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# Drought Effects on Germination Rate of Two Sagebrush Species, Artemisia cana and Artemisia arbusula, and Comparison of Seed Counts using a Photography App and Weighing

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# Drought Effects on the Germination Rate of Two Sagebrush Species, *Artemisia cana* and *Artemisia arbusula,* and Comparison of Seed Counts using a Photography App and Weighing

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**Abstract**

Sagebrush steppe ecosystems have been impacted by climate change, grazing, and invasive plants. While some widespread sagebrush species have been well-studied, including big sagebrush (*Artemisia tridentata*), others like silver sagebrush (*Artemisia cana*) and low sagebrush (*Artemisia arbuscula*) are understudied. To learn more about how to restore these important ecosystems, we conducted a germination study of two sagebrush species. We germinated seeds from *A. cana* in petri plates and in soil to compare the rates of germination in both methods, while *A. arbuscula* seeds were germinated only in petri plates. We also tested the efficiency of weighing and a photography app to estimate the number of seeds. The germination rate for both species was low, but there was a strong correlation between the manual seed counts and the app counts as well as the seed counts and seed weights. It is possible that *A. cana* and *A. arbuscula* naturally have low germination rates or the lack of pre-trial treatment of seeds could have contributed to the low germination rates. Though our results were limited, there is a possibility that drought could have an effect on seed germination, and subsequently the success of future restoration projects, but that more research is needed.

## Introduction

Sagebrush steppe is a widespread and important ecosystem type in North America that can be found within the Sierra Nevada Mountains, Columbia and Colorado Plateaus, and Mojave Desert (Kidesheva et al., 2019). Sagebrush steppe occurs in semi-arid areas and is dominated by sagebrush (*Artemisia spp.)* species, other species of shrubs, and grasses. The most widespread species in sagebrush steppe is the big sagebrush (*Artemisia tridentata),* followed by silver sagebrush (*Artemisia cana)* (Watkinson et al., 2020). Sagebrush communities provide habitat for many wildlife species, including the Greater Sage Grouse (*Centrocerus urophasianus).* This sagebrush obligate is a species of concern and is currently found in just 56% of its original range (Schroeder et al., 2004).

Sagebrush ecosystems are rapidly declining, mainly due to cattle grazing, introduction of invasive plants, land development, and climate change (Shaw & Jensen, 2014). Because of these changes, there is a growing need for restoration of sagebrush communities. Although some sagebrush species, such as *A. tridentata*, have been well studied, other species have not. *Artemisia cana* and *A. arbuscula* are understudied but make up a large portion of the plants in this ecosystem (Appendix A and B). There are many factors involved in the overall success of restoration efforts with dryland species, such as inherent site conditions and soil type, but seed germination is a major regulator of success (Wilder et al., 2019). There is also evidence indicating that drought conditions negatively affect seeds produced in other semi-arid communities, similar to those in which *A. cana* and *A. arbuscula* are found (Wagner et al., 2011). It is necessary to understand such characteristics as germination rate and seed production of these species in order to successfully restore sagebrush communities.

Photo counting software and weighing can be useful tools for counting small objects like sagebrush seeds. Software such as ImageJ (Wayne Rasband-National Institutes of Health) can be

used to count objects in photos for many fields, such as counting cells for medical studies, or counting corn kernels for agronomy studies (Choudhry, 2016; Zohaib et al., 2015). This technology has limited applications within the field of restoration but could prove useful to more efficiently count seeds to be used in restoration projects. The app "CountThings" is used professionally for counting objects in many different fields, specifically construction and medicine, and has the potential to be used for counting sagebrush seeds (CountThings, 2020). Another common method to estimate the number of seeds for restoration projects is by weighing them. It is likely that seed weights can give a general estimate of seed number for *A. cana* and *A. arbuscula*, but due to their small size, it is necessary to determine if this method is accurate.

The objectives of this study examined both seed counting methods and germination rates of silver and low sagebrush to help inform future restoration efforts of sagebrush habitat. We compared the reliability of the photo counting app "CountThings" for counting *A. cana* and *A. arbuscula* seed quantities with the accuracy of estimating seed numbers by weight. We wanted to determine the germination rate for these two species, without the use of pre-trial treatments typically used to increase low germination rates (Aeillo et al., 2017; Hou & Romo, 1998). For *A. cana*, we also compared germination rates between petri plates and soil. Many germination studies take place in petri plates, but soil as a medium is more reflective of actual restoration conditions (Palma & Laurance, 2015). Finally, we compared seeds exposed to drought conditions from each species to those that were not in drought conditions to see if there was a difference in size, germination rate, or number of seeds produced.

## Methods

*Site Description and Seed Storage*

Two species of sagebrush, *A. cana* and *A. arbuscula*, were studied in a three-year forced-drought experiment at a site near Gerber Reservoir east of Bonanza, Oregon, a small town 25.5 miles east of Klamath Falls (Figures 1-4). As part of the experiment, there were: 1) control plots and 2) drought plots that reduced ambient rainfall by approximately 40% through the implementation of rain shelters (Appendix C).

