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# Buoyancy and Emergence in Elymus Species

Sandor Jakab
swj5@uakron.edu

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**Abstract:**

It is important that native plants are able to successfully emerge in wetlands because they play an important role in maintaining diversity. The relationship between seed buoyancy and seedling emergence was analyzed for three wetland species of the same genus: *Elymus canadensis* (FACU), *Elymus macgregorii* (FACW), and *Elymus virginicus* (FACW). *E. macgregorii* seemed to be the most buoyant, and *E. virginicus* and *E. canadensis* demonstrated very similar, less buoyant levels. Based on the idea that more buoyant seeds will be deposited at a shallow depth, and less buoyant seeds will be deposited at a deeper soil level, a relationship between depth of the artificially placed seed and the ability to emerge was evident in the FACW species. *E. macgregorii* was less buoyant (with respect to the other two species) and more successful in emerging from a shallow depth. *E. virginicus* was more buoyant and more successful in emerging from a deep soil level.  This relationship was not observed in the FACU species. *E. canadensis* was more buoyant and more successful in emerging from a shallow depth.

**Introduction:**

Native plants are essential in maintaining wetland biodiversity. Unlike invasive plants, they do not rapidly spread or prevent others from growing. A persistent issue in the field of wetland restoration involves the struggle for these native plant species to emerge from the soil and establish. Emergence is defined as “the probability that a germinated seed in the seedbank will penetrate the soil/litter surface to commence autotrophic growth” (Larson & Funk, 2016). In order to emerge, several conditions must occur including arrival at a suitable location, receipt of germination cues, and appropriate environmental conditions. Furthermore the seedling must overcome “obstacles” which may include: being submerged in water/soil or under a rock, fighting for sunlight and area to grow, plant litter, sedimentation, soil compaction, etc.

While each of these challenges may play a role in the emergence of the plant, seed buoyancy and its relationship to emergence success will be the focus of this experiment. The term buoyancy refers to the ability or tendency of a seed to float. The extent to which seed buoyancy may impact the plant likely depends on where it is typically found, as a more buoyant seed may be more likely to flow with bodies of water (flood plain). This is important because “Floating time is a critical factor affecting the potential dispersal distances of water-dispersed propagules” (Zhao et al., 2021). A more buoyant seed attribute is likely the result of a trade-off the plant has endured over time. A trade-off in plants is indicated by the increase in performance of a certain trait which often has detrimental effects on another trait, given the resources and energy available. Because a more buoyant seed is likely to be deposited on the surface of the soil, it may be less successful if it is buried. These ideas lead to several research questions. The

primary question for this experiment is, “Is there a relationship between buoyancy and emergence in native *Elymus* species”. Secondary questions include: “Are more buoyant seeds less effective in emerging when artificially placed at a deep soil depth?”, “Are less buoyant seeds more likely to emerge when artificially placed at a deep soil depth?”.

To determine answers to these questions, I studied buoyancy and emergence, and their relationship in three different species. Each of the species are from the Poaceae family, which is more commonly known as the grass family. They are also members of the same genus, *Elymus*, and are therefore very similar. Because this experiment deals with the relationship of buoyancy and emergence, it is important to know and understand the wetland indicator status for each of these graminoids. This, along with a general description of each species, is discussed prior to the explanation of the experimental methods.

*Elymus canadensis,* also known as Canada wildrye, is native to most of North America. This includes the majority of Canada and all but seven U.S. states (LA, MS, AL, GA, FL, HI, AK). It is a short-lived perennial that grows about 2-4 feet tall. In the north-central northeast (NCNE) region of the United States, *E. canadensis* has a Facultative Upland plant (FACU) wetland indicator status. This means that it prefers to grow upland, but may be found in wetlands (USDA,NRCS, 2014).

**Figure 1:** This image shows *E. canadensis* as a seed and an adult plant, side by side. (Photo by John Hagstrom)

Native to the eastern half of North America, *Elymus macgregorii* is also a perennial. It typically grows 4 feet tall, and the NCNE wetland indicator status is Facultative Wetland (FACW). This means it prefers to grow in wetlands but can also be found in upland areas. As a result of this, early wild-rye (common name) is found in both floodplains and forests. An interesting detail to mention is that this particular species is rare in most states where it is found, and even endangered in New Hampshire (USDA,NRCS, 2014).

