received in the lab is provided. The samples were cleaned to obtain pure seed units as defined by the AOSA Rules for Testing Seeds.

A preliminary TZ test was conducted on 25 seeds to determine the viability of all the seed lots. The preliminary TZ test was conducted on 25 seeds due to the limited amount of seed available. Table 3 and Table 13 show the estimated viability from the preliminary TZ test for the two species. Four replications of 50 seeds were counted out for germination at each temperature regime used in the study. Six seed germinators and a thermogradient table were used to conduct the germination study. The six germinators were set to temperatures commonly used in seed testing, which include 15-25°C, 20-30°C, 15-30°C, 15°C, 25°C, and 20°C. For the alternating temperatures, the seeds

experienced nighttime temperatures for 16 hours and daytime temperatures for 8 hours. Lights, with a minimum 807 lumen, were on for 8 hours during the set daytime hours.

A thermogradient table was set up with a cold-water bath at one end and warm water bath at the other. The warm water bath had a 50/50 mixture of water and glycol. Figure 1 displays how the thermogradient table was setup. The thermogradient table had to be split into two experiments because setting the baths from 5°C to 40°C caused the warm end of the table to dry out extremely fast and condensation to be present on the germination box lids. To create the first temperature gradient, the baths were set at 5°C and 25°C. This produced six temperatures 24°C, 23°C, 22°C, 20°C, 17°C, and 13°C plus or minus one degree. The second temperature gradient was created with the baths set at 25°C and 40°C. This produced six temperatures 32°C, 31°C, 30°C, 28°C, 27°C, 26°C plus or minus one degree. AOSA Rules for Testing Seeds (2021) require germinator temperatures to be plus or minus 1°C. Location temperatures were determined by using a surface thermometer placed in the middle of the blotter on each "column/temperature line" on the thermogradient table. After the table was allowed to equilibrate for 24 hours, the replications of seed were planted in 4-inch x 4-inch boxes on top of two blotters saturated in deionized water. Due to limited space on the thermogradient table a randomized complete block design was used. Figure 2 demonstrates how the germination boxes were placed inside the thermogradient table. Lights were used in all the germinators and thermogradient table. The lights, with a minimum of 807 lumens, were on for 8 hours and off for 16 hours to designate day and night.

Germination was checked once a week for four weeks. The first count occurred after seven days. Normal seedlings, as defined by the ASOA Rules for Testing Seeds

(2021), were pulled off every week, and the blotters within the boxes were re-watered with deionized water. The boxes were watered as needed in between checks due to the condensation and warmer temperatures. After 28 days, a final count was performed. The ungerminated seeds were subjected to a TZ test to determine the percent dormancy. Since stiff goldenrod has a permeable seed coat, the ungerminated seeds were placed directly into 1% TZ solution without being nicked. The stiff goldenrod seeds were placed in an oven set at 35°C for 4-6 hours. The seeds were removed from the TZ solution and seed structure staining or lack of staining was evaluated under the microscope to identify whether the seed was dormant or dead. The ungerminated river bulrush seeds were cut lengthwise and placed in 1% TZ solution. The river bulrush seeds do not stain as readily as the stiff goldenrod, so they were left to stain overnight in the oven at 35°C. The seeds were removed from the solution and evaluated under the microscope. This whole process was repeated with the water baths set at 25°C and 40°C and in the six germinators.

A statistical analysis was conducted on the stiff goldenrod and river bulrush germination studies using R version 4.2.3 and the ANOVA command. The p-value for all analysis was equal to 0.05. The normality assumptions for ANOVA were met.

IV. RESULTS AND DISCUSSION

Weights for Analyses

Stiff Goldenrod

Table 1 displays all the lots and replications of stiff goldenrod used for determining the weights for a purity and noxious analysis. Table 1.1 shows the lots that had a CV higher than six and had an additional eight replications counted out. The CV greater than six could be explained by variation in seed size and missing pappus or seed coat from some seed lots analyzed. Figure 3 visually shows the variations in stiff goldenrod seeds. Some of the seeds have pappus, some have partial pappus, and others are missing it completely. There are differences in size. Variations between lots can be explained by growing conditions, harvesting conditions, and handling conditions.

Table 2 summarizes the minimum weights that were calculated for purity and noxious exams on stiff goldenrod. From the 29 samples of stiff goldenrod counted and weighed, the average weight of all the replications was 0.0548 grams. Using the formula provided, the minimum weight required for a purity analysis is 1.3701 grams and 13.7006 grams for a noxious analysis. The AOSA Rules for Testing Seeds (2021) notes that a weight less than five grams should be rounded to the nearest tenth. Following this statement, stiff goldenrod requires 1.4 grams for a purity analysis and 14 grams for a noxious exam. The average weight of seeds per gram was calculated to 1824.7391 seeds. One ounce of seed contains approximately 51,000 seeds. One study found 41,000 seeds/ounce (Brown, 2002). Another study suggests there is approximately 770,000 seeds/pound (USDA Planting Guide), which is approximately 48,000 seeds/ounce.

However, neither of these studies explain how this was determined or how many different lots were used to determine the number of seeds per ounce.

