Effects of Water Availability on Seed Production in Subalpine Wildflower Aster: *Hymenoxys hoopesii*.

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Abstract

In subalpine meadows surrounding Rocky Mountain Biological Laboratory, perennial wildflowers like *Hymenoxys hoopesii* are experiencing record drought conditions due to low snow pack. Since *H. hoopesii* are perennials, they are not obligated to reproduce each season and therefore must balance the tradeoffs between potentially reproducing in a drought year or waiting for better conditions. For plants that produce reproductive structures (flowers), there is a knowledge gap on how water is being allocated within the plant that this study sought to fill in order to better understand the ways in which plants respond to a manipulation of resource availability in environmental conditions (ambient drought conditions and additional water applied) and whether reduction in seed production is an important element of surviving in drought conditions. This study tested if water availability has a direct effect on the quantity and quality of seed production in the species *Hymenoxys hoopesii* in order to investigate the allocation of water towards seeding to determine seeding's benefit to plant survival. In order to do this, two groups of plants were studied: the manipulation cohort received water supplement once a week for ~7 weeks, and the control cohort experienced ambient rainfall conditions (in this year, a historic drought). At the end of the season senesced flower heads were collected, seeds were counted, and determined to be mature or immature. Overall allocation of resources to seeding did not change between the control and treatment, but the ability to successfully provision those reproductive structures did have a difference. The analysis of these counts found no significant difference between the total amount of seeds produced and being in either treatment group. A GLMM found that there was a ~4% increase in the amount of mature seeds produced by the manipulated group of plants. However it is important to consider that even for the control group there was a baseline ~91% chance of making a mature, successful seed. This means that even without the water supplement plants that invested in reproduction have a high success rate, and added water only slightly improves that chance, meaning nature is finding a way to persist even in a record drought year.

Introduction

Perennial plants grow for multiple seasons; dying back during winter, but sprouting again in spring. Perennial plants do not have to complete a reproductive cycle every year to continue their lineage because they can regrow from older root structures the next season. Drought conditions cause phenological shifts forward in flower timing due to water availability changing, which can force plants to complete their reproductive cycles earlier in the year, given that they reproduce (Inouye, 2008). But if plants are experiencing drought, yet still reproducing, that means some physiological trigger is being pulled that tells the plants to produce reproductive structures (flowers) despite not necessarily needing to. This trigger being pulled indicates that making flowers and reproducing, eventually making seeds, will have benefits for the plant's ongoing survival.

Spring and summer months mark the beginning of snow melt in alpine and subalpine environments in the Rocky Mountains. Snow melt serves as the primary source for hydrologic feedback in these environments, where melting increases availability throughout mountain systems (Bales et al., 2006). Water is a crucial contributor to the physical and physiological processes of all plants. For a seed to be produced, sperm that's contained in pollen of a plant must reach an ovary. Pollen carried by pollinators travels from one flower to another, but only in the presence of water can the exine (the outer shell of a pollen grain) be broken down to grow a pollen tube, releasing a plant's sperm to an ovule (Dresselhaus et al., 2016). But without attractive pollen, a pollinator may never visit the flower to transport pollen in the first place. Thus, a plant's reproduction depends not only on the successful transfer of pollen between individuals but also on having a substantial enough water source that 1) provides enough to support basic growth and development, 2) allows it to create attractive resources for pollinators to spread its gametes, and 3) lets the plant fully utilize inbound gametes to create seeds. The study of this balancing of finite water resources among these competing demands is an aspect of a process called resource allocation. Resource allocation is how scientists explain which processes an organism is putting its energy into to explain its growth or function.

Even after dry winters with very little snow pack like this past winter (2025-2026), many perennial wildflowers like *Hymenoxys hoopesii* are putting out reproductive structures. This would imply that the water availability in a record drought year at RMBL is still somehow a suitable reproductive year for *H. hoopesii*. Despite the production of reproductive structures, we don't know how this unprecedented drought will affect these plants' ability to produce healthy seeds. This study investigated seeding under drought conditions at the level of the reproductive unit (the seed), the quality of the reproductive structure (the flower), and the success of the individual (the plant itself). At these three levels of study we can fill the gap in our knowledge on whether or not water is a limiting resource on plant reproduction in our study species *Hymenoxis hoopesii*. But each level has multiple explanations for how water may be affecting the system.