**Figure 2:** This image shows *E. macgregorii* as a seed and an adult plant, side by side (Photo by Arthur Haines)

Similar to early wild-rye, *Elymus virginicus* has a FACW wetland indicator status. It's native to all but ten U.S. states (MT, ID, OR, WA, NV, CA, UT, CO, AK, HI), and most of Canada. Virginia wild-rye (common name) is a perennial that grows about 4 feet tall and is common in salt marshes, coastal beaches, and shores of rivers or lakes. Even though this species shares the same indicator status as *E. macgregorii*, they grow in different types of habitats which allows for a unique comparison (USDA,NRCS, 2014).

**Figure 3:** This image shows *E. virginicus* as a seed and an adult plant, side by side (Photo by Peter M. Dziuk)

**Objectives:**

The main goal/objective of this experiment is to determine the relative buoyancy level, the emergence success at a shallow and deep soil depth, and the relationship between seed buoyancy and emergence in the three *Elymus* species. Upon successful completion of the experiment, the results can be used to benefit the establishment of native plants in habitat restorations. In addition, further research ideas can be generated based on the acquired knowledge the experiment provides.

**Methods:**

Buoyancy Tests

To determine the relative buoyancy level of the three species, still water and moving water experiments were conducted using a water bin apparatus. The apparatus/procedure is similar to that conducted in an experiment regarding saltmarsh seeds in motion by Zhao et al. Three rectangular bins were filled with 2.5 liters of room-temperature tap water. Fifty dry individual *E. canadensis* seeds were poured into one bin (filled with water) and were left undisturbed. The same process was completed for fifty *E. macgregorii* and fifty *E. virginicus* seeds, in the other two bins. The number of seeds that sank to the bottom was recorded after 1 minute, 30 minutes, 1 hour, 1 day…7 days. The ratio of seeds floating to those which sank, was compared across the species. For the moving water experiment, a similar procedure was followed. However, after adding the fifty individuals, the water bin was shaken for 10 seconds, twice a day.

**Figure 4:** This image shows the apparatus used for the buoyancy tests.

Emergence: Single Seed Test

To determine the emergence success of the three species at a shallow and deep depth, single seed emergence tests were conducted. A lab environment and a greenhouse were utilized to effectively control the variables of the experiment. The initial step in the experiment involved the germination of seeds. About thirty *Elymus virginicus* seeds were spread out on a paper towel that had been soaked with water from the sink. With the seeds on it, the paper towel was folded three times and placed in a zip-lock bag. It was important to ensure the bag was locked, in order to maintain a moist environment. The same procedure was followed for *Elymus macgregorii* and *Elymus canadensis*. Each of the zip-lock bags were labeled according to the species name, number of seeds, and date. All three bags were taken to the greenhouse and placed on a windowsill. This process allowed for the efficient germination of the seeds. After about 10-14 days, the seedlings of all three species were ready to be used in the experiment. In a laboratory setting, seed trays and planters were utilized to effectively organize and separate the seeds. Twenty 4x4” square planters were filled to three centimeters (in height) with damp peat moss (purchased from the store) and two planters were placed in each tray. The trays were used for the sole purpose of organizing the planters into groups of two, where a non-germinated seed of a certain

species was paired with a germinated seedling of that same species, for a certain depth. The first step consisted of placing one *E. virginicus* seed in each planter. Ten of these seeds were initially germinated, and the other ten were non-germinated. From here, the groups were divided into the “deep” treatment and the “shallow” treatment. In the deep treatment, four centimeters of damp peat moss was added on top of the seed. In the shallow treatment, only one centimeter of peat moss was added. Five of the germinated seed planters received the deep treatment, and the other five the shallow treatment. This was the same for the ten non-germinated planters. Upon completion of this, labels were created for each planter, based on the treatment and the content. In the case of the first germinated *E. virginicus* seed that received the deep treatment, the label would read “EvDG1”. This entire process was repeated with *E. macgregorii* and *E. canadensis* seeds. Each of the sixty planters were simultaneously kept under “grow lights”, which were on from 7 a.m. to 7 p.m. each day. Every other day, emergence was noted and deionized water was added to the seed trays. In addition, the planters were rotated to ensure that each individual received a comparable amount of light. After 2 weeks, the planters were analyzed, and the emergence of individuals was recorded. If no emergence was observed, the seed was extracted to determine whether or not germination occurred. Following this, the root and shoot lengths of each individual were measured and recorded.