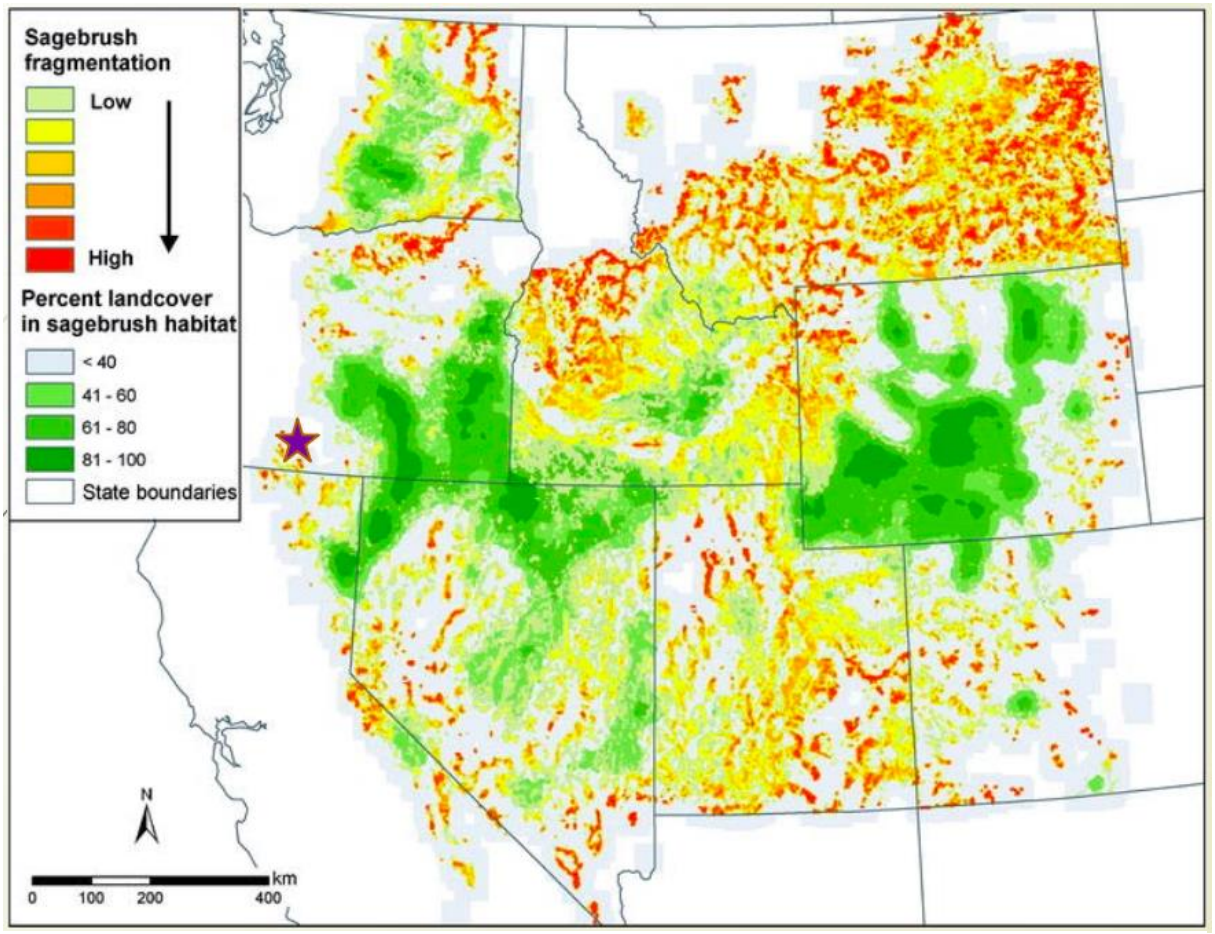


Figure 1. Map depicting location of Klamath Falls, Oregon just north of the California-Oregon border. This map also shows the intensity of sagebrush habitat fragmentation (disconnecting into patches) and the percent land cover of sagebrush steppe (adapted from Knick et al., 2003).