Seed formation can be heavily dependent on many factors. If pollen is available it doesn't always mean the ovule it reaches is compatible. Lower water availability can alter the efficiency by which pollen received is translated into seeds produced (Gonzalez & Campbell, 2021). Alternatively, it is possible the control and manipulated plants have a similar seed output, but with less water the seeds produced by the control cohort are not as high quality (quality defined here as lower maturity rate). It is important to consider that a lack of water could cause a wide variety of issues for flower heads that may impact seeding as well. If a plant lacks water and makes flowers that cannot attract pollinators to produce seeds, there's an immediate gap between controlled and manipulated plants with more water. If a flower was successful in making pollen, but the flowers get aborted due to environmental stressors, no seeds will be made (Saini et al., 2026). Or if the flowers and seed made are the same quality, there just happens to be a lower quantity overall, so seed count is lower. Together, information from the quality of the flower and the seed will allow us to fill the knowledge gap about how water is a limiting resource on plant reproduction rather than exclusively pollinator services like many studies do. We hypothesized that plants with more water would create both more seeds, as well as higher quality seeds, where quality is defined as a measure of success or failure (mature or immature respectively) of those seeds.

Methods

This work was conducted in four subalpine wildflower meadows around RMBL in which the Brosi lab conducted a watering experiment led by PJ Jones on three aster species: *Helianthella quinquenervis, Hymenoxys hoopesii, and Heliomeris multiflora*. These same *H. hoopesii* focal individuals were further evaluated for seed production and seed maturity rate by Omari Owens. At each site, our team identified forty focal plants of each species, placing a unique identifying flag at each, twenty of which received a water addition while the other twenty served as controls (no water addition). Once a week we traveled to each site, watering the manipulation plants with the equivalent of RMBL's wettest year's highest weekly rain average (2.36 Liters). To do this, we had many water storage containers to help transport water to each site in a lab vehicle, and specially measured pitchers to deposit the 2.36 L onto each plant receiving treatment. This watering pattern continued until the end of the field season when senesced flower heads were collected. The day following water addition, we returned to site to collect pollen and bee observations for other projects within the lab, and I conducted a survey that evaluated the lifecycle progression of the focal plants to determine if they were ready for collection. The survey that was conducted on each focal individual measured for number of flower buds/heads and individual florets, as well as height of tallest reproductive structure (flower, current or previous). This cycle continued until all the heads on a focal individual fully senesced. Once all flower heads had fully senesced, pollinator exclusion bags were added until the heads inside appeared ready for collection (yellow-orange color faded to brown, florets spread apart, round/microphone shaped head, firm/crisp rather than soft). Bags remained on these flowers through senescence in order to prevent loss of seeds to things like herbivory or dispersal. Once the flower head was notably senesced, it was ready for collection.

Heads of flowers were removed by cutting somewhere below the sepal, then placed into a ziploc bag, each head was in a separate bag to maintain organized data. Each focal individual had a larger ziploc bag full of its smaller head bags. Once back in the lab, each head was stripped of its seeds, and seeds were counted as either mature or immature. Where mature seeds were more silver, larger, firmer and easy to separate, while immature seeds were visibly smaller, green, or hard to separate from one another.

To analyze that data, we used RStudio to run generalized linear mixed effects models (GLMMs) which assessed the effects of the treatment (watered/control), number of seeds, number of mature and immature seeds, and total number of flower heads. We included site ID as a random effect in order to account for any variation between sites that was not due to our treatment.