River Bulrush

Table 11 summarizes information used to determine the weights for river bulrush. Table 11.1 presents the lots that had a CV greater than four, which required additional replications to be counted and weighed. The variability in replications causing the CV to be greater than four can be explained by the variation in seed size and unfilled seeds. According to the pure seed unit definition for river bulrush, a filled or unfilled seed is considered pure seed when there is not an opening present to determine that. Figure 4 displays the variation in seed size one seed lot can experience. The image also shows some seeds still have their bristles intact, and others are missing bristles most likely from being rubbed off during harvest or the handling of the seed.

Table 12 summarizes the minimum weights that were calculated for purity and noxious exams on river bulrush. From the 18 samples of river bulrush, the average weight for the replications was 0.6516 grams. The minimum weight for a purity analysis is 16.2888 grams and 162.8881 grams for a noxious analysis. The AOSA Rules for Testing Seeds (2021) suggests that any weight over five grams should be rounded to the nearest whole number. The minimum weight to be used by seed testing labs is 16 grams for a purity analysis and 160 grams for a noxious analysis. In one gram, river bulrush contained on average 153.4796 seeds.

With the purity and noxious weights determined for stiff goldenrod and river bulrush, rule proposals for the two species can be presented to the AOSA. The AOSA can accept these proposals to create standards for all seed testing labs to follow.

Germination

Stiff Goldenrod

Table 4 presents a summary of the mean percent germination, dormancy, and total viability for each of the four seed lots and the 18 temperatures. According to the AOSA Rules for Testing Seeds, the variation between replications were all within tolerance. The mean germination percentages varied from 0% to 79.5%. The mean dormancy percentage varied from 0% to 58%. The highest germination observed occurred at a constant temperature of 25°C.

ANOVA was used to evaluate if any of the temperatures were statistically different. The p-value was <2e-16, which is very small. This suggests there is a significant difference between the temperatures. Tukey's W was used to compare the germination means of the different temperatures. Table 7 summarizes the results from using Tukey's W. There was no significant difference ($p<0.05$) between the constant temperature 23°C, 24°C, 25°C, and the alternating temperatures 20-30°C, 15-25°C, and 15-30°C. This suggests that stiff goldenrod can germinate at constant or alternating temperatures. The six temperatures that were not statistically different all fall in the range of temperatures used in the study by Nuzzo (1976). Nuzzo (1976) germinated stiff goldenrod in a greenhouse with day-time temperatures ranging from 21-32°C and nighttime temperatures ranging from 4-21°C.

The low germination and dormancy at the constant temperatures 28°C, 30°C, 31°C, and 32°C is possibly due to the warmer temperatures adversely impacting seed longevity during the germination period. The low germination but high dormancy at the

temperatures 13°C, 15°C, 17°C, 19°C, 20°C, and 22°C suggest that these constant temperatures are too cool for germination to occur for this species.

The goal of seed testing laboratories is to provide accurate results in a timely manner. Performing a germination exam that lasts multiple weeks is not ideal for reporting germination to customers. Shorter germination periods can help limit the amount of mold growth on the blotters, which could kill viable seeds. The germination data at each count for the six temperatures that were not statistically different were evaluated to determine if there is a statistically significant difference between counts. Table 10 summarizes the comparisons of the four counts. There was no statistical difference ($p<0.05$) between counts at 28 days, 21 days, and 14 days. With that being said, seed testing laboratories can stop the germination exam at 14 days and still experience a high germination percentage. Nuzzo (1976) observed peak germination of stiff goldenrod at 14 days. Since there is no statistical difference, stopping the germination exam at 14 days would allow seed testing labs to provide customers with results in a timely manner compared to running the exam for 21 or 28 days.

While the four seed lots used in this experiment varied in age, none of the seed lots were freshly harvested. Further studies should be conducted to determine whether a pre-chill would be necessary for freshly harvested seed.

The AOSA also accepts rule proposals for germination methods. Before a rule proposal can be made for the germination methods for stiff goldenrod, a referee test needs to be conducted. A referee test involves multiple seed testing labs who voluntarily test the proposed germination methods. It is important to see if the identified methods can produce similar results in other labs.

River Bulrush

Table 14 presents a summary of river bulrush's mean percent germination, dormancy, and total viability. River bulrush experienced germination under the alternating temperatures 15-25°C, 15-30°C, and 20-30°C, and the species also germinated at the constant temperature of 30°C. Although there was germination, it was a very small percentage, basically insignificant. The low viability of the lots can be explained by the age of the lots, insect damage, and poor pollination causing empty seeds. Table 17 summarizes the mean comparisons for river bulrush. The only temperature significantly different ($p<0.05$) from the other temperatures was 20-30°C. Considering there was little to no germination, no comparison was conducted between counts.

It is not surprising to see germination at the alternating temperatures as previous studies have shown germination of related species using alternating temperatures. Wagner and Oplinger (2016) evaluated two different alternating regimes. The two *Schoenoplectus spp*. experienced the highest germination at 32-38°C and little to no germination at 40-46°C. The few seedlings that emerged at the temperature regime 40-46°C died, especially when exposed to 12 hours of light (Wagener and Oplinger, 2016). The germination at alternating temperatures suggests river bulrush may require shrinking and swelling of its seed coat to allow for the uptake of water. River bulrush might experience increased germination if the seed treatment included scarification or stratification techniques.

