

EFFECTS OF CLIMATE WARMING ON SEED TRAITS,
GERMINATION, AND EARLY LIFE TRAITS OF TWO PRAIRIE
ANNUALS ACROSS VARIABLE ENVIRONMENTS

by

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A THESIS

Presented to the Department of Biology
and the Robert D. Clark Honors College
in partial fulfillment of the requirements for the degree of
Bachelor of Science

May 2024

An Abstract of the Thesis of

Anna C. Easton for the degree of Bachelor of Science
in the Department of Biology to be taken June 2024

Title: Effects of Climate Warming on Seed Traits, Germination, and Early Life Traits of Two
Prairie Annuals Across Variable Environments.

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While it's known that warming influences species' reproduction, the interactive effects of warming with other abiotic and biotic factors are less clear. Successful reproduction in annual plants hinges on the production of viable seeds, yet few studies account for variation in seed quality by focusing on seed quantity. In this study, we explore how warming impacts seed and seedling traits across space and stages of fruit phenology. Understanding these traits is crucial for grasping the broader implications of warming on the dynamics of populations and communities, especially in annual plants.

We conducted a warming experiment at eight sites distributed across an elevational gradient to ask how warming affects seed characteristics across space. We studied two species of Willamette Valley prairie annuals, *Clarkia purpurea* and *Collinsia grandiflora*, and placed open-top warming chambers on half the plots at each site to simulate future environmental conditions. Mature fruits were collected from plots on two dates to examine the interaction of fruiting phenology and warming. We measured mean seed mass and number of seeds per fruit, followed by sowing the seeds in a greenhouse study to measure germination success and speed, and seedling dry root:shoot ratio and total dry mass.

We found that the timing of fruit collection had the most impact on seed and seedling traits overall and that warming effects were site specific. For both species, fruits collected later in

the season produced fewer and lighter seeds, while germination responses were species-specific. Seedlings from these seeds had decreased dry mass while showing opposing root:shoot ratio responses. Warming exacerbated the difference between early and late seeds for some traits and warming effects varied in magnitude and direction by site for all traits.

These findings emphasize the high variability of seed traits among populations and their nuanced sensitivity to shifts in climate. Although there were few consistent effects of warming on seeds, there was significant variability across sites and warming exaggerated the effects of timing of fruit production, indicating that subtle environmental differences could notably impact how these populations respond to climate change. Our findings highlight that predicting population dynamics under future climate conditions will necessitate a detailed understanding of plant functional traits across variable environments.

Acknowledgements

I would like to thank Dr. Jeff Diez for guiding me through this process and allowing me to be a part of the wonderful lab community he has built. I have thoroughly enjoyed my time in the Diez Lab, and I have learned so much over these past two years. Thank you to all the Diez Lab members, with a special shout-out to our lab manager, Katelin Kutella, for helping me set up and execute my project in the greenhouse. Thank you to my friends in the Diez Lab who have been an invaluable support and thank you to the Peter O'Day Fellowship for Biological Sciences for helping me support myself through this process. And finally, but not least, thank you Sarah Erskine for guiding me through every step of my project and for being there from day 1. This would not have happened without you, Sarah, so thank you for sharing your time and support.

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Introduction

As global temperatures escalate, the biological rhythms of Earth's ecosystems are experiencing profound changes, with uncertain implications for plants. In terrestrial environments, plant populations form the foundational basis of most ecosystems, making their response to climate change a critical area of ecological research (Mora et al., 2015). Population persistence depends on the successful reproduction of individuals, which is typically measured as fecundity, the number of seeds an individual produces, but the quality of seed produced is also important. To characterize the quantity and quality of a species reproductive output, one can measure various seed functional traits such as seed size or mass. Understanding how climate-driven changes affect plant functional traits is essential for predicting shifts in plant communities and developing effective conservation strategies (Saatkamp et al., 2019).

Increased temperatures can have many effects on plants, both directly and indirectly. Warming's impact on plants can manifest in various ways, and the timing, duration, and magnitude of the warming can affect how plants respond. Warming not only alters the direct physiological processes of plants, but also interacts with other abiotic variables, making it pivotal to dissect these interactions to predict and manage the future dynamics of plant communities effectively. To quantify the effects of increased temperatures on plants, ecologists measure functional traits across time or different scenarios to better understand how plants adapt and what strategies they use to persist through unfavorable conditions.