Results

There was no significant difference in the overall amount of seeds *Hymenoxys hoopesii* created between the two treatment groups (see **Figure 1a**), however according to our graph the control group had a higher average (but see potentially overlapping error bars, which represent the SE of each group). This non-significant trend continued for the amount of mature seeds created (see **Figure 1b**), as well as the amount of immature seeds created (see **Figure 1c**). When plotted to account and separate for the random factor of site/location, the proportion of mature seeds to total seeds was higher in the manipulated group. The control group was more variable for this ratio of mature:total seeds other than at the site AP (see **Figure 2**). We then statistically tested this proportion of mature:immature seeds with a binomial GLMM (with treatment as a fixed effect and site as a random effect). The baseline proportion of mature seeds to total seeds in the control group of plants was ~91%(90.5741%) (see **Figure 3, 4**). This value came from the result of our GLMM, where ~91% was converted from our estimated intercept of 2.26271 using a logistic sigmoid function: (1/(1+e^-2.26271))*100. The same sigmoid function was run to account for manipulated plants using the estimated "treatmentM" value of 0.62142 added to the intercept value of 2.26271 for a total of 2.88413, (1/(1+e^-2.88413))*100. This gave the Manipulated plants a baseline ~95% (94.7056) chance to create a successful mature seed (see **Figure 3, 4**). When analyzing the proportion of mature seeds to total seeds in the manipulated group a slight increase of ~4% was present (see **Figure 4**). The ~4% was calculated as the results of the two sigmoids: (0.9470563272 - 0.90574124923)*100.

Discussion

Results show that there is no significant difference between the control group and the manipulated group in terms of total seeds produced. When looking deeper into this phenomenon by comparing mature to immature seeds across sites we notice that manipulated plants more consistently made mature seeds. After running our GLMM we saw that the water addition did add a small, but significant, chance to create mature seeds; approximately 4%. But the baseline intercept of ~91% for control plants indicates there's a good chance of producing a mature seed already. What does this say about the drought conditions' effects on seeding in *Hymenoxys hoopesii*? It means that the plants who reproduce are allocating enough resources to seeding to ensure reproduction even in a drought year.

Results came from randomly selecting ten of the twenty individuals of each treatment group for each site after determining that half of the sample should still represent the total data. Overall, the data collected represents 22,566 seeds for the control group and 24,411 seeds for the manipulation group (46,977 seeds total). This was also decided in order to lighten the load of seed counting due to time constraints. While randomized, this selection of fewer than all plants treated could still have been involved in the insignificance of the data due to plant to plant variation.

The lack of significant difference in total seed count between the control and treatment (see **Figure 1a**) may be explained by the natural variation in flowerheads produced by *H. hoopesii* individuals. In this study some individuals had nine heads while others had one. If a plant with one head was really successful in that it only made mature seeds, it would show up as a really low, insignificant seeming point in something like **Figure 1a**. Whereas a plant with nine heads that only made half as many mature seeds per head would still shift a point on that same figure upward significantly in that its total seed count was still four and a half times higher. This variation between plants creates a skew in our data that is unaccounted for but should be mentioned as it could have impacted count results.

Some uncontrolled factors that may explain the phenomenon of the results include microclimate & variation between sites, date of harvest, bloom date, and species productivity. Microclimates are much more localized climate conditions that may have stronger effects on individuals rather than a larger area (macroclimate) due to proximity of environmental factors that can influence areas such as shade (Kemppinen et al., 2024). In our study, samples were collected across four sites each with a unique microclimate to one another or even within the site. Date of harvest is a variable that may have allowed later collected seeds more time to mature, creating a skew in the count distribution. Mature seeds of some heads of plants may have been dispersed by the time we were ready to collect the head shifting counts lower, however if a substantial amount of the head was dispersed, the head was typically excluded from collection due to the immeasurable productivity of that plant. For a future, longer study to control for some or all of these factors could prove to have different results from what was found in this study.

