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STINKNET (*ONCOSIPHON PILULIFERUM*) GERMINATION PLASTICITY

By

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A capstone project submitted for Graduation with University Honors

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ABSTRACT

Invasive plants – plants that are non-native to an ecosystem and having a negative impact – are a significant threat to biodiversity. Understanding what facilitates their establishment and spread is important for mediating these impacts. Germination rates of an invader can greatly impact their spread, as early germination may give them early access to resources. We investigated how germination for a new invader Stinknet (*Oncosiphon piluliferum*) varies compared to other invasive and native species from Riverside County. Stinknet (*Oncosiphon piluliferum*) is an invasive plant native to South Africa that is spreading in Southern California and Arizona; Riverside County has one of the greatest infestations of Stinknet. It was initially discovered in Riverside County in 1981 and has since been competing with Riverside County's native plants. Germination rates of Stinknet can greatly impact its spread, but the range of conditions under which it germinates are unknown. We assessed germination under a variety of watering conditions to see if germination differences were dependent on rainfall patterns. We applied four watering conditions (watered daily, every other day, once a week, and every day and once a week) onto Stinknet seeds and seeds of other local (n=9) and invasive (n=4) species to collect data on their germination times and percentages. We expected Stinknet to germinate quicker than the native species, but at a similar time to other invasive species. Understanding the germination time of Stinknet in varying conditions is critical in predicting good establishment conditions for the plant and identifying windows for invasive species management actions.

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INTRODUCTION

Biological invasions are recognized as major environmental and economic problems and are a great threat to the biodiversity of a region (Vilà & Weiner, 2004). Although there are many discussions regarding the impacts of invasive species, there is little quantitative data that exists to measure the environmental impacts of most invasive species. Economic impacts of invasive species have been further researched as data may be easily more accessible than that of environmental impacts (Forseth & Innis, 2004). The U.S. Forest Service states that invasive species have contributed to the decline of 42% of the United States of America's endangered or threatened species. As this decline continues, the diversity of plants and animals can be diminished; this would lead to a plant dominating the biodiversity. Invasive species can influence a decline by competing directly with native species for resources (*Invasive Plants*, n.d.).

But what is considered an invasive species? An invasive species has been federally defined in Executive Order 13312 to be an "alien species whose introduction does or is likely to cause economic or environmental harm or harm to human health" (Executive Order 13112: Invasive Species, 1999).

Our study mainly is primarily centered around the Coastal Sage Scrub environment's plant species. The Coastal Sage Scrub vegetation of the Motte Rimrock Reserve turned out to be of great influence on the species list used in this study. The Coastal Sage Scrub is a drought-adapted community with hot and dry summers and rainfall predominantly occurring during the winter months. Motte Rimrock Reserve is one of the many reserves of the University of California Natural Reserve System that is managed by the University of California, Riverside. The location is an ecological reserve and biological field station that is located on a small plateau overlooking the Perris Valley in west-central Riverside County, California, United States. Coastal Sage Scrub communities have fewer fire-adapted plants than Chaparral and may take longer to recover after a fire, but this environment is still called a Soft Chaparral. Indicator species of the Coastal Sage Scrub include California Sagebrush (*Artemisia californica*), White sage (*Salvia apiana*), Black Sage (*Salvia mellifera*), California Buckwheat (*Eriogonum fasciculatum*), Lemonadeberry (*Rhus integrifolia*), and California Brittlebush (*Encelia californica*).

There is one plant that is observed to be the dominating species of areas in the Coastal Sage Scrub environment. Hillsides can be seen covered in a blanket of small yellow globes. Stinknet (*Oncosiphon piluliferum*), which may also be known as globe chamomile, is a new invasive species in the Southern California area. It has been damaging to the Coastal Sage Scrub, a shrub-dominated Mediterranean-type ecosystem found in California (Wainwright & Cleland, 2013). Currently, controlling Stinknet's invasiveness is a pressing objective for land managers in Riverside County (Rodriquez & Larios, 2021).