The variability of dormancy between the lots can possibly be explained by the age of the seed lots, insect damage, and empty (unfilled) seed units in some lots. However, ANOVA was performed on the dormancy of river bulrush at different temperatures. The

p-value was 0.937, which means none of the temperatures are significantly different in breaking dormancy.

Future Work

Further investigation needs to occur for river bulrush's germination methods before a proposal can be made to the AOSA. Since river bulrush is found growing on the banks of rivers and lakes, river bulrush could experience more germination with moist soil compared to just blotters. Soaking the seeds in water before planting showed promising results in other published studies. Common methods typically used in seed testing labs are using a pre-chill and KNO_3 as a wetting agent.

Many of the published studies involving related species used warmer temperatures than the temperatures established in this experiment. Testing warmer constant temperatures and warmer alternating temperature regimes may improve germination. 20-35°C is another common temperature used in seed testing labs, which river bulrush could germinate at. Using a pre-chill is a common practice in seed testing labs, testing different lengths of pre-chill.

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APPENDIX

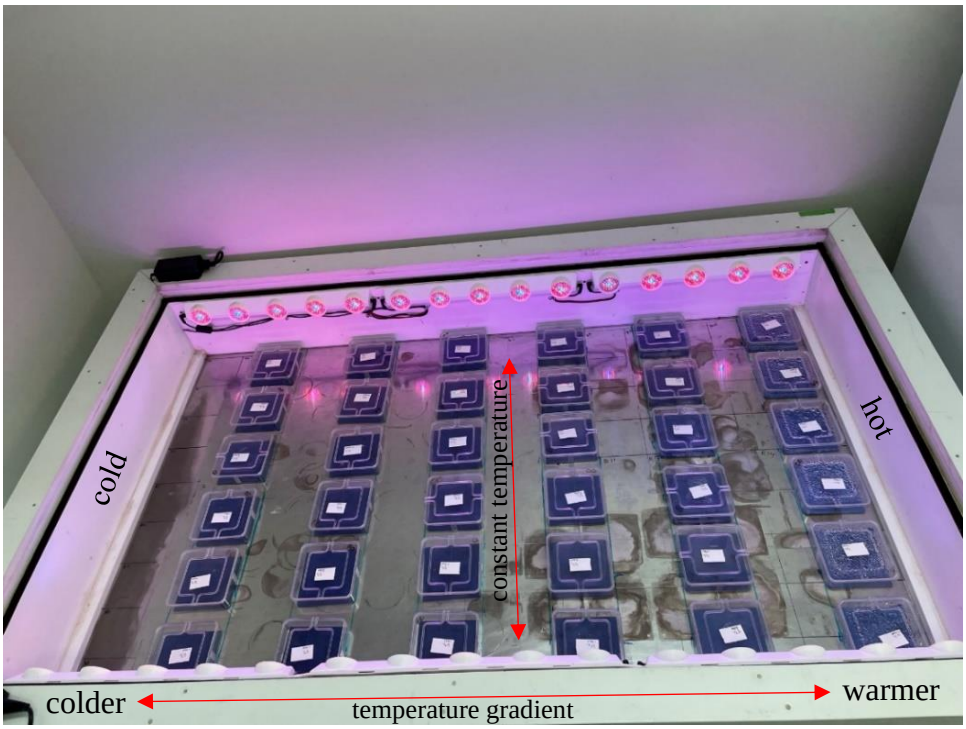

Figure 1: The interior view of the thermogradient table. The temperature gradient is created from left to right. Each column is a constant temperature.