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Tables and Figures

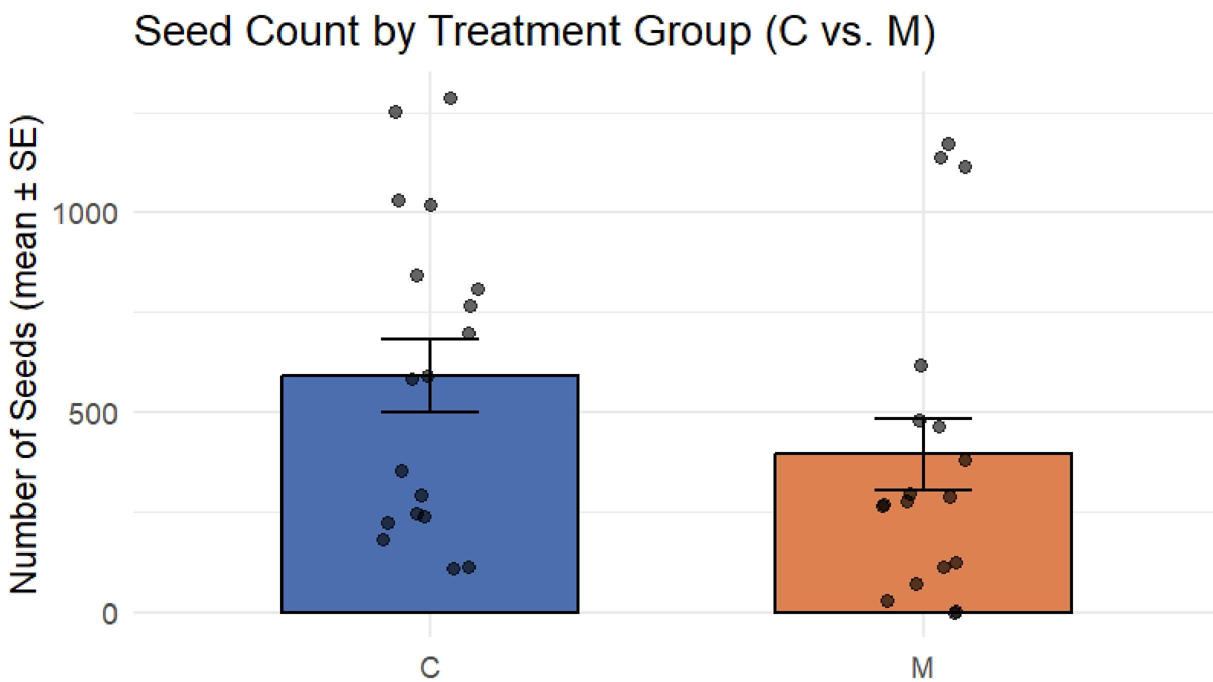

Figure 1a: Total seed count per plant by treatment group, where C is the control group, and M is the manipulated group that received water treatment.