The plant offers low-value nutrition and has been threatening to the Stephens' Kangaroo Rat (*Dipodomys stephensi*); the Stephens' Kangaroo Rat is an endemic species to Riverside County. As of 2020, the Stephens' Kangaroo Rat has been reclassified from endangered to threatened by the Fish and Wildlife Service (*Endangered and Threatened Wildlife and Plants; Reclassification of Stephens' Kangaroo Rat From Endangered To Threatened With a Section 4(d) Rule*, 2020). A Stephens' Kangaroo Rat foraging study was conducted at active burrows across a gradient of Stinknet invasion. For each of the trials, the Stephens' Kangaroo Rat were offered 4 different seed species and Stinknet. A ratio of preference to avoidance was calculated for each of the species and for Stinknet at the different levels of invasion, dependent on the proportion of seed removal. The study concluded that Stinknet foraging by Stephens' Kangaroo Rat did not differ in the different levels of invasion. Stinknet can possibly outcompete the plant species that dominate the Stephens' Kangaroo Rat's diet, thus decreasing their ability to sustain with the changing plant biodiversity if Stinknet invades their habitats. Not only does Stinknet invasion influence the Stephens' Kangaroo Rat's diet, invasion of Stinknet can lead to the reduction and/or removal of the open spaces that are preferred by the Stephens' Kangaroo Rat (Service, 1997). With the Stephens' Kangaroo Rat being endemic to Riverside County, the research and control of Stinknet spread is a pressing management objective in Riverside County.

Stinknet plants can be found in their native home of South Africa, but Stinknet is found to be an invasive plant in Riverside County, San Diego County, the state of Arizona, and even in Australia. Since the early 1980s, Stinket has been flourishing as a widespread weed in Riverside County (Chamberland, 2020).

This project revolves around the question: "How does stinknet's germination time compare to other invasive and native species under a variety of water conditions?" I will do so by collecting the seeds of stinknet plants, other invasive plants, and native plants of the Motte Rimrock Reserve and germinate the various seeds under differing watering conditions. We will investigate if stinknet's germination rate matches that of other invasive plants and is faster than the germination rates of native species in response to favorable watering conditions (plasticity).Although there is not much information on the germination times of Stinknet. Field researchers have observed that Stinknet is part of the sunflower family (*Asteraceae*) and is a cool-season annual plant. Stinknet seeds tend to germinate after cool-season rains.

Germination can begin in late October or November and can continue through early winter if the season rains can provide moist soils for a longer time (Chamberland, 2020).

Climate is the main factor that controls and regulates the phenological events in plants (Menzel et al., 2006). Phenology is the timing of recurrent biological events related to climate, such as bird migration, frog calling, and leafing, flowering and fruiting of plant populations (Rosenzweig et al., 2008). Because climate affects plant phenology, differing annual precipitation can result in years with greater or fewer observations of certain plant species (Went, 1949).

Plasticity in germination cues with rain seasons may be key traits that facilitate the invasion of exotic plant species in new environments (Wainwright et al., 2012). When non-native species germinate earlier, they can procure early access to space and resources over native species (Belyea & Lancaster, 1999). Therefore, allowing the non-native species to become considered invasive if they can outcompete and act as a threat to the invaded ecosystem.

The result of this study is intended to help land managers with their efforts in controlling the spread of Stinknet. Plasticity in phenology can be used to predict a plant's invasiveness (Ren & Zhang, 2009). Thus, understanding Stinknet's phenology provides the ability to predict the possible increase or decrease of Stinknet's population from seasonal rain patterns, which may help land managers in being proactive against Stinknet's invasiveness. Therefore, maintaining the diversity of Riverside County's Coastal Sage Scrub ecosystem.

MATERIALS AND METHODS

DESIGN

The experimental design was to collect seeds of species that coexist with Stinknet and conduct a watering treatment based experiment to test germination plasticity in the different watering treatments. There are four treatments used: watered once everyday (W1), watered once every other day (W2), watered once once a week (W3), and watered once everyday for a week and then once once a week (W4).

The watering treatments were decided by taking into account the possible water saturation in soil due to the precipitation pattern of the Coastal Sage Scrub environment, also known as a soft chaparral environment. Coastal sage scrub is generally found below 3,000 feet in elevation and on dry, rocky slopes of mountains and hillsides. The Coastal Sage Scrub climate's annual rainfall generally is 10 to 20 inches that generally occurs during the winter. During the summer, the climate is typically hot and dry.

13 species were used in the experiment to assess germination under a variety of watering conditions to see if germination differences were dependent on rainfall patterns. We applied four watering conditions (watered daily, every other day, once a week, and every day and once a week) onto Stinknet seeds and seeds of other local (n=9) and invasive (n=4) in 5 repetitions (noted as Blocks in the study). There are 13 cones per watering treatment in each block. Each cone had 10 seeds of the species that was randomly assigned to it. Please refer to Figure 1 for a drawing of one block for more information.