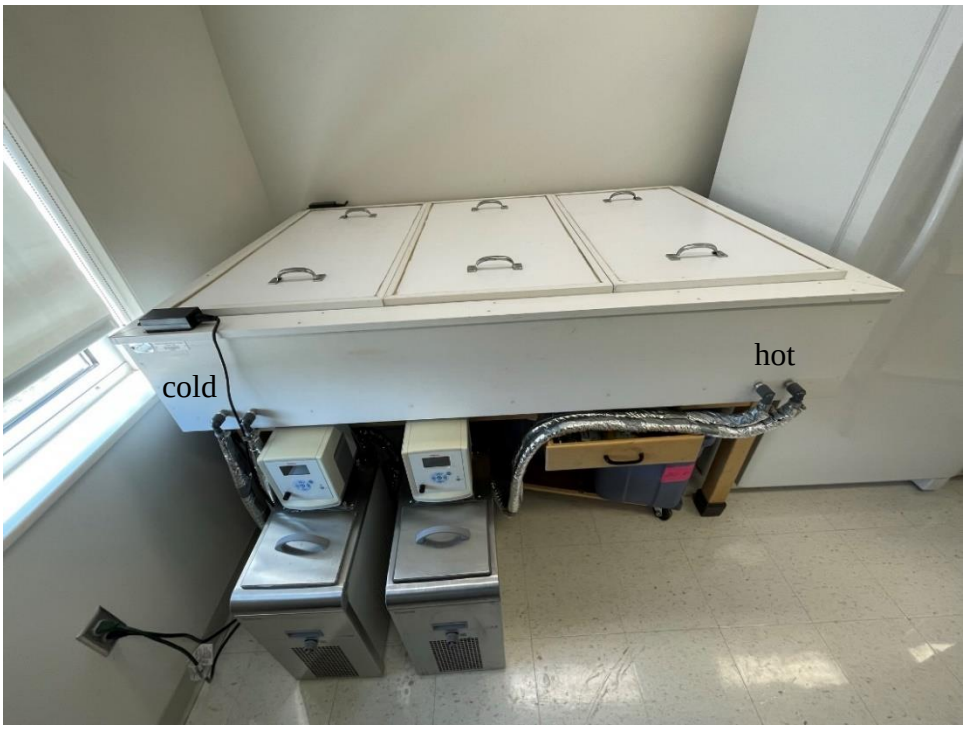

Figure 2: The exterior view of the thermogradient table with the insulated lids in place. To create a temperature gradient, the cold water enters the table on the left side, and the hot water enters the table on the right side.

Figure 3: An image of stiff goldenrod seed unit variation under the microscope.

Figure 4: An image of river bulrush seed unit variation under the microscope.

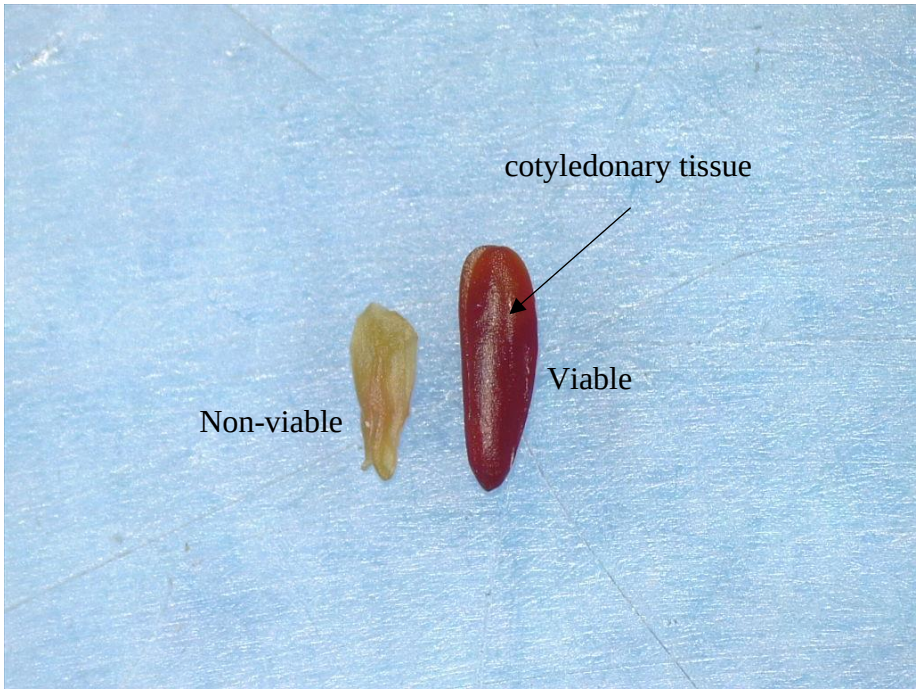

Figure 5: The staining of a non-viable and viable stiff goldenrod seed when subjected to tetrazolium.

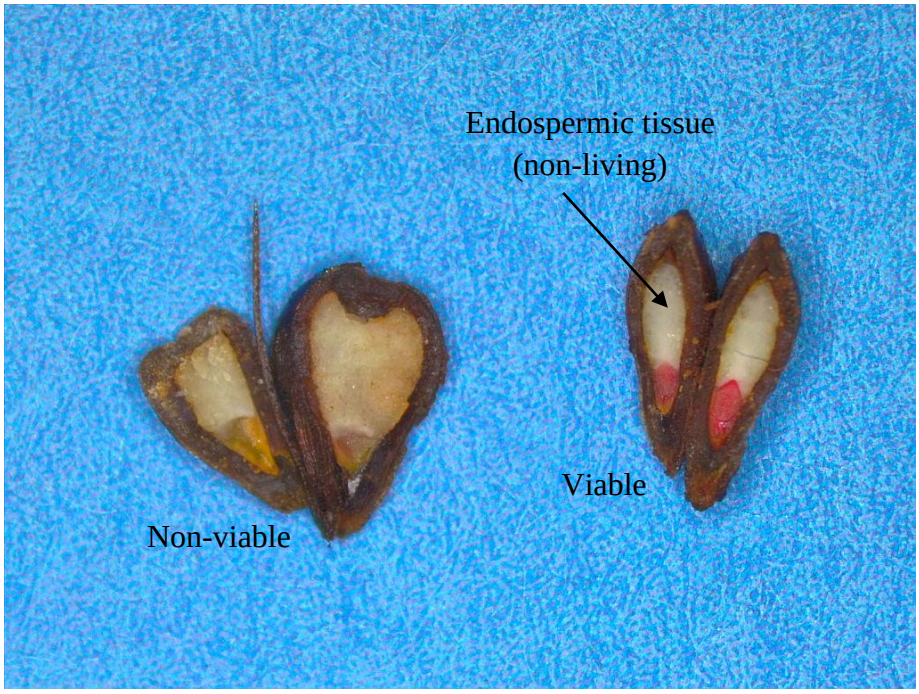

Figure 6: The staining of a nonviable and viable river bulrush seed when subjected to tetrazolium.