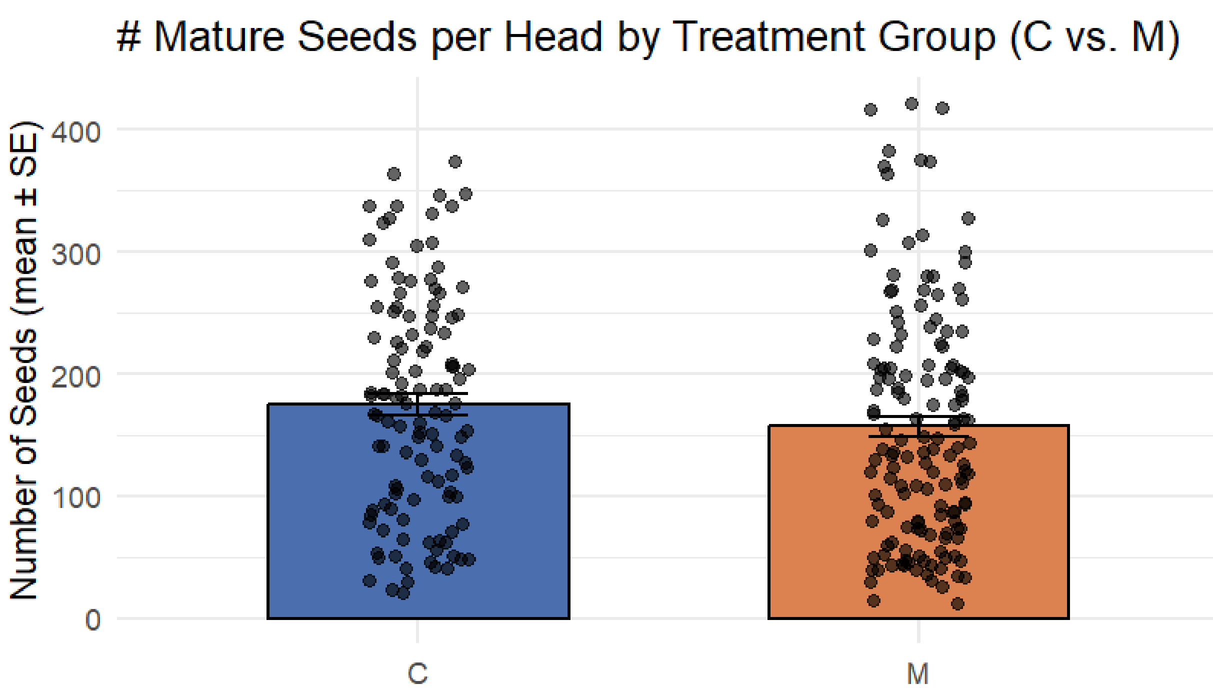

Figure 1b: Total mature seed count per head by treatment group, where C is the control group, and M is the manipulated group that received water treatment.

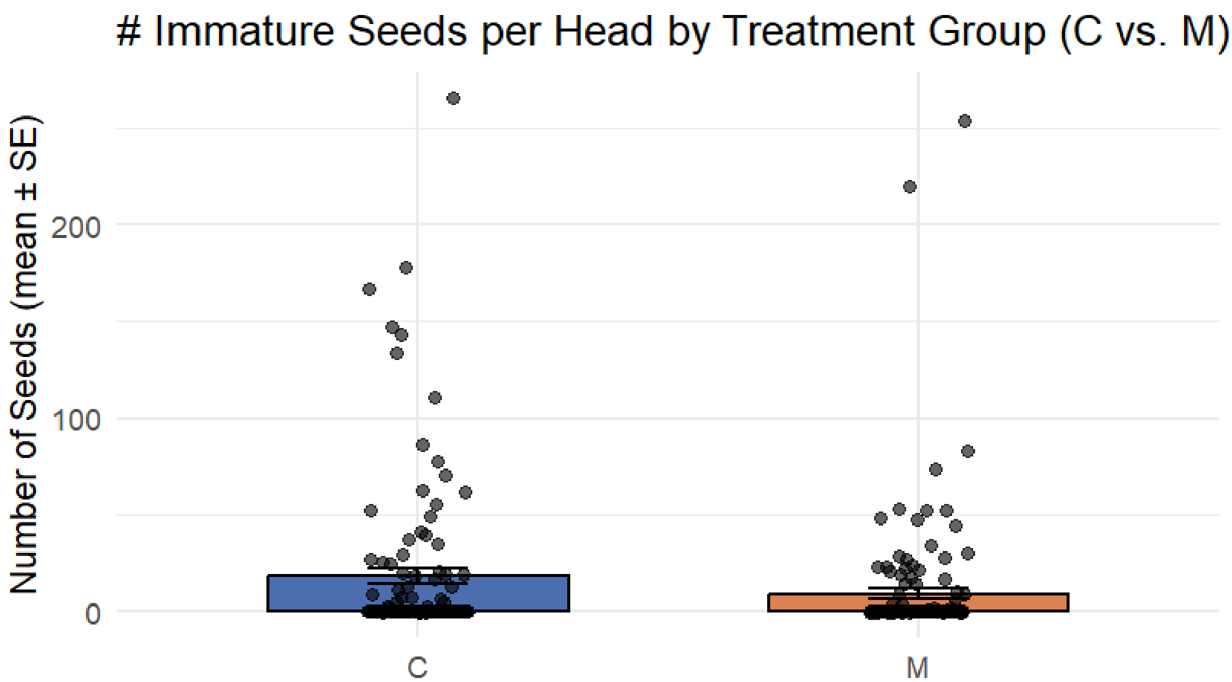

Figure 1c: Total immature seed count per head by treatment group, where C is the control group, and M is the manipulated group that received water treatment.