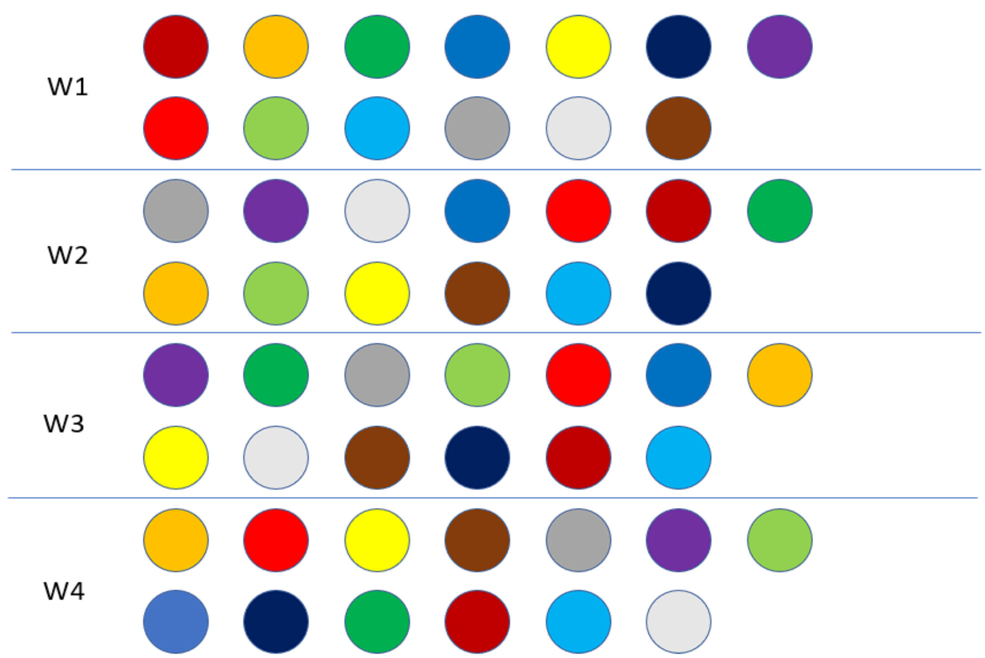

Figure 1. An example of a possible configuration of the cone racks for a singular repetition. Each color represents one species (for 13 total) and there are 13 species per watering treatment.

The study was conducted in a greenhouse at the University of California Riverside where the temperature and humidity could be controlled. To account for greenhouse effects, the blocks were rotated once a day.

This proposed research method is appropriate for a project such as this because it has been successfully conducted with other invasive plants in the Coastal Sage Shrub ecosystem. We drew inspiration for this project from a research paper from 2013. In this research, they recorded time to germination and percentage germination in response to variation in three environmental cues: temperature, day length, and soil moisture (Wainwright and Cleland 2013).

SOIL

Soil used was Soil Mix III from Agricultural Operations of the College of Natural and Agricultural sciences. Please refer to Table 1 for more information regarding the soil mix.

Ingredient	**Per Cubic Yard of Soil**
Plaster Sand	15.50 cu. ft.
Peat Moss	11.50 cu. ft.
KNO3	0.25 lb.
Limestone Flour	1.50 lb.
Phosphate	1.25 lb.
Dolomite	3.75 lb
Magnesium	0.07 lb.

Iron	0.13 lb.
Manganese	0.03 lb.
Zinc	0.05 lb.
Copper	0.11 lb.

Table 1. Soil Mix Recipe for Soil Mix III.

SEEDBANK

The seedbank used in this experiment was collected from Motte Rimrock Preserve in 2019 and in 2021 or they were commercially bought. There was an effort to use seeds collected in the Motte Rimrock Preserve as the dominant type of seeds used in the study. This is due to the notion that a species' seeds can differ in the population. There can be genetic variation and diversity in every species' seeds (Walker, Hodder, Bullock, & Pywell 2004). The differentiation between seeds in a species can be due to local adaptations which can result in a site advantage for the offspring. Therefore, we tried to continue to use seeds collected from the Motte Rimrock Preserve to minimize differences in seeds that may have been imported from a different location with slightly different adaptations that would have aided in their germination at their home site.