Seeds, being produced by plant reproductive structures, are important to study as they are the crux of the next generation and are profoundly influenced by their environmental conditions (Saatkamp et al., 2019). Many different seed traits can be measured, and although seed mass is the most common, other traits such as germination speed, light requirements, and seedling

growth rates can be measured (Saatkamp et al., 2019). Several studies have assessed the effects of warming on plant size, fecundity, and phenology, but fewer have investigated these effects on the resulting seeds. Of the studies that have examined the effects of warming on seeds produced, many report contrasting results and hypothesize that the effects of warming may be strongly species- and site-specific (Zi et al., 2023), indicating the need to study various species to get accurate predictions of future populations and communities. For example, in an experimental warming study on a short-lived alpine plant, plants grown in warmer temperatures produced fewer and smaller seeds but had increased germination (Notarnicola et al., 2023a) while a meta-analysis of 61 manipulative warming studies found an overall increase in seed mass due to warming (Zi et al., 2023).

Annual plants are convenient study organisms for studying the effects of environmental conditions on seed production because they complete their entire lifecycle within one year (Fig. 1), making the relationship between environmental conditions and responses more straightforward. As an annual plant dies at the end of the summer, the majority of its resources are used to produce fruits, which contain seeds ready to be dispersed, and the temperatures experienced by an annual plant over the course of its whole lifecycle can impact the future success of these seeds (Penfield & MacGregor, 2017) suggesting that increased temperatures due to climate warming could have severe impacts on annual plant populations over time. Additionally, a literature review on the effects of the parental environment on seed germinability found that increased temperatures and drought is associated with higher germinability, but acknowledge that the literature on this topic is scattered and inconsistent, prompting further research (Fenner, 1991).

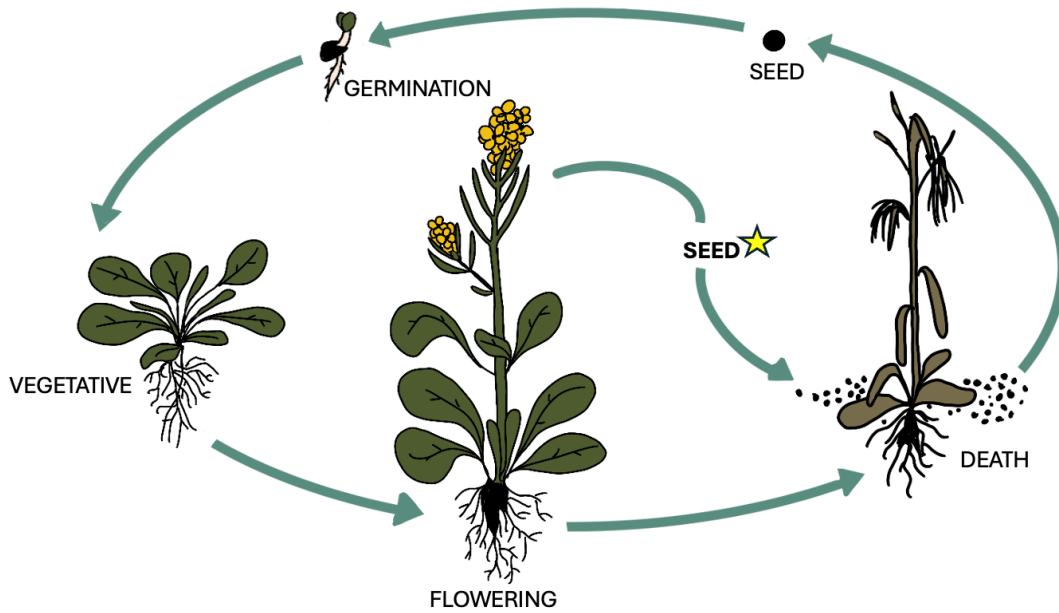


Figure 1: Diagram of the annual plant lifecycle, highlighting the production of seeds as a key component. (Diagram adapted from the Montana Department of Agriculture, 1998)

While annual plants complete their life cycles within a year, their seeds can persist across years in the soil seed bank waiting for the right combination of environmental factors to cue germination (Baskin & Baskin, 1998). For annual plants with varying reproductive successes due to unpredictable effects from the environment, some seasons or years may not provide adequate conditions for the seeds to germinate and succeed, therefore, producing seeds with a variety of germination requirements ensures at least some successful germination and establishment in the face of different environmental conditions (Tielbörger et al., 2012). With this in mind, it is important that we study not only the quantity of seeds produced but also the quality, as it can impact germination and therefore recruitment of the next generation.

In the present study, we chose to examine the effects of warming on the seed and seedling traits of two prairie annuals native to the Willamette Valley of Oregon. Before Euro-American settlement in the Pacific Northwest region of the United States, prairies and savannas made up 49% of Oregon’s Willamette Valley ecoregion, but due to alterations in fire regimes

allowing the encroachment of conifers and shifting land-use towards agriculture, less than 4% of these prairies and savannas remained in 2011 (Bachelet et al., 2011) (Fig. 2). In the past century, annual mean temperatures have increased by 0.5° - 1.5° C and annual precipitation has increased by 10% (Bachelet et al., 2011), further threatening the remaining fragments of prairies. Many restoration and conservation efforts are in place, but more research needs to be conducted on how warming will affect the seeds used in such efforts to maximize the limited resources available.