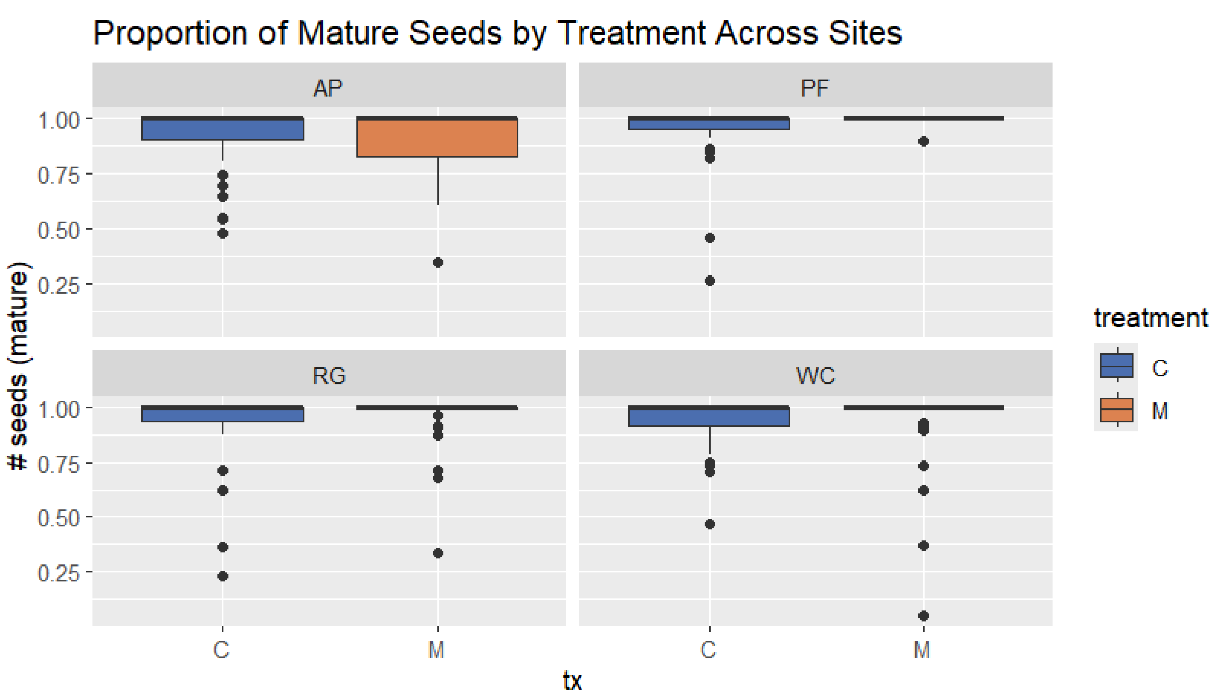

Figure 2: Proportion of mature seeds to total seeds per plant by treatment group, divided by site. Where C is the control group, and M is the manipulated group that received water treatment.