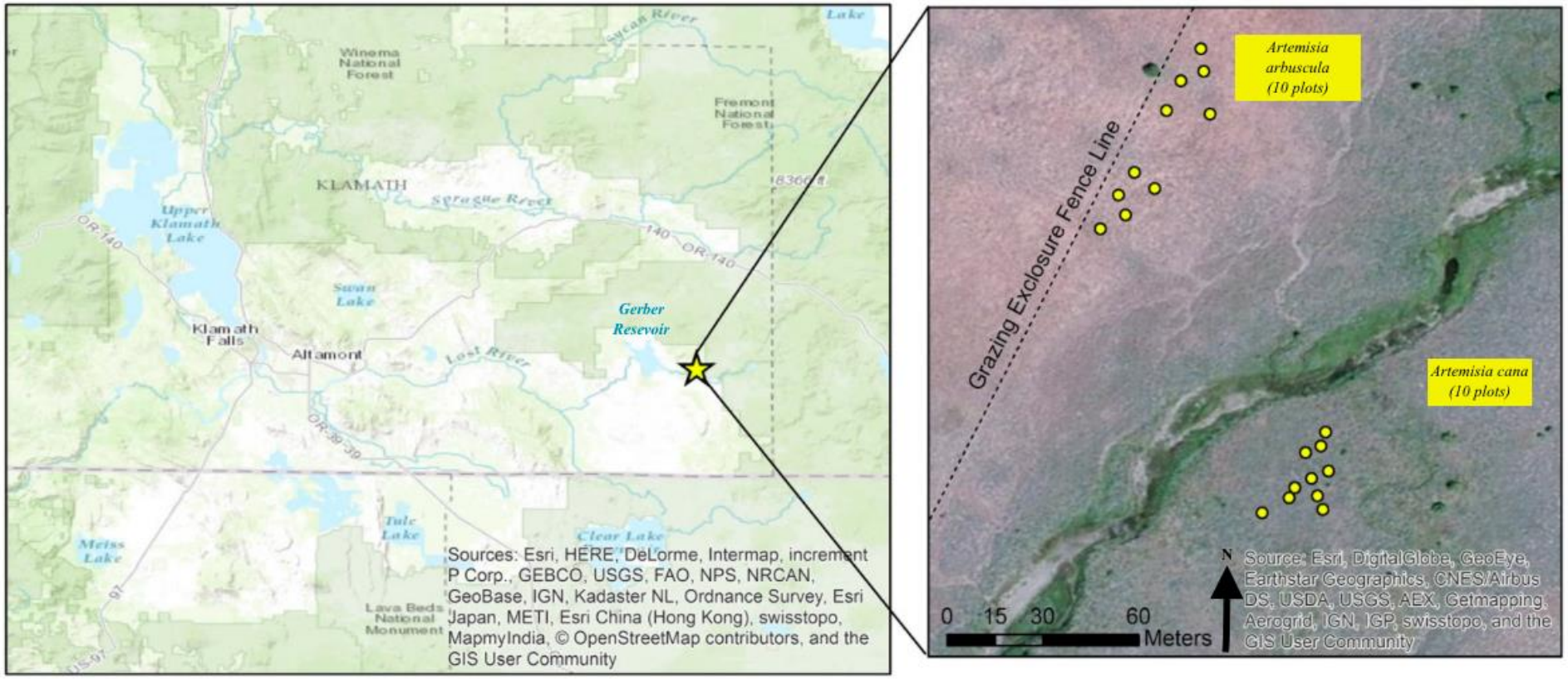


Figure 2. Map showing location of study site east of Klamath Falls, Oregon (left). The study site is east of Gerber Reservoir with the *Artemisia arbuscula* plots on the northwest side of the site and the *Artemisia cana* plots on the southwest side of the site (right) (Source: Kaczynski, 2017).


Figure 3. The project site is delineated by the yellow star. Gerber Recreation Area and Gerber Reservoir are the nearest landmarks to the project site besides some forest service roads (Figure 4). The project site is about 15 miles southeast from Gerber Recreation Area (Google Maps).


Figure 4. This is a satellite image of the project site, again delineated by the yellow star. The *Artemisia cana* plots can be seen inside the red circle. The coordinates of the project site are 42.184, -121.015 and the site can only be accessed by dirt forest roads and Gerber Dam Road which runs northeast from Gerber Recreation Area (Figure 3) (Google Maps).

The study site and surrounding area is currently managed by the Bureau of Land Management (BLM) and is dominated by sagebrush, different species of perennial and annual grasses, western juniper (*Juniperus occidentalis*), and ponderosa pine (*Pinus ponderosa*). It is also inhabited by a variety of different animals including garter snakes (*Thamnophis sirtalis*), black-tailed deer (*Odocoileus hemionus*), and coyotes (*Canis latrans*) (Oregon Forest Resources Institute, 2020 and Juillerat et al., 2007). The Greater Sage Grouse (*Centrocerus urophasianus)* resides within the site area as well (Schroeder et al., 2004). North Africa grass (*Ventenata dubia),* an annual invasive grass species from Europe and Africa, has recently increased in abundance and is encroaching on native vegetation (Scheinost et al., 2008). Before this project began, the site locations and surrounding area was used for cattle grazing and there are still many cows around the site area and closer to Gerber Reservoir.

Seeds were collected in September 2019 from a total of nine *A. arbuscula* plants (six in control plots and three in drought plots) and ten *A. cana* plants (five in control plots and five in drought plots) by a researcher on the project and were stored at room temperature until this study began in August of 2020. Not every plot had sagebrush plants and not every plant produced racemes with seeds on them at the time of collection, which is why *A. arbuscula* seeds were only collected from three plants in the drought plots.

*Pre-Germination Study*

We manually counted and photographically estimated the number of seeds collected from the sagebrush plants in the study. We first extracted seeds from the racemes and then spread them out on a blank piece of paper to manually count and then photograph them using the app "CountThings" (Version 2.61.0, Build 6).

In order to take accurate pictures, we had to first pick a suitable template for the size and shape of the seeds and then make adjustments as needed. Since the seeds of these sagebrush species are variable in shape and size, we used several different templates depending on which worked best for that particular subset of seeds. We used the "Baby Tilapia Fingerlings," "Potato Cyst Nematodes," and "Cattle" templates. The app would then detect the seeds laid out on the paper. Due to the sensitivity of the app, some things that were not seeds were counted and often many seeds were not counted. After the initial photo count, we then manually increased or decreased the "counting sensitivity" by using the "adjustment" tool which allowed us to click on and "add tags" or "subtract tags" (the "tags" represent the seeds in this case).

Lastly, we weighed the seeds grouped by plant before the germination study began (Appendix D). These seeds were "cleaned," so no other plant material mixed in with them obscured the weights.

*Seed Germination Study*

In order to better understand the potential factors that could affect the success of germination for sagebrush species, we conducted a two-part study comparing the incorporation of petri plates and direct seeding in soil. The seed germination study began September 25, 2020 and concluded on October 31, 2020. For our study we planted 1,000 seeds in soil and placed 1,517 seeds in petri plates, with both methods subjected to 24-hour stable light and temperature conditions. An automatic lighting system was on between 18:00-6:00. Both the petri plates and soil studies took place in the experimental greenhouse at Humboldt State University in Arcata, California.

The number of seeds per plant for *A. cana* differed widely from the number of seeds in each *A. arbuscula* plant. Due to the large number of seeds produced per *A. cana* plant, we had enough seeds to study them in both petri plates and soil conditions.

We monitored seeds in the soil and petri plates daily between September 25 and October 31, 2020 to check for germinates. When seeds germinated, we removed them to ensure that no double counting occurred. Seeds were considered germinated when the radicle reached 1 mm long (Meyer & Monsen, 1992).

*Petri Plates*

We set up the petri plates for both *A. cana* and *A. arbuscula* in a laboratory using sterilized conditions (Appendix E). Seeds were collected from ten *A. cana* plants, and for each plant we germinated 100 seeds per individual in four replicates of 25 seeds, for a total of 40 petri plates. Because *A. arbuscula* plants had far fewer seeds than *A. cana,* we only tested the germination rate of these seeds in petri plates. We counted four replicates of 25 seeds for each *A. arbuscula* plant that had at least 100 seeds, and for those with fewer than 100 we planted all of the seeds, using a total of 22 petri plates (Table 1). Each petri plate had one layer of filter paper that was moistened with deionized water until the filter paper was wet to the touch (Bai et al., 1995). We placed the seeds of both species inside petri plates and labeled the top lid of each plate by plant number and treatment (control or drought) (Figure 5).