<u>Emergence: Four Seed Tests:</u>

To determine the emergence success of the three species at a shallow and deep depth with an increased sample size, four seed emergence tests were conducted. The

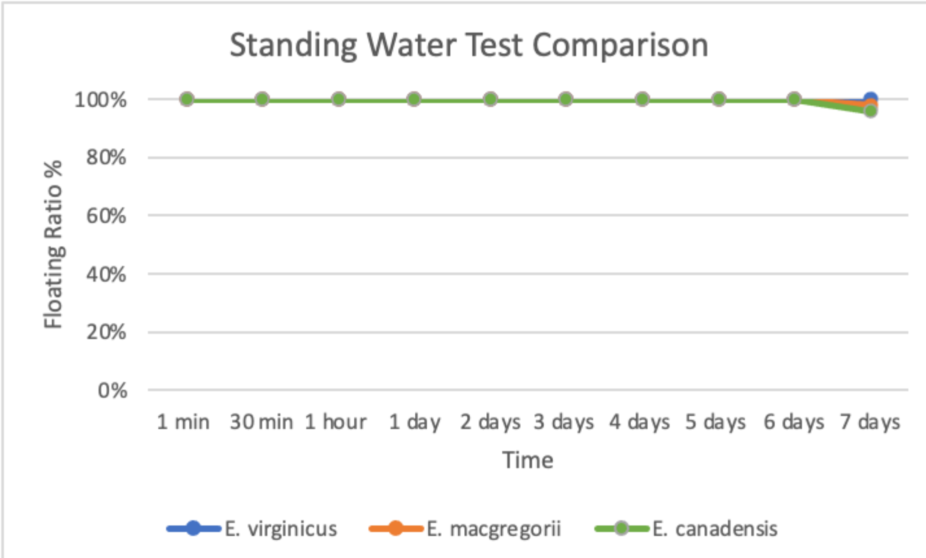
procedure for the four seed tests was exactly the same as the single seed tests, with the exception of using four seeds per planter instead of one. Each seed was placed about 1 cm away from the walls of the planter.

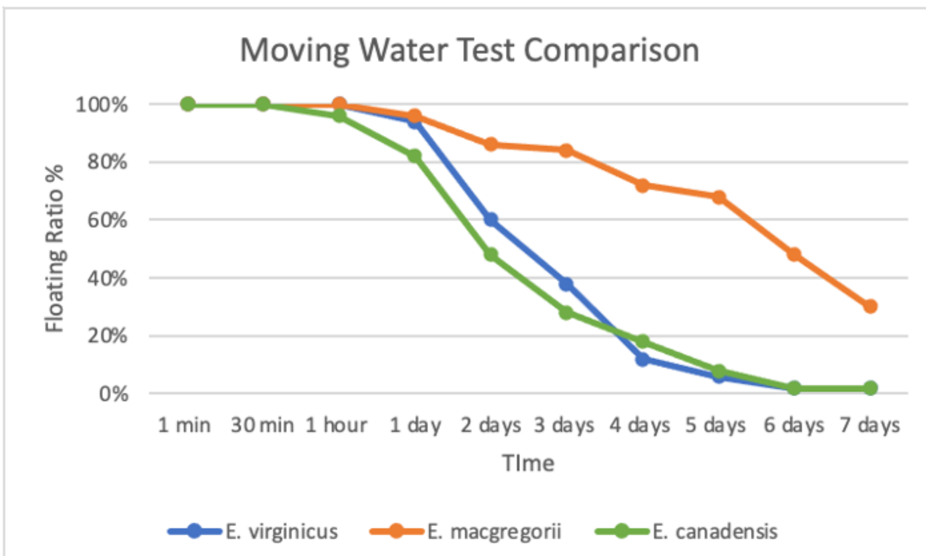

**Figure 5:** This image is an example of the labeling system mentioned in the methods section. EcDG1 stands for *Elymus canadensis* deep germinated 1.