Species were chosen on the criteria that the flowering season is within a one month difference of Stinknet, meaning that the species could be seen to coexist with Stinknet and that the species is an annual species. We excluded any collected seeds that were legumes as the nitrogen fixing characteristics could provide the species with an advantage in germination.

Please refer to Table 2 for more information on the seedbank used in the study.

Species	Family	Flowering Season	Native or Exotic
Oncosiphon piluliferum	Asteraceae	Mar. - June	Exotic
Layia platygossa	Asteraceae	Feb. - May	Native
Lasthenica californica	Asteraceae	Feb. - June	Native
Rafinesquia californica	Asteraceae	Apr. - July	Native
Amsinckia intermedia	Boraginaceae	Feb. - June	Native
Phacelia minor	Boraginaceae	Mar. - June	Native
Nemophila menziesii	Boraginaceae	Mar. - June	Native
Eucrypta chrysanthemifolia	Boraginaceae	Mar. - June	Native
Avena barbata	Poaceae	Mar. - June	Exotic
Erodium cicutarium	Geraniaceae	Feb. - June	Exotic
Bromus madritensis	Poaceae	Feb. - Mar.	Exotic
Escholzia californica	Papaveraceae	Mar. - June	Native

Salvia columbariae	Lamiaceae	Mar. - June	Native

Table 2. Seedbank species and their family, flowering season, and whether they are native or exotic to California.

WATERING TREATMENT

Each instance of watering was timed at 5 minutes per block. The 5 minutes of hand misting time was tested prior to the experiment. 10 cones were filled with soil to the amount that would be used during the experiment and hand misted until the water dripped out from the soil in the cone.

A gravimetric soil moisture was then calculated on 10 samples from each cone.

$$\%Moisture = \frac{(soil_{wet} - (soil_{drysoil+tin} - tin))}{(soil_{drysoil+tin} - tin)} * 100$$

The average soil moisture percentage of the 10 samples concluded to be 37.07%.

ANALYSIS

Data was collected initially in an excel spreadsheet and then imported into RStudio for statistical analysis and data visualization. Transformation of data was initially done to meet the requirements. This is to ensure that all requirements are met to conduct a one way ANOVA (Analysis of Variance): normality, sample independence, variance equality, and dependent variable.

The one-way ANOVA was done as a days to emergence is a function of the watering treatment. This was done to determine if there was a significant difference between the watering treatments. A Tukey’s post-hoc test would then be conducted if there was evidence that there is a difference between the four different watering treatments to see the relationship and differences between each treatment.

A total germination proportion was also conducted to overall analyze the end result of germination rate between the species.

RESULTS

When plotting the graphs, the data set was not normalized. Normal Q-Q plots that exhibit an unnormalized distribution will have heavy tails in the distribution usually mean that the data have more extreme values than would be expected if they truly came from a Normal distribution. Therefore, outliers that were highlighted by RStudio were eliminated to transform the data to have a more normal distribution. Points 39, 48, and 142 are outliers and can be removed from the data set: 39 was a cone that had Stinknet, 48 was a cone that had *Avena barbata*, and 142 was a cone that had *Salvia columbariae* (Figure 2.).