Table 1: Weights and Calculations for Determining the Weights for Stiff Goldenrod

Sample #	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Ave.	Min. Wt. for Purity	Min. Wt. for Noxious	Seeds/ Gram	Variance	Std. Dev.	CV
11466	0.0601	0.0564	0.0591	0.0585	0.0589	0.0601	0.0595	0.0559	0.0586	1.5	15	1708	2.54E-06	0.0016	2.7
10954	0.0506	0.0505	0.0557	0.0529	0.0522	0.0526	0.0519	0.0551	0.0527	1.3	13	1898	3.56E-06	0.0019	3.6
6893	0.0615	0.0622	0.0560	0.0594	0.0555	0.0578	0.0592	0.0576	0.0587	1.5	15	1705	5.77E-06	0.0024	4.1
1877	0.0526	0.0507	0.0533	0.0541	0.0543	0.0536	0.0523	0.0503	0.0527	1.3	13	1899	2.23E-06	0.0015	2.8
4731	0.0491	0.0537	0.0485	0.0476	0.0501	0.0503	0.0504	0.0513	0.0501	1.3	13	1995	3.48E-06	0.0019	3.7
9392	0.0506	0.0525	0.0489	0.0508	0.0480	0.0486	0.0514	0.0450	0.0495	1.2	12	2021	5.60E-06	0.0024	4.8
9826	0.0603	0.0629	0.0594	0.0611	0.0600	0.0628	0.0660	0.0587	0.0614	1.5	15	1629	5.70E-06	0.0024	3.9
2389	0.0488	0.0500	0.0454	0.0464	0.0460	0.0447	0.0460	0.0447	0.0465	1.2	12	2151	3.68E-06	0.0019	4.1
9183	0.0527	0.0483	0.0513	0.0515	0.0551	0.0478	0.0511	0.0519	0.0512	1.3	13	1953	5.43E-06	0.0023	4.6
5955	0.0610	0.0554	0.0558	0.0556	0.0560	0.0568	0.0540	0.0507	0.0557	1.4	14	1797	8.20E-06	0.0029	5.1
2265	0.0549	0.0508	0.0528	0.0533	0.0549	0.0586	0.0513	0.0539	0.0538	1.3	13	1858	6.00E-06	0.0024	4.5
1431	0.0455	0.0466	0.0447	0.0442	0.0418	0.0426	0.0445	0.0438	0.0442	1.1	11	2269	2.32E-06	0.0015	3.4
11730	0.056	0.0551	0.0543	0.0562	0.0564	0.0585	0.0566	0.0569	0.0563	1.4	14	1778	1.55E-06	0.0012	2.2
5828	0.0534	0.0521	0.0548	0.0543	0.0518	0.0517	0.0565	0.0487	0.0529	1.3	13	1890	5.67E-06	0.0024	4.5
1260	0.0477	0.0473	0.0463	0.0461	0.0473	0.0474	0.0463	0.0455	0.0467	1.2	12	2140	6.17E-07	0.0008	1.7
9521	0.0568	0.0579	0.0564	0.0552	0.0555	0.0555	0.0580	0.0592	0.0568	1.4	14	1760	2.07E-06	0.0014	2.5
3081	0.0602	0.0573	0.0566	0.0595	0.0589	0.0576	0.0606	0.0614	0.0590	1.5	15	1695	2.95E-06	0.0017	2.9
6743	0.0507	0.0538	0.0487	0.0478	0.0522	0.0499	0.0501	0.0530	0.0508	1.3	13	1969	4.36E-06	0.0021	4.1
2674	0.0416	0.0434	0.0442	0.0426	0.0448	0.0448	0.0431	0.0420	0.0433	1.1	11	2309	1.49E-06	0.0012	2.8
1505	0.0433	0.0461	0.0460	0.0419	0.0438	0.0416	0.0404	0.0438	0.0434	1.1	11	2306	4.12E-06	0.0020	4.7
2390	0.0511	0.0515	0.0533	0.0541	0.0511	0.0520	0.0522	0.0514	0.0521	1.3	13	1920	1.19E-06	0.0011	2.1
6411	0.0852	0.0802	0.0838	0.0822	0.0815	0.0854	0.0801	0.0824	0.0826	2.1	21	1211	4.21E-06	0.0021	2.5
7054	0.0806	0.0836	0.0836	0.0809	0.0833	0.0828	0.0878	0.0891	0.0840	2.1	21	1191	9.12E-06	0.0030	3.6
2939	0.1020	0.1011	0.1019	0.1003	0.1033	0.1027	0.1028	0.1021	0.1020	2.6	26	980	9.34E-07	0.0010	0.94
775	0.0490	0.0472	0.0483	0.0501	0.0474	0.0491	0.0484	0.0492	0.0486	1.2	12	2058	9.36E-07	0.0010	2
9104	0.0476	0.0465	0.0461	0.0473	0.0477	0.0482	0.0486	0.0454	0.0472	1.2	12	2120	1.19E-06	0.0011	2.3
7612	0.0482	0.0460	0.0484	0.047	0.0522	0.0457	0.0455	0.0446	0.0472	1.2	12	2119	5.83E-06	0.0024	5.1