**Figure 6:** This is an image of the lab environment utilized in the experiment. The planters (green) are placed in trays (dark gray).

**Results:**

<u>Buoyancy Tests:</u>



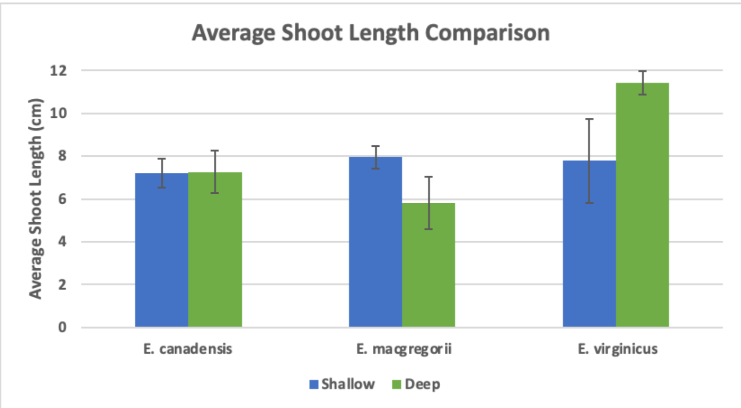



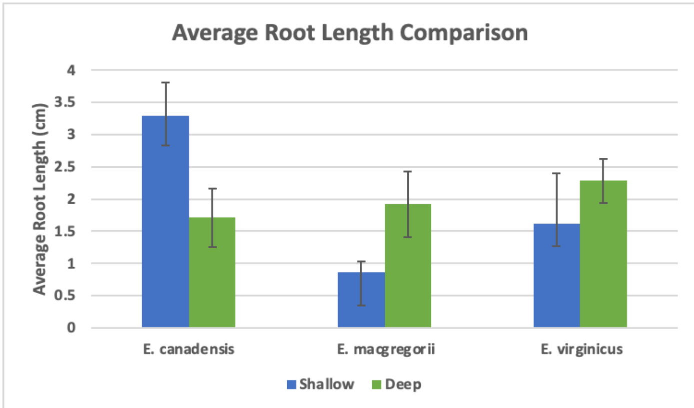

**Figures 7 and 8:** These graphs show the standing water test and moving water test comparisons across the three species, where the floating ratio % is plotted against time. *E. canadensis*: N=1, *E. macgregorii*: N=1, *E. virginicus*: N=1

In the standing water tests, there was no notable difference in the floating ratio of the seeds. After 7 days almost 100% of the seeds, for each species, remained floating.

On the other hand, the moving water tests demonstrated different results. Based on *Figure 8,* it seems that *E. macgregorii* is more buoyant than the other two species. However, sample sizes (N=1 bin per species) were not sufficient to allow for statistical tests.

<u>Single Seed Tests:</u>




**Figures 9 and 10:** These graphs display the mean shoot and root lengths (whiskers represent SE) at different depths, for each species after 2 weeks of growth (1 seed per planter). *E. canadensis* Shallow: N=10, *E. canadensis* Deep: N=9, *E. macgregorii* Shallow: N=7, *E. macgregorii* Deep N=6, *E. virginicus* Shallow: N=6, *E. virginicus* Deep: N=5.