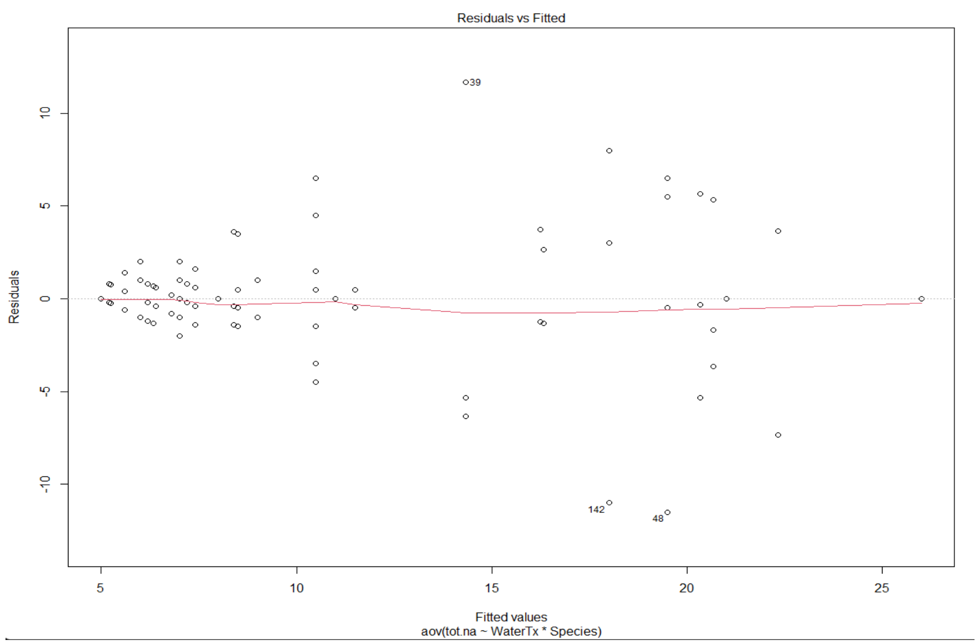

Figure 2. Residual vs. fitted plot for the untransformed data set.

Once the three points were removed, a more normal distribution was observed (Figure 3.).

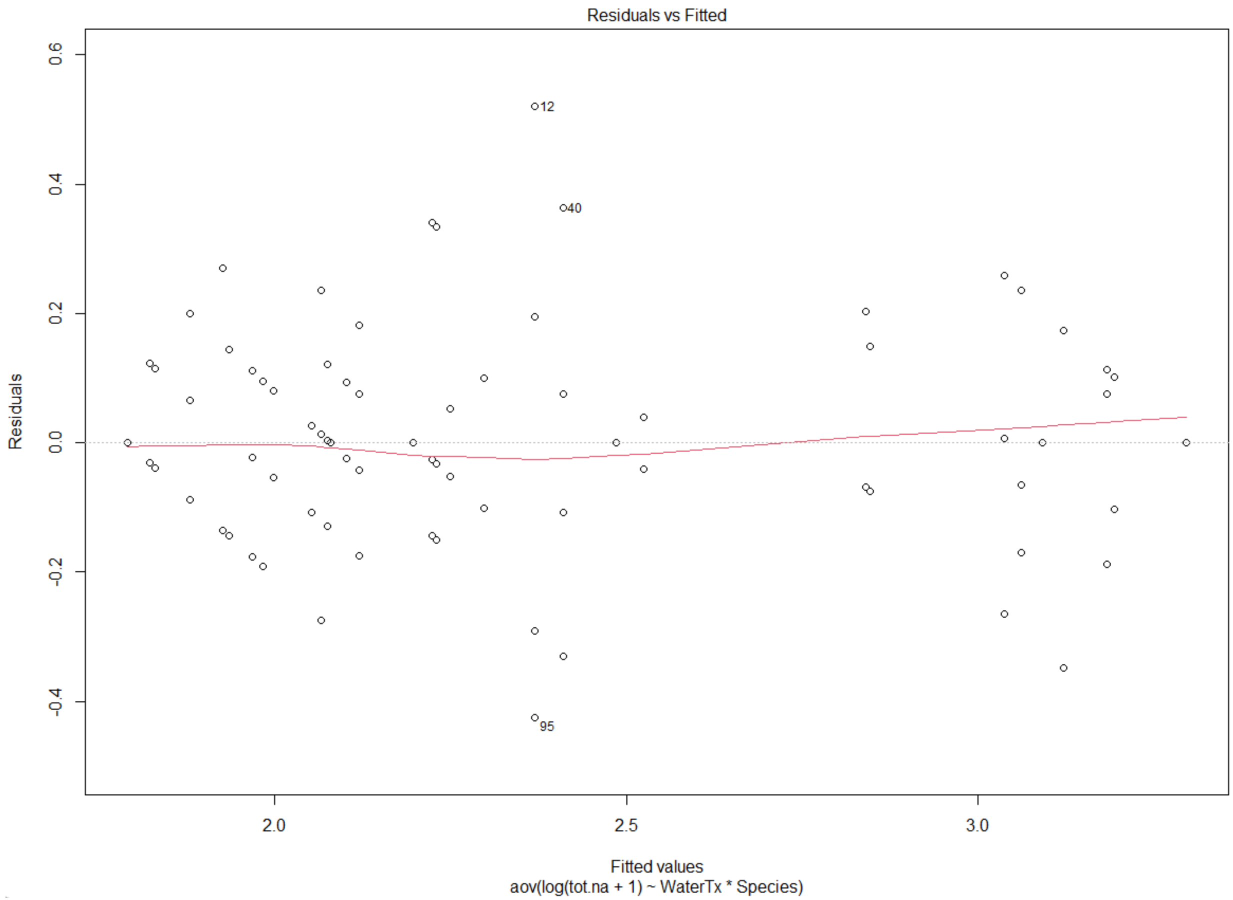

Figure 3. Residuals vs fitted plot for the transformed data plot. There are less extreme outliers than the pre-transformed dataset.