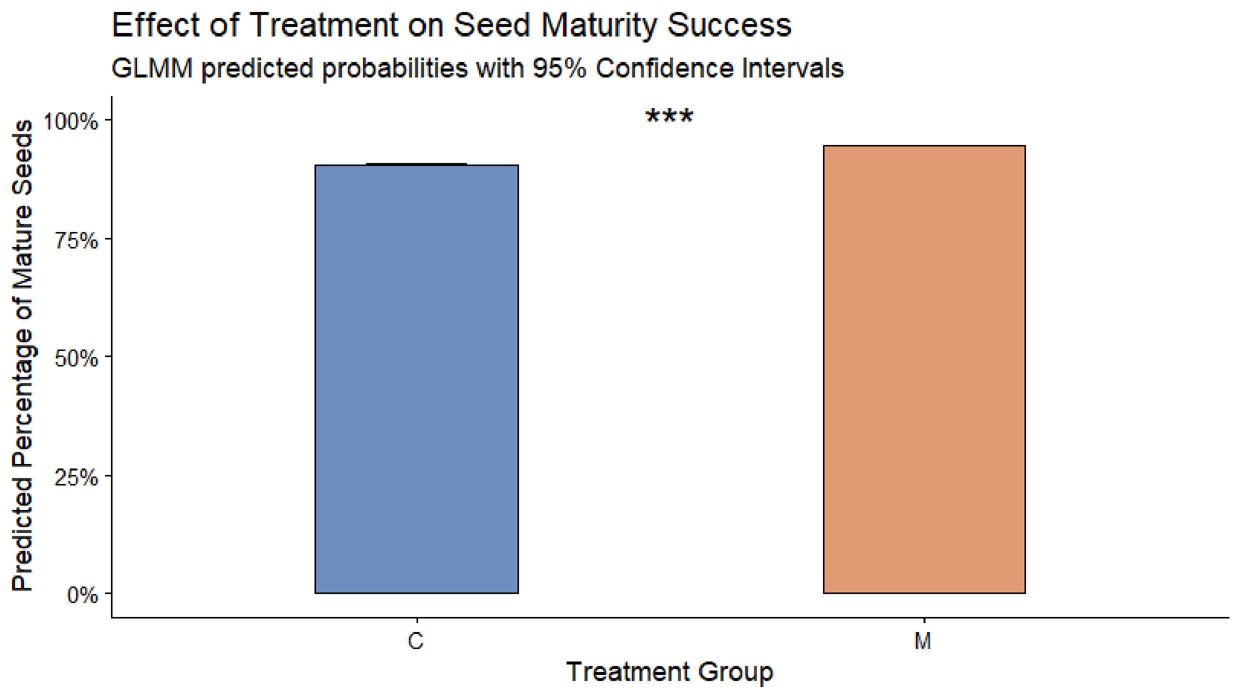

Figure 3: Baseline chance of a plant's ability to produce a mature seed based on its respective treatment group, where C is the control group, and M is the manipulated group that received water treatment.