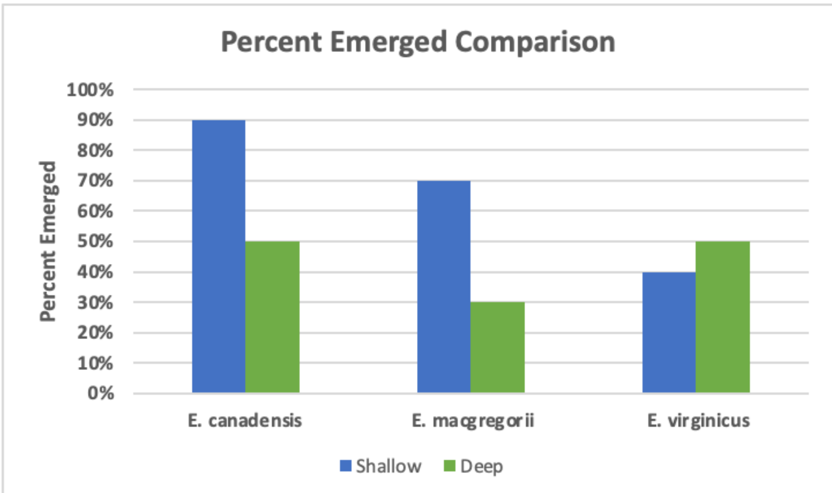



**Figure 11:** The graph shows the percent of emerged seeds at the shallow and deep depth, for each species after 2 weeks of growth (1 seed per planter) *E. canadensis* Shallow: N=10, *E. canadensis* Deep: N=10, *E. macgregorii* Shallow: N=10, *E. macgregorii* Deep N=10, *E. virginicus* Shallow: N=10, *E. virginicus* Deep: N=10

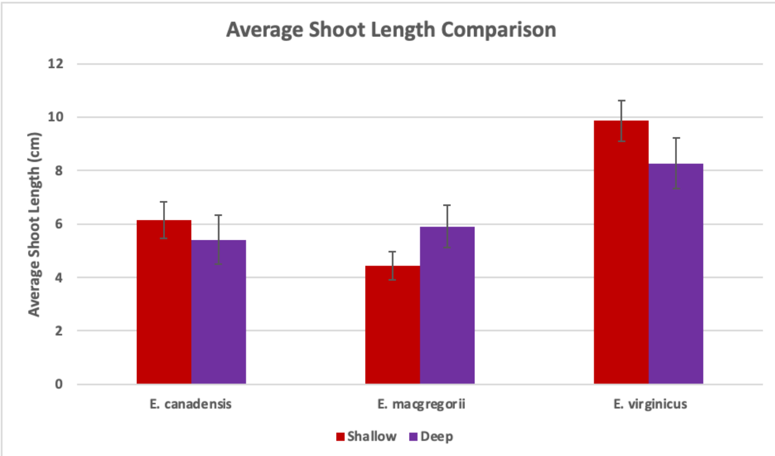
| Single Seed Shoot Length | | | |
|---|---|---|---|
| Source | df | F | P |
| Species | 2 | 2.82 | 0.07 |
| Depth | 1 | 0.29 | 0.6 |
| Interaction | 2 | 2.7 | 0.08 |
| Error | 37 | | |

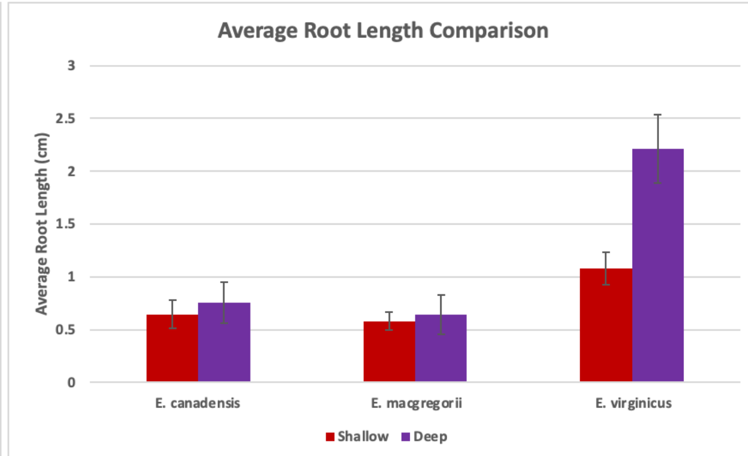
| Single Seed Root Length | | | |
|---|---|---|---|
| Source | df | F | P |
| Species | 2 | 2.27 | 0.12 |
| Depth | 1 | 0.01 | 0.92 |
| Interaction | 2 | 3.81 | 0.03 |
| Error | 37 | | |

**Figures 12 and 13:** These tables show the statistical analysis of the shoot and root data collected in the single seed tests.