Table 1. *Artemisia arbuscula* petri plate organization delineating number of petri plates and the number of seeds per petri plate. Petri plates 1 and 7 had fewer than 25 seeds placed in them because those plants only had 46 seeds total (petri plate 1) and 21 total (petri plate 1) and 21 total (petri plate 7) seeds. The rows that have "N/A" are for plants that no seeds were found.

| *Artemisia arbuscula* | | | |
|---|---|---|---|
| **Petri Plate Number** | **Treatment** | **# of Petri Plates (21 Total)** | **# of Seeds per Petri Plate** |
| 1 | control | 2 | 25, 21 |
| 2 | control | N/A | N/A |
| 3 | control | 4 | 25, 25, 25, 25 |
| 4 | control | 4 | 25, 25, 25, 25 |
| 5 | control | 4 | 25, 25, 25, 25 |
| 6 | control | 4 | 25, 25, 25, 25 |
| 7 | drought | 1 | 21 |
| 8 | drought | 2 | 25, 25 |
| 9 | drought | N/A | N/A |

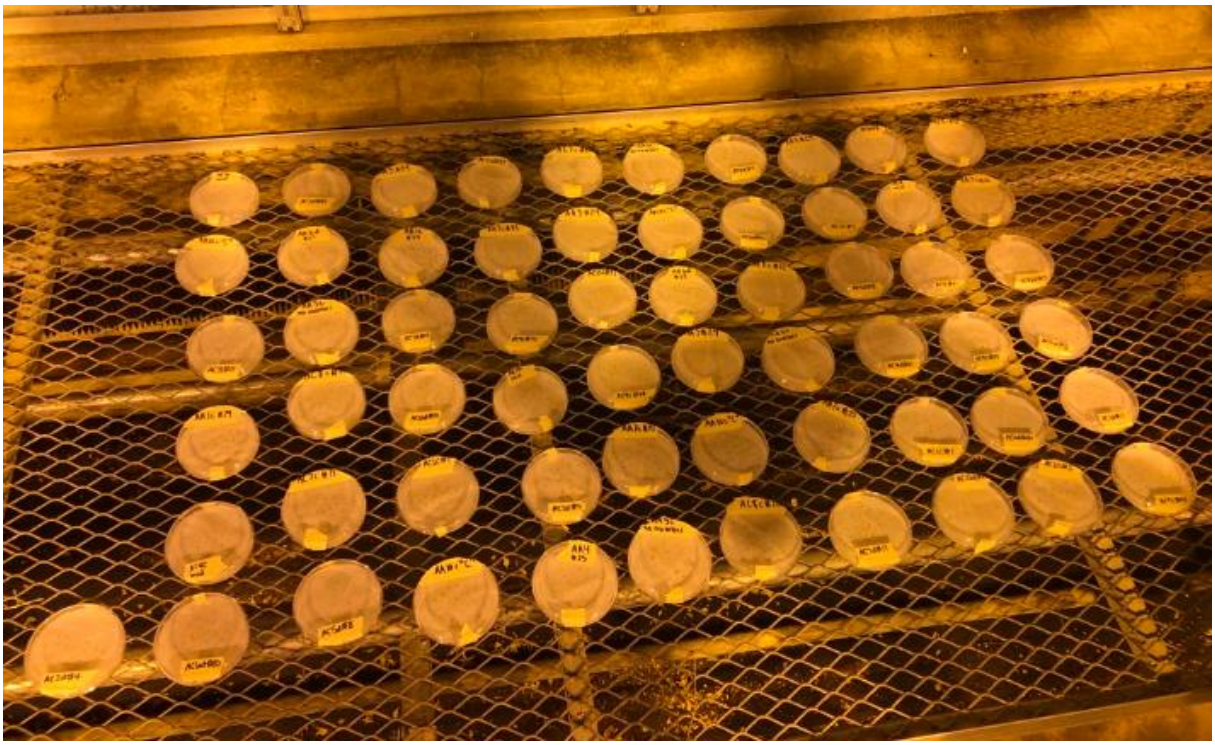

Figure 5. The original petri plate set-up in the greenhouse that was done on the first day of the study, September 25, prior to moving them into bags.

Because petri plates left on the greenhouse bench initially dried out quickly, we stacked them and put them inside 1-gallon ziplock bags with an empty petri plate on the top of the stack in order to keep them moist (Figure 6). The empty petri plate had a dry piece of filter paper in it so the top petri plates with seeds were not receiving more sunlight than the petri plates at the bottom of the stack (Meyer & Monsen, 1992). We checked the petri plates daily and remoistened with deionized water as needed. We randomly rotated the bags every three days but did not change the position of the petri plates in the stacks. If we observed mold in any of the petri plates, we carefully changed the filter papers in the moldy petri plates and moved the moldy seeds into a new petri plate, keeping them labeled by plant (Appendix F).

Figure 6. Petri plates stacked in gallon zip lock bags to help keep the filter papers moist. This transition was made on September 29, 2020 in the Humboldt State University Experimental Greenhouse. Petri plates were kept in the bags until the study ended on October 31, 2020.

*Planting Seeds in Soil*

*Artemisia cana* seeds were planted in flat trays using "Royal Gold Tupur" soil. The bottoms of the trays were lined with gravel and mesh metal sheets on top. Approximately 2 cm of soil was added on top of the mesh sheets. These trays were split in half and labeled with the plant name and treatment (control or drought) (Appendix G). The addition of the control tray was to make sure that there were no non-sagebrush seeds germinating from contamination via seeds blown into the greenhouse.

We delineated four sections in each half of the planting trays and 25 seeds were planted in each section (for a total of 100 seeds) (Figure 7). We sprinkled seeds onto the surface of the soil and then covered with a light dusting of soil on top of the seeds (Limón & Peco, 2016).