DAYS TO EMERGENCE

Days to Emergence is considered the days before the first instance of emergence. The day counted as emergence would be marked when the cotyledons are present.

Block	**WaterTx**	**Cone**	**Species**
B1	W3	28	Nemo. men.
B1	W3	31	Pha. min.
B1	W3	32	Av. bar.
B1	W3	38	Eu. chrys.
B2	W2	21	Onco. pil
B2	W3	27	Nemo. men.
B2	W3	33	Esch. cali.
B2	W3	34	Sal. cali.
B2	W3	36	Las. cali.
B3	W3	28	Sal. cali.
B3	W3	29	Las. cali.
B4	W4	40	Sal. cali.

Table 3. Species that did not emerge at all in their respective watering treatments.

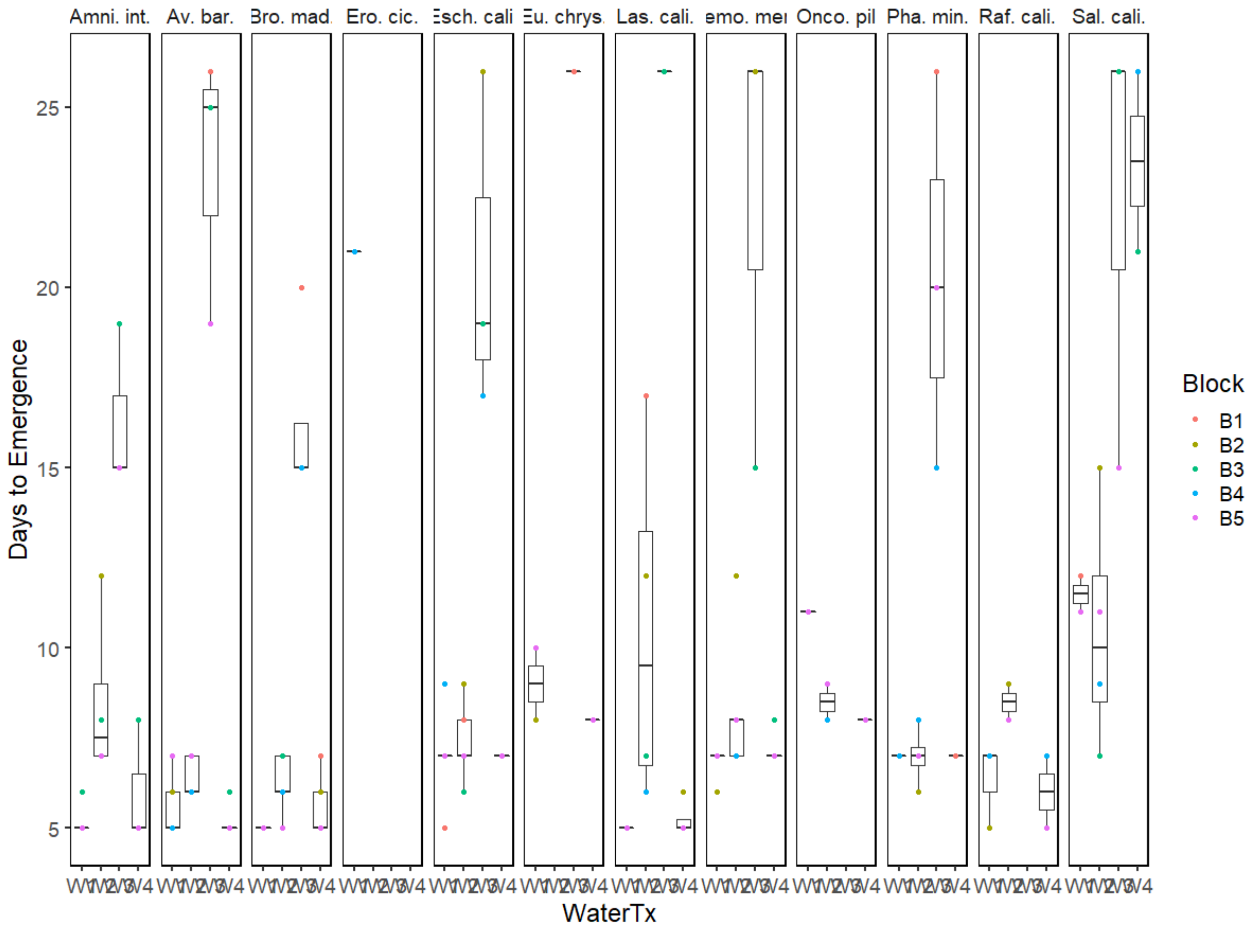

Figure 4. Days to Emergence separated by watering treatment and by species.