Table 1.1: Weights and Calculations for Determining the Weights for Stiff Goldenrod for Seed Lots that had a CV Greater than 6

Sample Number	1878	2673
Rep 1	0.0393	0.0476
Rep 2	0.0395	0.0486
Rep 3	0.0387	0.0435
Rep 4	0.0408	0.0454
Rep 5	0.0368	0.0408
Rep 6	0.0351	0.0421
Rep 7	0.0390	0.0387
Rep 8	0.0323	0.0395
Rep 9	0.0395	0.0450
Rep 10	0.0391	0.0434
Rep 11	0.0359	0.0465
Rep 12	0.0386	0.0434
Rep 13	0.0381	0.0421
Rep 14	0.0375	0.0451
Rep 15	0.0412	0.0422
Rep 16	0.0311	0.0407
Mean	0.0377	0.0434
Std. Dev.	0.0028	0.0028
Std. Dev. *2	0.0057	0.0057
Mean -	0.0320	0.0378
Mean +	0.0433	0.0491
New Mean	0.0381	0.0434

Table 2: Minimum Weights Calculated for Testing Purity Analysis and Noxious Weed Seed Exams on Stiff Goldenrod

Calculated Weights (grams)			
Average Weight of 100 Seeds	Min. Weight for Purity	Min. Weight for Noxious	Seeds Per Gram
0.0548	1.37	13.7	1825
Weights Required for Testing (grams)			
Minimum Weight for Purity		Minimum Weight for Noxious	
1.4		14	

Table 3: Preliminary TZ Results for Stiff Goldenrod

Seed Lot	Viability %
10954	40
11466	44
5955	72
9183	72

Table 4: Mean percentage of germination, dormancy, and total viability for Stiff Goldenrod at various temperature regimes

Seed Lot	Lot 10954			Lot 11466			Lot 5955			Lot 9183		
Temp.	Mean Germ	Mean Dormancy	Total Viability	Mean Germ	Mean Dormancy	Total Viability	Mean Germ	Mean Dormancy	Total Viability	Mean Germ	Mean Dormancy	Total Viability
13°C	1	58	59	0	33	33	0	54	54	6	45	51
15°C*	14	45	59	6	45	51	3	44	47	50	19	69
17°C	10	50	60	2	44	46	1	48	49	42	22	64
19°C	10	22	33	17	28	45	9	30	39	58	9	67
20°C*	44	14	48	30	21	51	24	36	60	68	2	70
22°C	47	15	62	31	19	50	21	21	42	59	4	63
23°C	60	4	64	39	11	50	41	15	56	62	4	66
24°C	45	4	49	39	8	47	45	9	54	63	1	64
25°C*	64	3	67	48	17	65	80	5	85	65	8	73
26°C	53	3	56	19	5	24	50	4	54	56	1	57
27°C	32	4	36	17	6	23	46	4	50	41	5	46
28°C	17	6	23	13	6	19	31	4	35	35	5	40
30°C	11	4	15	3	5	8	20	2	22	23	4	27
31°C	1	5	6	0	1	1	4	3	7	5	5	10
32°C	0	1	1	0	0	0	0	5	5	0	0	0
15-25°C*	58	11	69	32	27	59	55	22	77	68	5	73
15-30°C*	51	11	62	21	9	30	57	12	69	63	4	67
20-30°C*	66	4	70	26	8	34	59	6	65	67	2	69

[1]Percentages were rounded according to the AOSA Rules

[2]Samples with an * represent the germination chambers used. The rest of the temperatures were established on the thermogradient table.

Table 5: ANOVA for Stiff Goldenrod Germination

Source	DF	Sum of Squares	Mean Squares	F Value	Pr>F*
Model	17	27331	1608	28.71	<2e-16
Residuals	270	15120	56		
Corrected Total	287	42451			

*statistically significant <0.05

Table 6: ANOVA for Stiff Goldenrod Dormancy

Source	DF	Sum of Squares	Mean Squares	F Value	Pr>F*
Model	17	13657	803.3	51.4	<2e-16
Residuals	270	4220	15.6		
Corrected Total	287	17877			

*statistically significant <0.05

Table 7: Mean Comparison using Tukey's W for Stiff Goldenrod Germination

Temperature	Germination*
25°C	31.88 a
20-30°C	27.19 ab
15-25°C	26.56 ab
23°C	25.06 abc
24°C	23.88 abcd
15-30°C	23.88 abcd
26°C	22.19 bcd
20°C	20.56 bcde
22°C	19.63 bcde
27°C	17.00 cdef
19°C	15.38 defg
28°C	11.88 efg
15°C	9.00 fgh
30°C	7.00 gh
17°C	6.69 gh
31°C	1.19 h
13°C	0.81 h
32°C	0.00 h

*Means with the same letter are not statistically different at the $p>0.05$

[1]Mean germination listed in this table is not a percent, but the average number of seeds germinated out of 50 seeds.