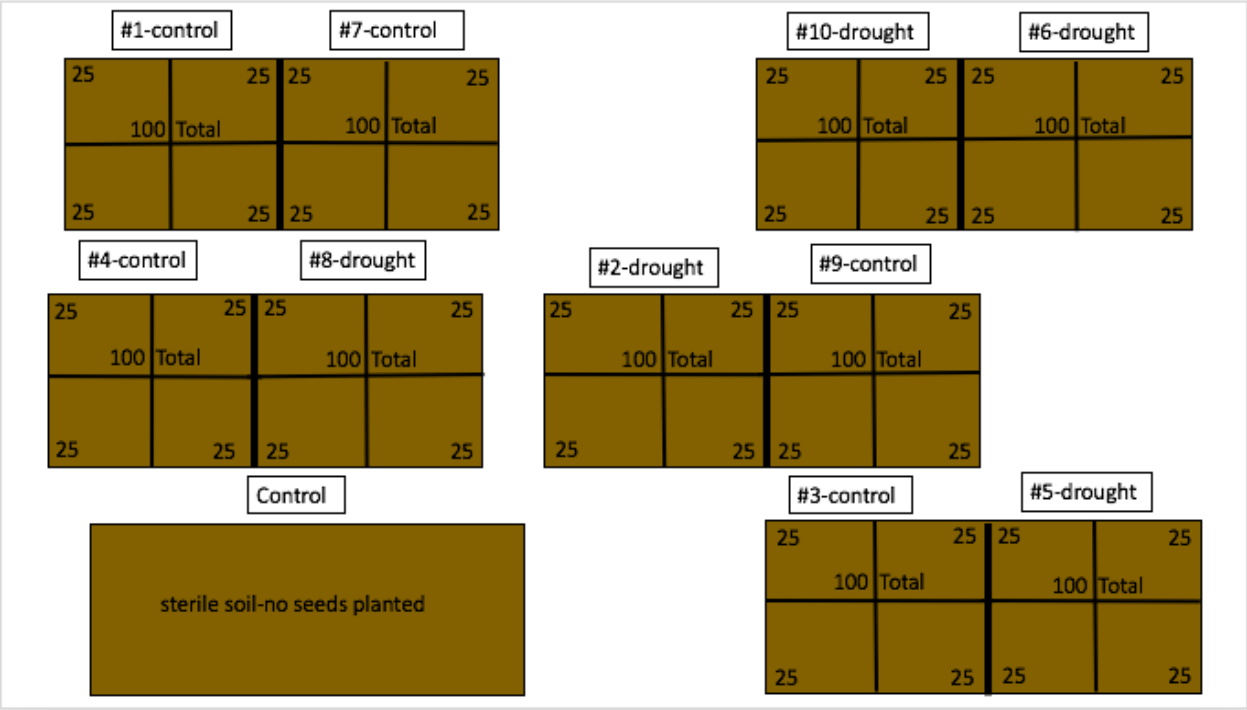


Figure 7. Layout of the *Artemisia cana* seed planting set-up in the Humboldt State University Experimental Greenhouse. The study began on September 25 and concluded on October 31, 2020. Each of the brown rectangles represent the soil trays that were split in half and then in quarters so that 25 replicates could go in 4 different sections. Each half had 100 total seeds. Every section had the seeds of one plant in it (as you can see by the labels above each of the halves). We incorporated one tray of sterile soil that had no seeds planted in it.

We used an automatic watering system to water the plants each day. The system was set to water the plants for one minute every morning at 9:30 am and the trays were manually watered when the soil was dry to the touch (Appendix H). We examined soil trays once per day to make sure the watering system was working appropriately and to check for new germinates. Every three days we randomly rotated the trays.

*Data Analysis*

We compared the manual seed counts to the estimated counts from the "CountThings" app for both species using linear regression analysis in Microsoft Excel. We also analyzed the seed weights versus the manual seed counts with linear regressions. We compared the weight and number of seeds separately for *A. cana* and *A. arbuscula.*

## Results

*Germination Study*

The 36 day-long study of seeds in the greenhouse in October 2020 yielded very low germination rates. We stopped the study after about a month because a previous study showed that seeds typically stopped germinating after 11 days with 54% germinated by the third day, 81% by the fourth, and 92% by the fifth (Watkinson et al., 2020). Of the 1,517 total seeds that were placed in petri plates, only seven seeds germinated (0.46%). None of the *A. cana* seeds in the petri plates germinated, nor did any of the seeds in the soil. The seven seeds that did germinate were all *A. arbuscula* seeds, and they were all from the control plants (non-drought conditions). *Artemisia arbuscula* plants had a germination rate of 1.35%. Figure 8 shows on which days the seeds germinated during the study. Due to the lack of germinating seeds, we were not able to analyze a difference between drought and control plants.

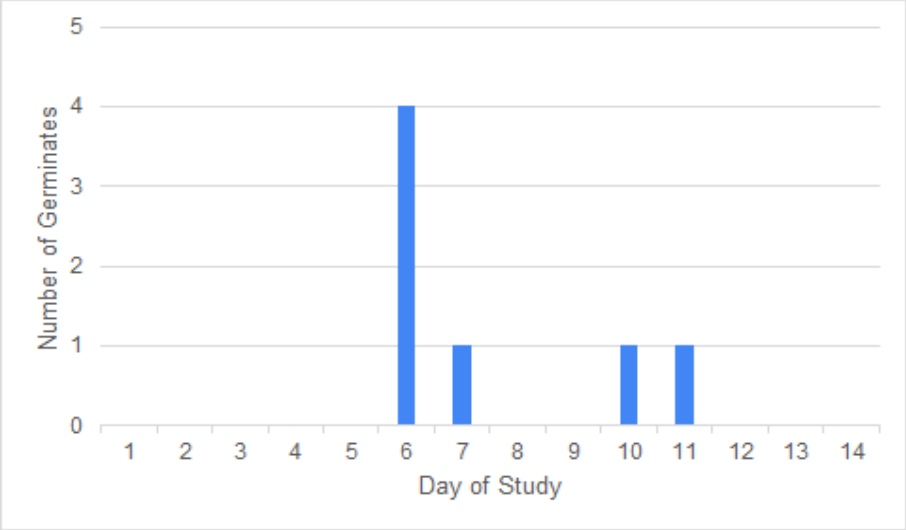


Figure 8. Number of *A. arbuscula* seeds germinated per day from control plants over the first fourteen days of the study (September 25-October 8, 2020). No seeds germinated after day 11 to the end of the study (October 5-October 31, 2020) of the study and no seeds from *A. cana* germinated.