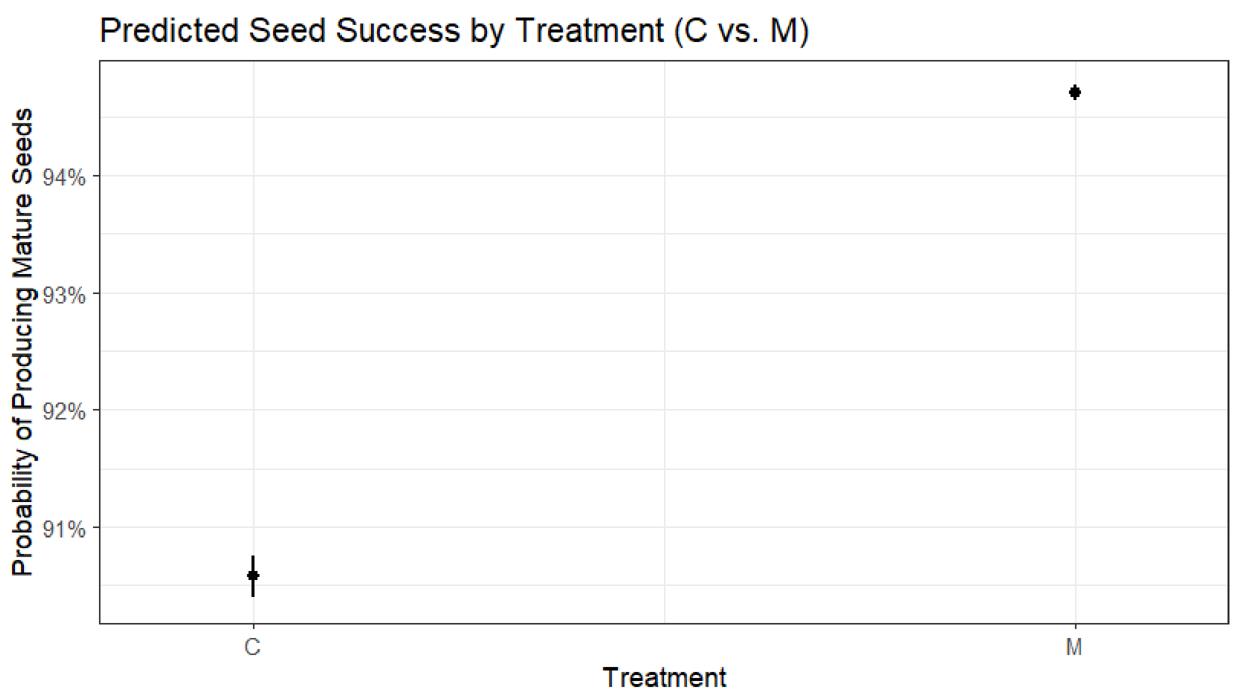

Figure 4: Baseline chance of a plant's ability to produce a mature seed based on its respective treatment group, where C is the control group, and M is the manipulated group that received water treatment. Emphasized on specific ranges of error bars to better view percentages.

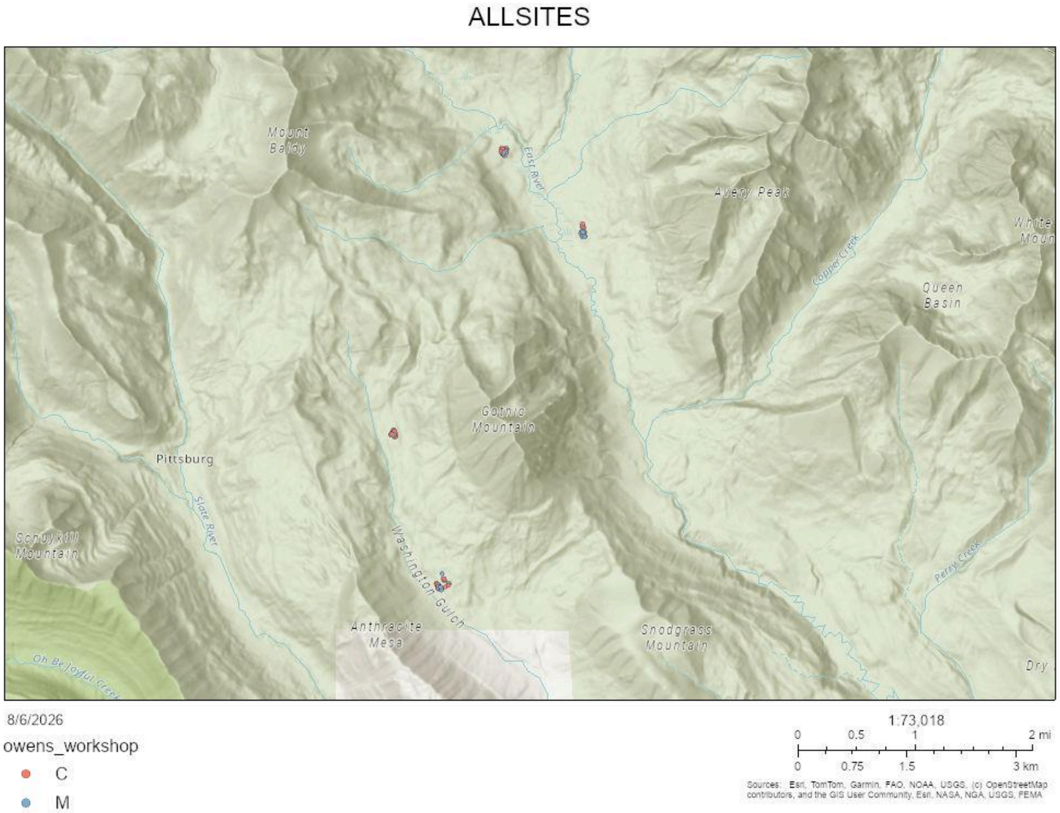

Figure 5: Zoomed out map showing all four sites of the study, from top down: RG, AP, PF, WC. Red dots are the control group (C), and blue dots are the manipulated group (M).