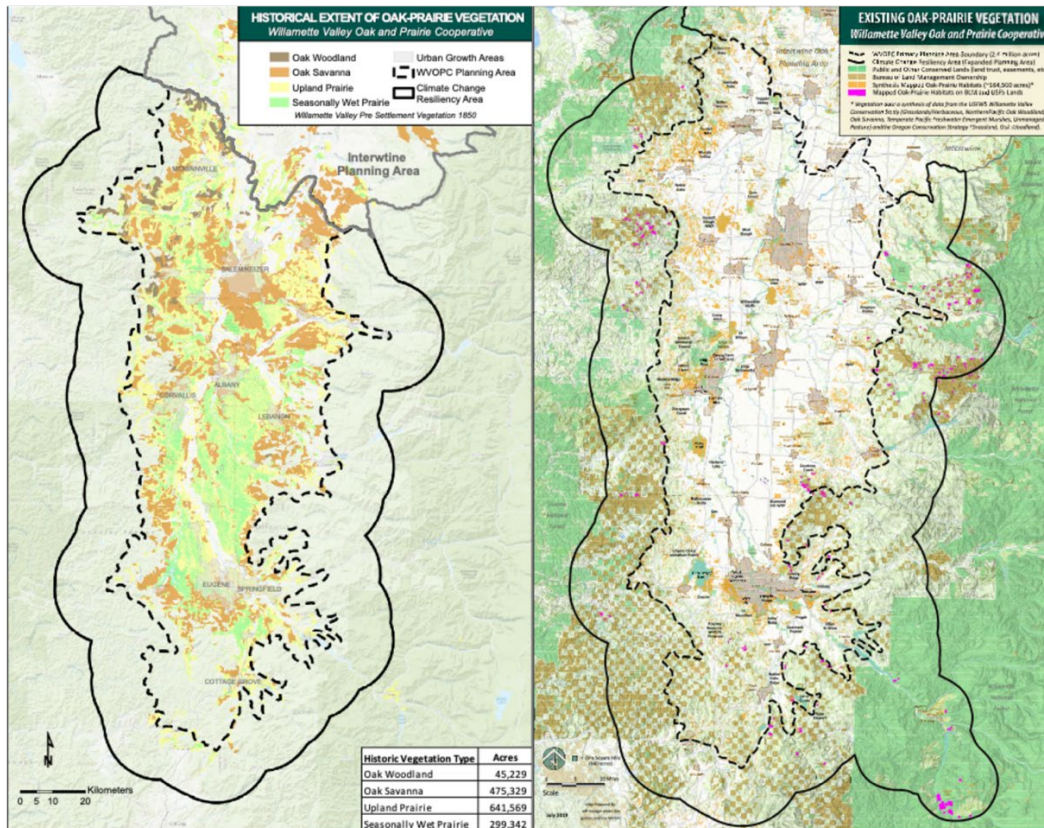


Figure 2: Historical extent (left) and existing (right) oak-prairie vegetation in the Willamette Valley (Images from the Willamette Valley Oak and Prairie Cooperative).

We investigated how climate warming will affect seed traits, germination responses, and early-life history traits across variable environments by using *Clarkia purpurea* and *Collinsia grandiflora* as a case study to answer the following questions: (1) How does warming and the

timing of fruit maturation influence the mass and number of seeds produced across variable sites? (2) How does warming on the mother plant and the timing of fruit maturation influence the germination success and speed of seeds produced across variable sites? (3) How does warming on the mother plant and the timing of fruit maturation influence the initial growth of seeds produced across variable sites?

We hypothesized that our plants under warming treatments experience greater stress due to the increased temperatures and therefore produce seeds with decreased mass while producing a greater quantity of seeds to maximize germination under different environmental conditions. We also hypothesized that the lighter seeds from warming plots have decreased germination rates and initial growth of seedlings due to smaller amounts of resources stored in the seeds. Additionally, we hypothesize that the seeds from warmed plots would grow into seedlings that allocate more initial resources toward their roots to have more access to water in the dry season, but overall, the seedlings originating from warming conditions are smaller due to less initial resources stored in the seed. We hypothesized that the strength of the seed and seedling responses would vary across sites but remain consistent across the fruit maturation season.