*Seed Counting Comparison*

The number of seeds estimated using "CountThings" was similar to the numbers of seeds counted manually. We compared our actual count versus the app using a linear regression analysis. This comparison showed an $R^2$ value of 0.92 and a p-value of <0.001 (Figure 9).

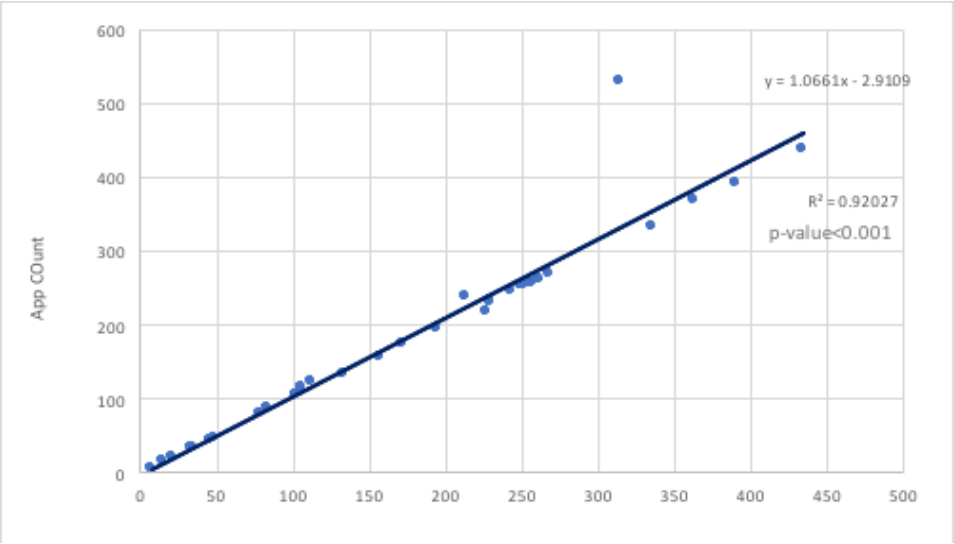


Figure 9. Comparison of actual seed counts for both *A. arbuscula* and *A. cana* compared to the app count with a linear regression trend line (R2 =0.92, p-value <0.001).

The number of seeds manually counted per plant was also highly correlated with the weight of the seeds. The results were quite similar in *A. arbuscula* and *A. cana*, with $R^2$ values of approximately 0.93 and 0.97 and p-values of <0.0001 (Figures 10 & 11). For *A. arbuscula*, the more seeds there were, the less accurate the weight was as a predictor of seed number (Figure 10). For *A. cana*, higher seed numbers were not less accurate (Figure 11).

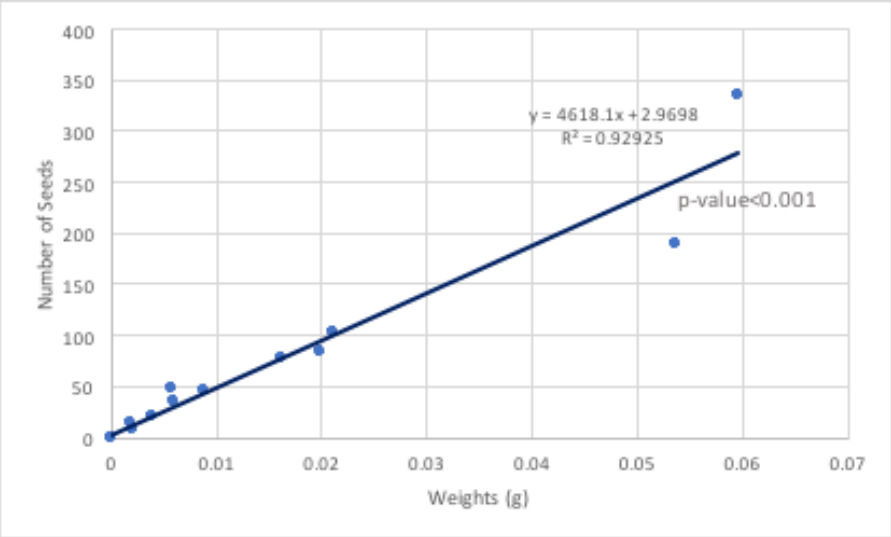


Figure 10. Comparison of seed weight (g) and seed count for *A. arbuscula* with a linear regression trendline (R2 = 0.92925, p-value <0.001).

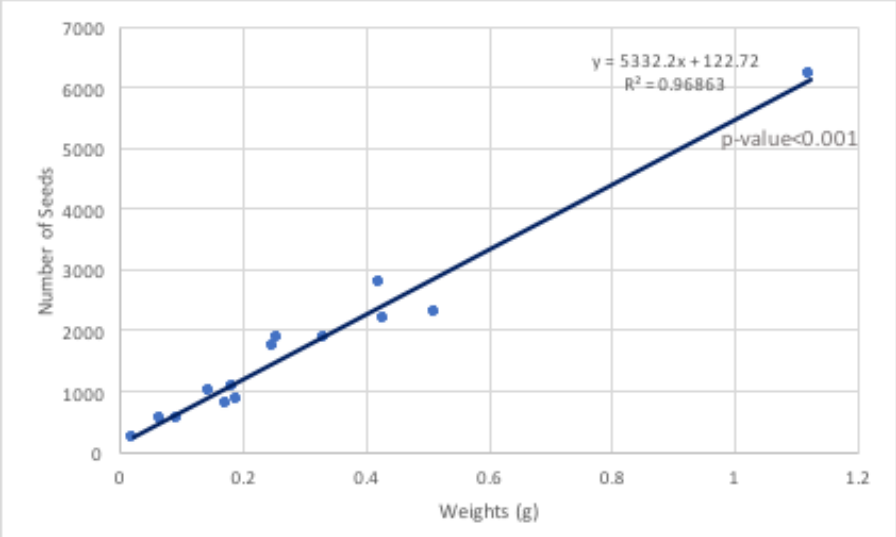


Figure 11. Comparison of seed weight (g) and seed count for *A. cana* with a linear regression trendline (R2 = 0.96863, p-value <0.001).