Table 8: Mean Comparison using Tukey's W for Stiff Goldenrod Dormancy

Temperature	Dormancy*	
13°C	23.6	a
17°C	20.4	a
15°C	19.0	a
19°C	11.0	b
20°C	8.9	bc
15-25°C	8.1	bcd
22°C	7.2	bcde
15-30°C	4.3	cdef
23°C	4.2	cdef
25°C	4.0	def
24°C	2.6	ef
28°C	2.4	ef
20-30°C	2.4	ef
27°C	2.3	f
30°C	1.8	f
31°C	1.7	f
26°C	1.6	f
32°C	0.6	f

*Means with the same letter are not statistically different at the $p>0.05$

[1]Mean dormancy listed in this table is not a percent, but the average number of seeds dormant out of the number of ungerminated seeds.

Table 9: ANOVA for Stiff Goldenrod Counts

Source	**DF**	**Sum of Squares**	**Mean Squares**	**F Value**	**Pr>F***
Model	3	12772	4257	59.42	<2e-16
Residuals	380	27227	72		
Corrected Total	383	39999			

*statistically significant <0.05

Table 10: Mean Comparison using Tukey's W for Stiff Goldenrod Counts

Count	Germination*
28 days	26.4 a
21 days	25.8 a
14 days	23.5 a
7 days	12.1 b

*Means with the same letter are not statistically different at the p>0.05

[1]Mean germination listed in this table is not a percent, but the average number of seeds germinated out of 50 seeds.

Table 11: Weights and Calculated Results for Determining the Weights Required for River Bulrush

Sample #	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Ave. Wt.	Min. Wt. for Purity	Min. Wt. for Noxious	Seeds/ Gram	Variance	Std. Dev.	CV
2324	0.6758	0.6245	0.6368	0.6434	0.6756	0.6437	0.6828	0.6600	0.6553	16	164	153	0.0005	0.0213	3.2
11655	0.6408	0.6611	0.6701	0.6755	0.6683	0.6616	0.6667	0.6869	0.6664	17	167	150	0.0002	0.0132	2.0
2643	0.7523	0.7467	0.7463	0.7440	0.7483	0.7337	0.7550	0.7689	0.7494	19	187	133	0.0001	0.0101	1.3
11732	0.6316	0.6038	0.5964	0.6365	0.6272	0.6338	0.5960	0.6122	0.6172	15	154	162	0.0003	0.0171	2.8
7470	0.6679	0.6612	0.6655	0.6979	0.6199	0.6673	0.6563	0.6504	0.6608	17	165	151	0.0005	0.0217	3.3
8259	0.6701	0.6989	0.6815	0.6842	0.6714	0.6808	0.6647	0.6287	0.6725	17	168	149	0.0004	0.0206	3.1
8761	0.6236	0.6460	0.5963	0.6642	0.6230	0.6433	0.6433	0.6219	0.6327	16	158	158	0.0004	0.0207	3.3
7614	0.6068	0.6204	0.6002	0.6219	0.6046	0.6096	0.6122	0.6094	0.6106	15	153	164	0.0001	0.0074	1.2
1912	0.6562	0.6518	0.6883	0.6310	0.6504	0.6017	0.6272	0.6502	0.6446	16	161	155	0.0006	0.0253	3.9
9376	0.6685	0.6595	0.6194	0.6477	0.6708	0.6757	0.6274	0.6854	0.6568	16	164	152	0.0006	0.0235	3.6
9377	0.6320	0.6259	0.6255	0.5910	0.6171	0.5973	0.6346	0.6494	0.6216	16	155	161	0.0004	0.0194	3.1
9388	0.7371	0.7298	0.7346	0.7253	0.7355	0.7533	0.7203	0.7538	0.7362	18	184	136	0.0001	0.0121	1.6
2684	0.5765	0.5728	0.6190	0.6188	0.5899	0.6056	0.5783	0.6123	0.5967	15	149	168	0.0004	0.0195	3.3
5880	0.5893	0.6422	0.6317	0.6507	0.6650	0.6316	0.6312	0.6104	0.6315	16	158	158	0.0005	0.0234	3.7
AgRB	0.6568	0.6564	0.6388	0.6750	0.6832	0.6866	0.6832	0.6289	0.6636	17	166	151	0.0005	0.0219	3.3
7715	0.6830	0.6861	0.6727	0.6795	0.6650	0.6616	0.6646	0.6984	0.6764	17	169	148	0.0002	0.0127	1.9

Table 11.1: Weights and Calculations for Determining the Weights for River Bulrush for Seed Lots that had a CV Greater than 4