GERMINATION PROPORTION

There were found to be significant differences between the 4 watering treatments (Pr<F: < 2e-16) and between the 13 species (Pr<F: < 2e-16). Therefore a Tukey's Post-Hoc test was needed to determine the differences between the 4 watering treatments. Through the Tukey's test, we found that watering treatment 3 differed between the other 3 watering treatments. We also found that the species also differed between one another (refer to Table 4).

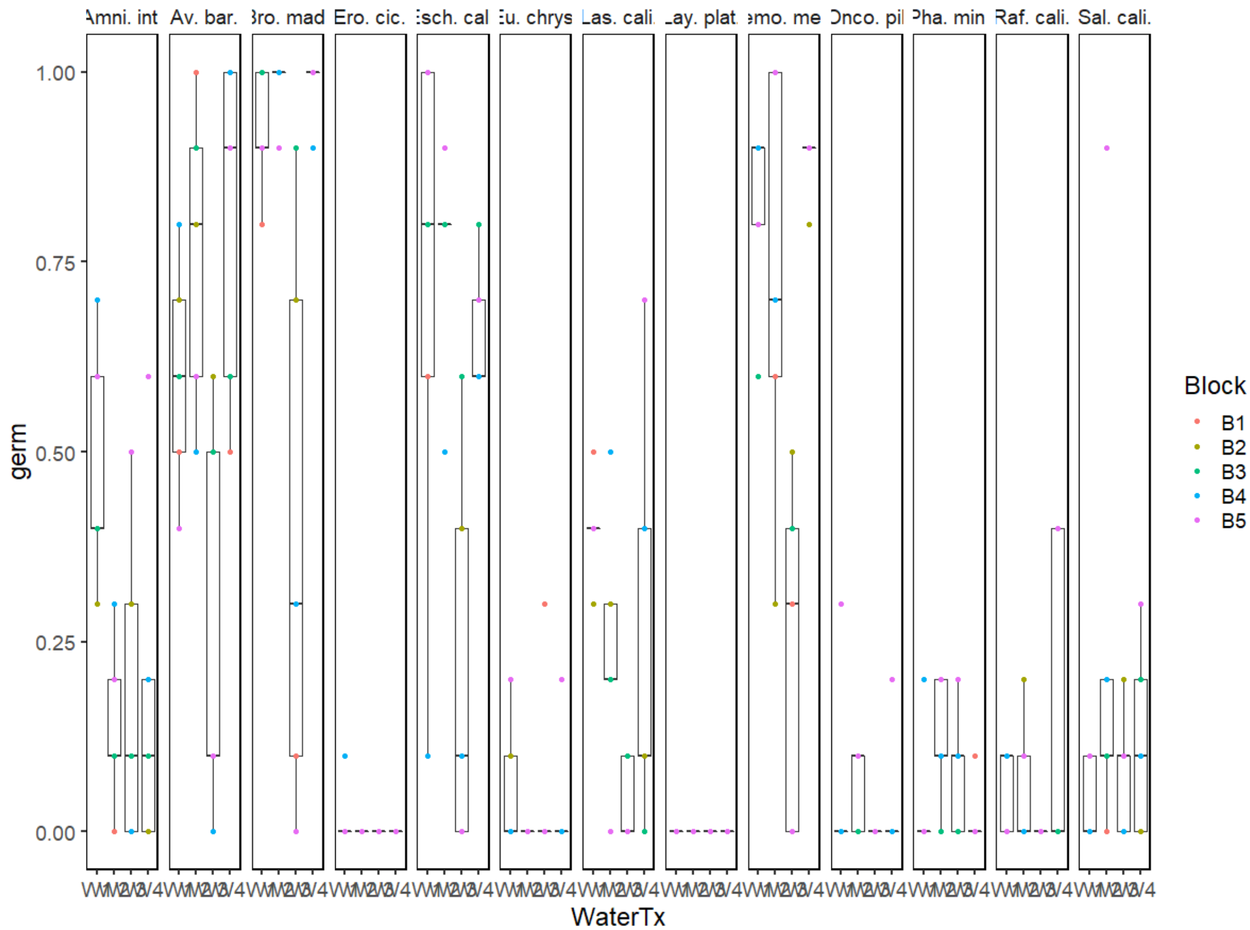

Figure 5. Germination proportion by watering treatment for each species.