*E. canadensis* emerged most successfully at a shallow depth and demonstrated the largest root growth at this depth. There was no notable difference regarding shoot growth at either depth, for this species. *E. macgregorii* emerged most successfully at a shallow depth and showed greater shoot growth when placed at this depth. *E. virginicus* emerged most successfully at the deeper depth and the root and shoot growth was greatest at this depth. ANOVA indicates that the only significant relationship is the interaction of species and depth for root length. This results from contrasting responses among species when planted deep. *E. canadensis* demonstrated a strong reduction in root length (50%), while the other two species showed increases in root length. Shoot length did not vary significantly among species or depths, although the species and interaction effects were close to significance. Error bars are not included in *Figure 11* as the graph is simply a comparison of the number of individuals that emerged for each species.

Four Seed Tests:




**Figures 13 and 14:** These graphs display the mean shoot and root lengths (whiskers represent SE) at different depths, for each species after 2 weeks of growth (4 seeds per planter). *E. canadensis* Shallow: N=16, *E. canadensis* Deep: N=13, *E. macgregorii* Shallow: N=20, *E. macgregorii* Deep N=15, *E. virginicus* Shallow: N=10, *E. virginicus* Deep: N=13

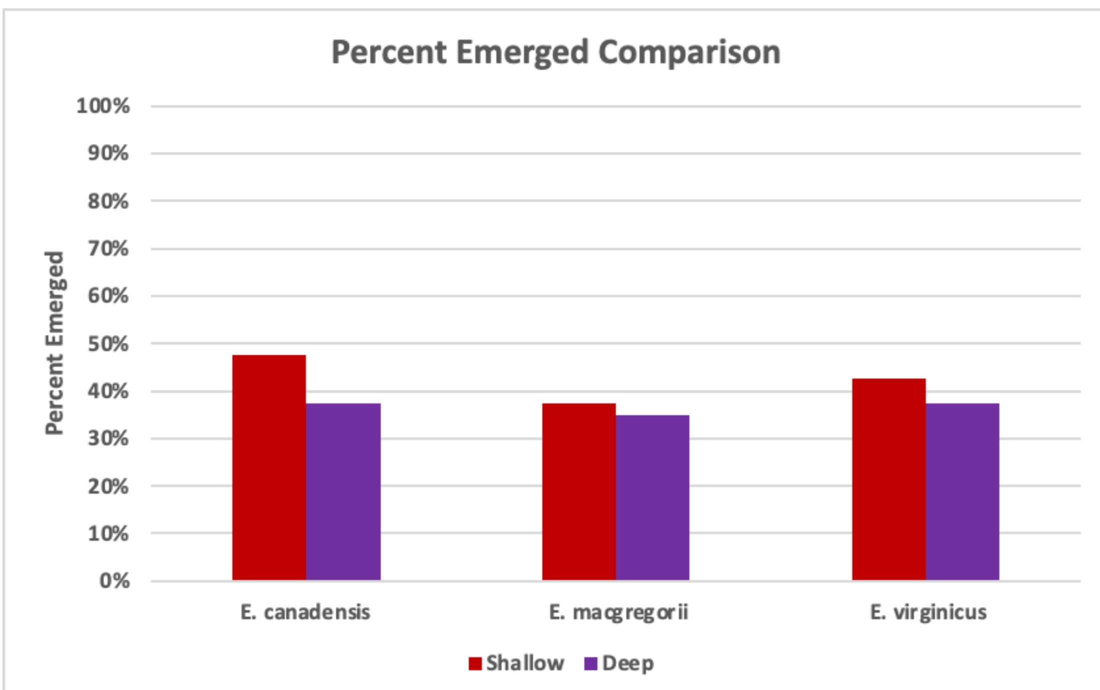



**Figure 15:** The graph shows the percent of emerged seeds at the shallow and deep depth, for each species after two weeks of growth (4 seeds per planter). *E. canadensis* Shallow: N=40, *E. canadensis* Deep: N=40, *E. macgregorii* Shallow: N=40, *E. macgregorii* Deep N=40, *E. virginicus* Shallow: N=40, *E. virginicus* Deep: N=40