Sample Number	11381	3028
Rep 1	0.5964	0.7206
Rep 2	0.5698	0.7325
Rep 3	0.5560	0.6995
Rep 4	0.5404	0.6607
Rep 5	0.6093	0.6599
Rep 6	0.5624	0.6392
Rep 7	0.5974	0.6052
Rep 8	0.4981	0.6511
Rep 9	0.6159	0.6934
Rep 10	0.5612	0.7017
Rep 11	0.5535	0.6878
Rep 12	0.5131	0.7416
Rep 13	0.5968	0.6538
Rep 14	0.6514	0.6512
Rep 15	0.5283	0.6644
Rep 16	0.5364	0.6102
Mean	0.5679	0.6733
Std. Dev.	0.0409	0.0401
Std. Dev. *2	0.0819	0.0801
Mean -	0.4860	0.5932
Mean +	0.6498	0.7534
New Mean	0.5623	0.6733

Table 12: Minimum Weights Calculated for Purity Analysis and Noxious Weed Seed Exams on River Bulrush

Calculated Weights (grams)			
Average Weight of 100 Seeds	Min. Weight for Purity	Min. Weight for Noxious	Seeds Per Gram
0.6516	16.3	162.9	153
Weights Required for Testing (grams)			
Minimum Weight for Purity		Minimum Weight for Noxious	
16		160	

Table 13: Preliminary TZ Results for River Bulrush

Seed Lot	Viability %
3028	36
8259	24
9377	46
AgRB	44

Table 14: Mean percentage of germination, dormancy, and total viability of River Bulrush at various temperature regimes

Seed Lot	Lot 3028			Lot 8259			Lot 9377			Lot AgRB		
Temp.	Mean Germ	Mean Dormancy	Total Viability	Mean Germ	Mean Dormancy	Total Viability	Mean Germ	Mean Dormancy	Total Viability	Mean Germ	Mean Dormancy	Total Viability
13°C	0	33	33	0	13	13	0	37	37	0	17	17
15°C*	0	21	21	0	13	13	0	28	28	0	11	11
17°C	0	37	37	0	8	8	0	37	37	0	14	14
19°C	0	39	39	0	5	5	0	37	37	0	15	15
20°C*	0	35	35	0	5	5	0	37	37	0	13	13
22°C	0	35	35	0	9	9	0	34	34	0	18	18
23°C	0	36	36	0	4	4	0	39	39	0	17	17
24°C	0	41	41	0	5	5	0	39	39	0	22	22
25°C*	0	35	35	0	7	7	0	38	38	0	14	14
26°C	0	37	37	0	9	9	0	40	40	0	14	14
27°C	0	40	40	0	8	8	0	33	33	0	13	13
28°C	0	33	33	0	6	6	0	34	34	0	15	15
30°C	0	31	31	0	5	5	1	41	42	0	15	15
31°C	0	33	33	0	5	5	0	37	37	0	9	9
32°C	0	28	28	0	3	3	0	28	28	0	6	6
15-25°C*	0	38	38	1	9	10	2	43	45	0	11	11
15-30°C*	1	34	35	2	7	9	1	43	44	1	19	20
20-30°C*	3	27	30	4	4	8	4	40	44	0	12	12

[1]Percentages were rounded according to the AOSA Rules

[2]Samples with an * represent the germination chambers used. The rest of the temperatures were established on the thermogradient table.

Table 15: ANOVA for River Bulrush Germination

Source	DF	Sum of Squares	Mean Squares	F Value	Pr>F*
Model	17	26.28	1.5460	13.49	2e-16
Residuals	270	30.94	0.1146		
Corrected Total	287	57.22			

*statistically significant <0.05

Table 16: ANOVA for River Bulrush Dormancy

Source	DF	Sum of Squares	Mean Squares	F Value	Pr>F*
Model	17	479	28.16	0.53	0.937
Residuals	270	14339	53.11		
Corrected Total	287	14818			

*not statistically significant p<0.05

Table 17: Mean Comparison using Tukey's W for River Bulrush Germination

Temperature	Germination*
20-30°C	1.25 a
15-30°C	0.50 b
15-25°C	0.25 bc
30°C	0.06 c
22°C	0.00 c
23°C	0.00 c
24°C	0.00 c
26°C	0.00 c
27°C	0.00 c
28°C	0.00 c
31°C	0.00 c
32°C	0.00 c
25°C	0.00 c
15°C	0.00 c
20°C	0.00 c
13°C	0.00 c
17°C	0.00 c
19°C	0.00 c

*Means with the same letter are not statistically different at the $p>0.05$

[1]Mean germination listed in this table is not a percent, but the average number of seeds germinated out of 50 seeds.

Table 18: Mean Comparison using Tukey's W for River Bulrush Dormancy

Temperature	Dormancy*	
24°C	13.31	a
15-30°C	12.75	a
15-25°C	12.56	a
26°C	12.38	a
13°C	12.38	a
23°C	11.94	a
17°C	11.94	a
19°C	11.94	a
22°C	11.88	a
25°C	11.69	a
27°C	11.63	a
30°C	11.31	a
20°C	11.13	a
28°C	11.00	a
31°C	10.38	a
20-30°C	10.19	a
15°C	8.94	a
32°C	8.06	a

*Means with the same letter are not statistically different at the $p>0.05$

[1]Mean dormancy listed in this table is not a percent, but the average number of seeds dormant out of the number of ungerminated seeds.