	diff	lwr	upr	p adj
Ero. cic.-Amni. int.	1.193708966	0.619390158	1.76802777	0.0000000
Sal. cali.-Amni. int.	0.518571062	0.297829872	0.73931225	0.0000000
Ero. cic.-Av. bar.	1.205577405	0.634257671	1.77689714	0.0000000
Eu. chrys.-Av. bar.	0.367161632	0.059775855	0.67454741	0.0065618
Sal. cali.-Av. bar.	0.530439501	0.317623107	0.74325590	0.0000000
Ero. cic.-Bro. mad.	1.280773817	0.710245934	1.85130170	0.0000000
Esch. cali.-Bro. mad.	0.211672155	0.028766772	0.39457754	0.0099604
Eu. chrys.-Bro. mad.	0.442358044	0.136446548	0.74826954	0.0002915
Nemo. men.-Bro. mad.	0.253049400	0.070144017	0.43595478	0.0006596
Onco. pil-Bro. mad.	0.394866003	0.088954506	0.70077750	0.0021067
Sal. cali.-Bro. mad.	0.605635913	0.394954530	0.81631730	0.0000000
Esch. cali.-Ero. cic.	-1.069101662	-1.640421396	-0.49778193	0.0000007
Eu. chrys.-Ero. cic.	-0.838415773	-1.460134119	-0.21669743	0.0010293
Las. cali.-Ero. cic.	-1.126097212	-1.700416020	-0.55177840	0.0000002
Nemo. men.-Ero. cic.	-1.027724417	-1.599044151	-0.45640468	0.0000020
Onco. pil-Ero. cic.	-0.885907815	-1.507626161	-0.26418947	0.0003851
Pha. min.-Ero. cic.	-1.120112965	-1.706274644	-0.53395129	0.0000004
Raf. cali.-Ero. cic.	-1.101018929	-1.695495373	-0.50654248	0.0000009
Sal. cali.-Ero. cic.	-0.675137904	-1.255946396	-0.09432941	0.0094098
Sal. cali.-Esch. cali.	0.393963759	0.181147364	0.60678015	0.0000009
Sal. cali.-Las. cali.	0.450959309	0.230218119	0.67170050	0.0000000
Sal. cali.-Nemo. men.	0.352586513	0.139770119	0.56540291	0.0000158
Sal. cali.-Pha. min.	0.444975061	0.195034882	0.69491524	0.0000026
Sal. cali.-Raf. cali.	0.425881025	0.157018937	0.69474311	0.0000443

Table 4. Significant differences between two species in the germination proportion.

DISCUSSION

We were able to find that there was a significant difference between watering treatment 3 and the other three watering treatments. There was a much lower germination proportion in watering treatment 3 across the 5 blocks. This would make sense as there would have not been the correct amount of water needed for a species to germinate. There were a few that did emerge in watering treatment 3, but the species that did emerge were the species that were quicker to emerge in the other three treatments.

Stinknet actually had a lower germination proportion and longer days to emergence than we expected due to the high observance of Stinknet in the Coastal Sage Scrub environment. We believe that this could have been an issue with nonviable seeds used in the study. Therefore, more repetitions of this study should be done with a different sample of Stinknet seeds. This is also the case for *Erodium cicutarium*, *Eucrypta chrysanthemifolia*, and *Layia platygossa*.

CONCLUSION

We assessed germination under a variety of watering conditions to see if germination differences were dependent on rainfall patterns. We applied four watering conditions (watered daily, every other day, once a week, and every day and once a week) onto Stinknet seeds and seeds of other local (n=9) and invasive (n=4) species to collect data on their germination proportion and days to emergence. We expected Stinknet to germinate quicker than the native species, but at a similar time to other invasive species. However, we were unable to confidently say that Stinknet would germinate at a higher proportion and have shorter days to emergence. To understand the germination time of Stinknet in varying conditions, more research is needed as the seedbank in this study did not have the most viable seeds for Stinknet and its other coexisting species.

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