| Four Seed Shoot Length | | | |
|---|---|---|---|
| Source | df | F | P |
| Species | 2 | 15.5 | <0.0001 |
| Depth | 1 | 0.14 | 0.71 |
| Interaction | 2 | 4.1 | 0.02 |
| Error | 115 | | |

| Four Seed Root Length | | | |
|---|---|---|---|
| Source | df | F | P |
| Species | 2 | 19.98 | <0.0001 |
| Depth | 1 | 9.99 | 0.002 |
| Interaction | 2 | 5.1 | 0.0075 |
| Error | 115 | | |

**Figures 16 and 17:** These tables show the statistical analysis of the shoot and root data collected in the four seed tests.

Overall, results from the four seed tests differed from what was observed in the single seed tests. The interactions between seedlings may have played a role in this, however, it is more likely that this was due to a technical error (light malfunction, discussed in the conclusion). Nonetheless, *E. canadensis* and *E. macgregorii* demonstrated similar results to the single seed tests, in regard to emergence. On the other hand, *E. virginicus* showed greater emergence success at a shallow depth, which is the opposite of what was observed in the single seed tests. ANOVA indicates a significant relationship in the interaction of species and depth for both root and shoot length. It also suggests a significant relationship among species in shoot and root length. However, further testing must be done to determine the reliability of these results. Error bars are not included in *Figure 14* as the graph is simply a comparison of the number of individuals that emerged for each species.

**Conclusion:**

The two FACW species (*E. macgregorii* and *E. virginicus*) were expected to be more buoyant than the FACU species (*E. canadensis*) because they are typically found in a more “wet” habitat. The data collected from the buoyancy test experiments was analyzed to determine trends among the three species. As shown in *Figure 7,* there were no notable differences in regard to the standing water test. For each species, almost 100% of the seeds remained floating after 7 days. The data obtained from the moving water tests presented differing results. The most buoyant seed was determined to be *E. macgregorii,* with 30% of the seeds floating after 7 days. *E. virginicus* and *E. canadensis* followed a similar pattern throughout the experiment, with only 2% of the seeds floating at the conclusion of the time period. This can be observed in *Figure 8.* The results indicated that the hypothesis was only partially supported. While *E. macgregorii* (FACW) seemed to be more buoyant than *E. canadensis* (FACU), *E. virginicus* (FACW) did not. An interesting detail to note is that *E. virginicus* and *E. canadensis* have very similar seed structures, while *E. macgregorii* differs greatly because it lacks glumes. This is shown in *Figures 1, 2,* and *3*. It is likely that this plays a role in buoyancy along with the interactions between the seeds. To determine the significance of these results, it’s important to repeat this experiment using a larger sample size, and observe whether or not the trends hold. This was not completed due to a lack of time. Another improvement may involve conducting a single seed test to determine whether or not interactions with other seeds impacts buoyancy.

An interesting difference noted in the buoyancy tests involved the germination and shoot growth across the 7 day time period. For the standing water tests, 14 of the

50 *E. virginicus* seeds germinated with 11 of them growing shoots. In *E. macgregorii,* 5 germinated with shoot growth and in *E. canadensis,* 44 seeds germinated with 40 of them growing shoots. For the moving water tests, only 1 *E. virginicus* seed germinated, and the same seedling grew a shoot. For *E. macgregorii,* 6 seeds germinated with 3 growing shoots. For *E. canadensis* 38 seeds germinated with 13 growing shoots. While this was not investigated further, it's fascinating that a FACU seed germinates most successfully in the extremely wet conditions. These differences may be important for FACW species that are dispersed in areas near bodies of water. It is possible that the seeds may possess a trait that prevents them from germinating under these conditions as it may lead to unsuccessful growth. Further research must be done to test this potential hypothesis.