## Discussion

Because of their significant ecological role and the decline of their ecosystem communities, sagebrush species like *A. cana* and *A arbuscula* are critical to study and understand. Our study investigated the germination rate of these species as well as the effectiveness of photographic counting software to estimate seed numbers. Determining germination rates provides useful background knowledge for future restoration projects. Counting software, if accurate, can be used to estimate numbers of seeds for a variety of species. We found very low germination rates for both species of sagebrush *A. cana* and *A arbuscula*. The "CountThings" app was accurate for counting seeds.

Low germination rates are not unusual for sagebrush species. Despite the high number of seeds that are produced, seed bank studies have shown low numbers of germinates (Hassan & West, 1986; Martyn et al., 2016). A recent study testing the germination rate of several California grassland and scrub species yielded no *Artemisia californica* germinates (Ginn et al., 2020). These studies indicated that our results are not uncommon, and that sagebrush species in general have low germination rates.

One factor that could have contributed to the low germination rate observed in this study was the presence of mold on the unsterilized seeds. It is common in germination studies to sterilize seeds using a bleach or thiram solution (Robert & Wilson, 1982), but due to the small size of *Artemisia* seeds, they are not usually sterilized before germination on petri plates (Meyer & Monsen, 1992; Watkinson, 2020). Because the seeds were not sterilized, we encountered an issue with mold growing on the seeds in the petri plates (Appendix F), despite the petri plates and filter paper being sterile. Mold may have reduced the germination potential of seeds (Lukseviciute & Luksiene, 2020).

Another potential cause for low germination rates was seed dormancy. While dormancy is relatively unstudied in *A. cana* and *A. arbuscula,* it is a prevalent trait in many shrub species within the Great Basin (Kildesheva, 2019). Studies of *A. tridentata* indicate that big sagebrush becomes dormant and cold stratification can be necessary to break this dormancy and germinate the seeds (McDonough and Harniss 1974; Eddleman 1977; Meyer and Monsen 1992).

It is possible, especially because of the long storage time (>1 year) of the seeds used in our study, that dormancy was a cause of lower germination rates. Further research can determine the dormancy traits of *A. cana* and *A. arbuscula.*

The low germination results of our study and others suggest that pre-germination treatments may be pivotal for restoration success with *A. cana* and *A. arbuscula.* Another study had high germination rates using pretreatments such as cold stratification and gibberellic acid on *A. cana* seeds (Watkinson, 2020). Future studies should investigate whether these treatments have a similar positive effect on *A. arbuscula* germination rates.

Although we do not have enough data to draw conclusions about the effects of the drought conditions on the plants, it is noteworthy that the only seeds to germinate were all from the non-drought control plants. This result suggests that drought could potentially affect the viability of the seeds and their germination rates. Past studies that came to this conclusion did extensive studies on the abiotic conditions of the habitat and the effects that lack of water in maternal plants had on seed production and viability (Wagner et al., 2011). A similar habitat assessment of our site would have been important in determining if drought had anything to do with our results.

We found that the photo app "CountThings" had a high level of accuracy in estimating seed quantities ($R^2 = 0.92$). However, there were also flaws with the counting app. In some cases,

"CountThings" dramatically overestimated or underestimated the number of seeds present, and it was necessary to use the adjustment features to get a more accurate count. While the adjustment features were somewhat time consuming, overall using the photo counting app saved time and still provided accurate results. It is also important to note that although we referred to our manual count as the "actual count," it is likely that there was some human error with manually counting tens of thousands of seeds. This could be the cause of some of the discrepancies between the "actual count" and "app count." Seeds that have been counted by the app are indicated with a circle around them, which makes the app useful for ensuring that no seeds are recounted.

The use of photography software to count small items has not yet been widely used for restoration purposes. Its use has mostly been for counting things like cells and seeds in agricultural settings (Mussadiq et al., 2015). Because we found that "CountThings" can reliably count small *Artemisia* seeds, it could also be used for counting any small seeds and has broad restoration applications. In restoration projects where a large number of seeds need to be counted, this method is more accurate and less time consuming than the traditional method of manually counting by hand.

Our study indicates that seed weights are also an accurate way to estimate the number of seeds for both *A. cana* and *A. arbuscula.* The $R^2$ values comparing seed weight and seed count for both species was close to 1.0, indicating a very high correlation. These results illustrate that weight can be used to estimate large numbers of seeds, which can prove to be a useful tool for sagebrush restoration projects. Projects often use small native seeds by hundreds or thousands to seed restoration plots (Bucharova & Krahulec, 2020). Estimating seed numbers by weight would be one of the quickest ways to count seeds for such projects.

Both the "CountThings" app and weighing seeds can have a broader impact on the success of restoration projects. The ability to quickly estimate seed numbers for restoration projects would save time and therefore money and would make larger scale revegetation more plausible. The success of "CountThings" for counting A. *cana* and *A. arbuscula* presents the possibility that the app could be used for any small seeded species. Further research can analyze the success of the app for other species and using other templates. The low germination rates we discovered for both species indicate a need for pretreatments of seeds in order to have successful germination (Watkinson, 2020). Future studies should further analyze the effect of drought on germination rate, and the effectiveness of pretreatments like gibberellic acid for germinating *A. cana* and *A. arbuscula* seeds. Ideally, a future restoration project could use this data, combined with the utility of the "CountThings" app, to create a successful sagebrush restoration project.