A more buoyant seed is more likely to float on water and therefore be deposited near the surface of the soil. Based on this idea, and the results of the buoyancy tests, *E. macgregorii* is expected to emerge best at shallow depths. This was observed in both the single and four seed tests. Emergence directly corresponds to shoot and root growth as the plant devotes resources to these components in order to grow under different conditions. *E. canadensis* demonstrated very similar shoot growth averages at a shallow and deep depth, and *E. virginicus* showed the largest shoot growth at a deep depth. *E. canadensis* had the best overall emergence at a shallow depth, with 90% of the seeds emerging (N=10). This species also showed the greatest emergence at a deep depth (tied with *E. virginicus*). Based on this, it is likely that this species is effective in growing under all conditions. This makes sense because as a FACU plant, *E. canadensis* is mostly deposited near the surface of the soil. But also, it seems to be

fairly buoyant (relative to the species studied) and therefore it may possess the ability to effectively emerge when deposited at a deeper depth. *E. macgregorii* was more effective in emerging from a shallow depth (as predicted), and *E. virginicus* was more effective in emerging from a deeper depth. This relates to the results obtained in the buoyancy tests and will be discussed later in this conclusion. Upon the evaluation of the seedlings which did not emerge, the reason as to why is unclear. It's possible that the soil moisture may have impacted the growth. To ensure the consistency of moisture level, an improvement for the future would include adding the same amount of soil in each planter. In addition to this, I would not include the non-germinated group. In doing this, I added another variable which did not specifically pertain to the research questions being addressed.

Slightly different results were observed in the 4 seed per planter test. However, there was a technical malfunction with the grow lights. About halfway through the experiment, the lights would shut off at different times due to a broken timer. This may have skewed the results as the amount of light was not consistent. In addition, the interactions between the seeds, and their impact on emergence was not initially considered. To effectively increase the sample size, more single seed tests should have been done. Nonetheless, *E. canadensis* showed the same results, with more overall emergence at a shallow depth. On the other hand, the results from *E. virginicus and E. macgregorii* were slightly different from what was observed in the 1 seed tests. *E. virginicus* demonstrated greater shoot growth at a shallow depth and showed a contrasting overall percent emergence. This can be observed in *Figures 12* and *14.* It was still evident that individuals of the *E. macgregorii* species had more emergence

success at a shallow depth, however, there appeared to be less of an advantage (*Figure 14*). Unfortunately, due to a time constraint, this experiment could not be redone.

To enhance the establishment of native species in wetlands, it is essential to understand the variables that impact emergence. The data collected only partially supported the hypothesis that the seeds of the FACW species, *E. virginicus* and *E. macgregorii,* are more buoyant than the FACU species (*E. macgregorii*). *E. macgregorii* demonstrated a much higher floating ratio percentage after 7 days, relative to the two other species. While further testing must be done, these results lead to the idea that *E. macgregorii* is deposited at a higher depth than *E. virginicus and E. canadensis.* The emergence patterns, based on both the 4 seed and the 1 seed tests, corresponded to this idea as it was more successful at emerging from the shallow depth. This was also the case for *E. virginicus*, emerging best from a deeper depth (single seed test). However, for *E. canadensis,* despite being a more buoyant seed, it emerged most successfully from shallow depth (single seed and four seed tests). It is possible that this relates to the wetland indicator status, and where these species are typically deposited. For the FACW species, which are found in very wet areas, buoyancy could be a major factor in the emergence success. This may be a possible explanation for the trends that are observed. Whereas FACU species are not typically deposited in a wet area, and therefore it’s not likely that buoyancy is a major factor. This may be why there is no evident relationship between the results of the buoyancy and emergence tests for *E. canadensis.* While this experiment exposed some trends, further research and testing must be done to confirm these suspicions.

It’s important to look back and reflect on what went well in the experiment, and some areas that can be improved. The obvious improvements include redoing the four seed tests (making sure the lights work), and increasing the buoyancy test sample sizes. I think it's also necessary to conduct a single seed buoyancy test to determine the extent of which the interaction of seeds affects buoyancy. With that being said, I thought the buoyancy tests and the single seed tests went really well. The results suggested interesting trends that can be utilized in later experiments. Overall, as an honors student, I learned a lot from this experiment and believe it was a very positive experience.

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