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## Appendices

*Appendix A.* Photograph of *Artemisia arbuscula* racemes, taken by Sam Kelly on October 2, 2020 in Bonanza, Oregon.

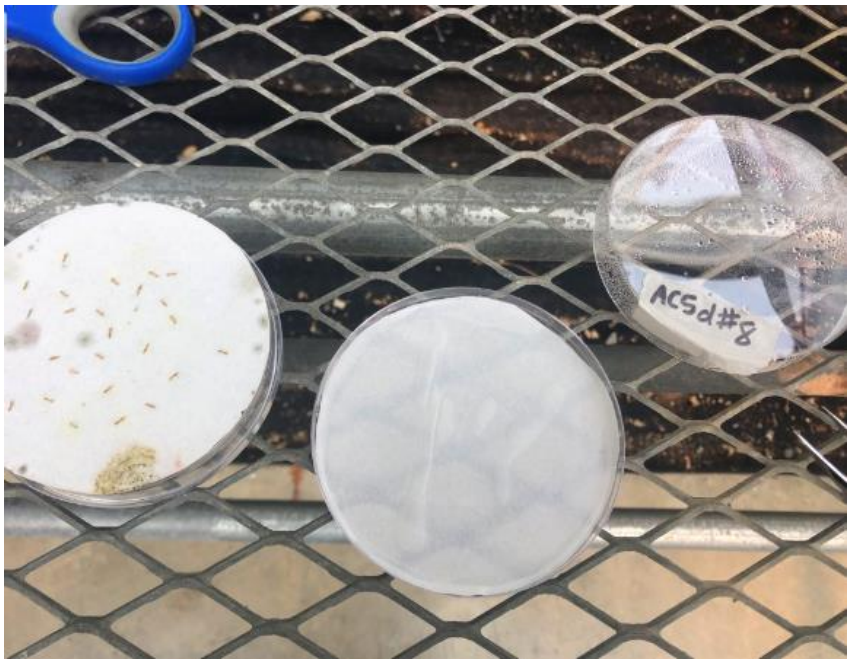
*Appendix B.* Photograph of *Artemisia cana* racemes, taken by Sam Kelly on October 2, 2020 in Bonanza, Oregon.

*Appendix C.* The red circle delineates the control plot, and the arrow is pointing to the rain shelter and the drought plot for the *Artemisia cana* site in Bonanza, Oregon. This photo was taken by Sam Kelly on October 2, 2020.

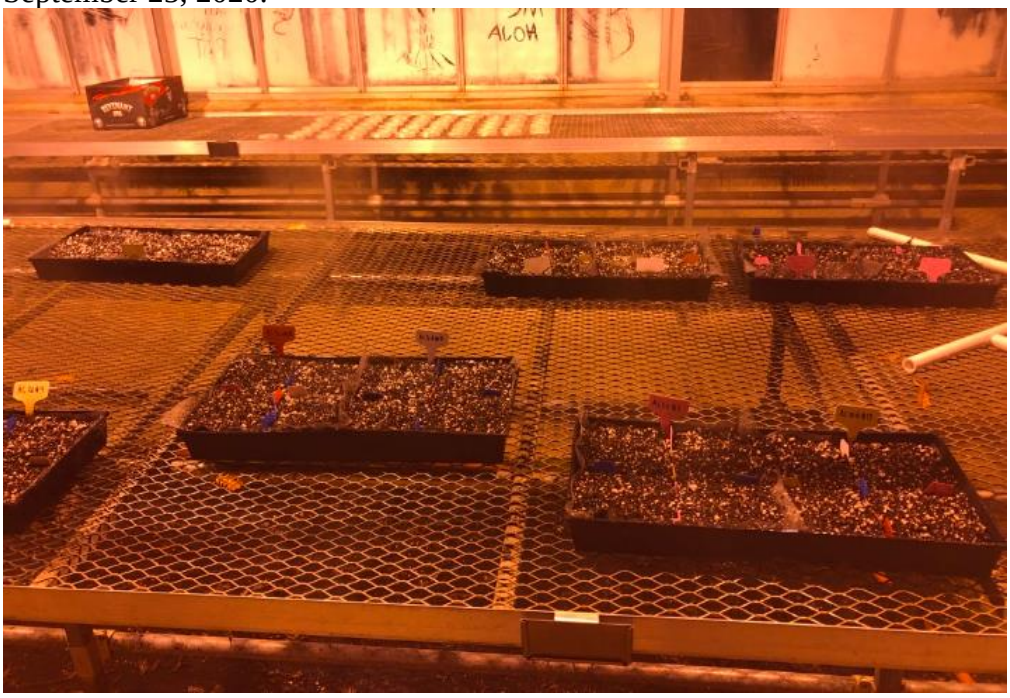
*Appendix D.* Weighing Seeds in lab. Photos taken by Cessair McKinney on September 12, 2020 in a lab at HSU. We placed seeds from each plant in small trays and weighed them on a Mettler AE 163 Analytical Balance Scale.

*Appendix E.* Petri plate preparation in a sterilized lab done on September 25, 2020. We put the filter papers inside the petri plates and then wet with deionized water before setting the seeds inside and labelling. Photo taken by Sam Kelly.

*Appendix F.* Changing the filter paper from a moldy petri plate (left) onto a clean filter paper (right) in the HSU Experimental Greenhouse on October 5, 2020. Photo taken by Sam Kelly.



*Appendix G.* Soil tray setup in the HSU experimental greenhouse, taken by Sam Kelly on September 25, 2020.



*Appendix H.* The automatic watering system in the HSU Experimental Greenhouse. Photo taken on September 25, 2020 by Sam Kelly.