Methods

Study System

We conducted our field experiment near Eugene, OR, in Willamette Valley prairies, a geographical region formed by receding ice sheets in the late Pleistocene epoch and characterized by a Mediterranean climate with wet mild winters and dry summers (Russel, 2011). Willamette Valley prairies are typically dominated by low-stature perennial bunchgrasses and forbs, with small topographical differences creating a mosaic of plant communities (Vesely & Rosenberg, 2010). The area received on average 108 cm of precipitation annually from 1990 to 2020, and the mean winter temperatures were 5.85°C while the mean summer temperatures were 18.0°C.

We seeded two winter annual forbs native to Willamette Valley prairies across the eight sites: *Clarkia purpurea* (Onagraceae; Winecup clarkia) and *Collinsia grandiflora* (Plantaginaceae; Giant blue-eyed Mary). Both species germinate in the fall with *C. grandiflora* flowering from March to June and *C. purpurea* flowering from May to September. The eight sites were selected to capture significant environmental variability with sites differing in elevation, soil type, slope, and aspect. Our experiment was done at eight sites within a 2-square-km area, ranging from 284m to 495m above sea level, with slopes ranging from 4% to 18%, and aspect ranging from 138° to 268° (Fig. 3).

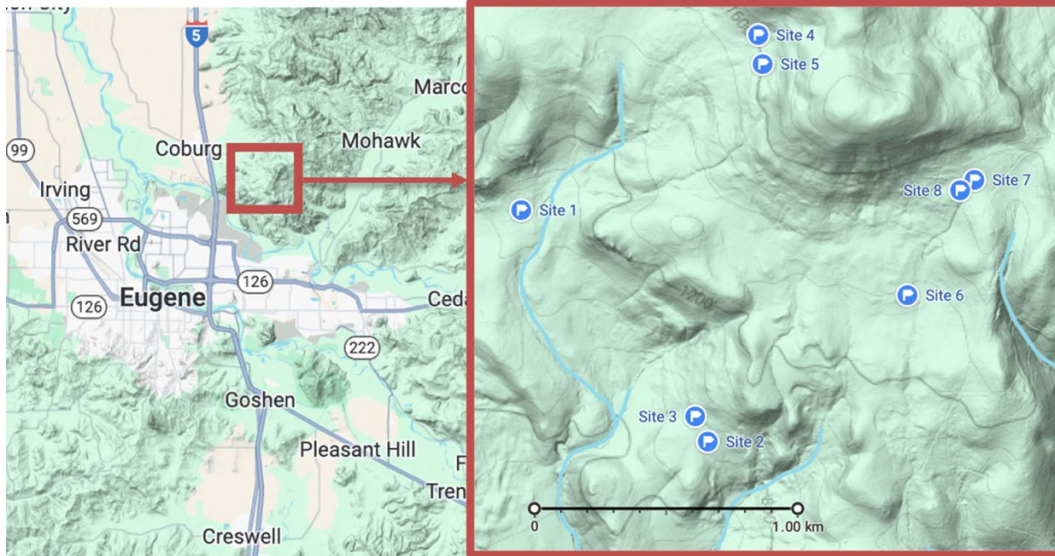


Figure 3: Location of sites within the greater Eugene area (left) and a topographic map of specific site locations (right). (Images from Google Maps)

Plots were mowed, solarized, and burned before seeding. Half of the plots were covered with open-top warming chambers modified from the International Tundra Experiment (ITEX) (Fig. 4). Plots were initially seeded on November 18th, 2022, but due to herbivore damage, sites one, two, three and six were re-seeded in January 2023. At each site, we collected up to 10 fruits per warming and ambient plot at two points during the fruiting stage. Fruit phenology will be used to denote to the timing of fruit collection and will be referred to as ‘early’ and ‘late’ phenology fruits/seeds.



Figure 4: Photo of Site 5 plots, with open-top warming chambers on the left and ambient plots to the right of the chambers. (Image from Sarah Erskine)

Seed Collection and Trait Measurements

Up to ten capsules of each species were collected from each plot twice and sealed in paper coin envelopes. Capsules, dry fruits containing seeds, were deemed ready to collect when they appeared brown and dry but had not yet split at the seams to release the seeds. This occurred in May and June for *C. grandiflora* and in June and July for *C. purpurea*. All plots, except some with low success, were collected from once when capsules first started browning, and once at the end of the fruiting season. Envelopes of fruits were stored in a lidded plastic container, at room temperature, out of direct sunlight.

After one to two weeks of storage, fruits in envelopes were opened and all the seeds were separated from other plant material (Fig. 5). For each envelope, the total number of seeds was counted and divided by the number of fruits collected to get a mean number of seeds per fruit, and the total mass of the seeds was found using a balance and divided by the number of seeds to get an estimate of mean seed mass.

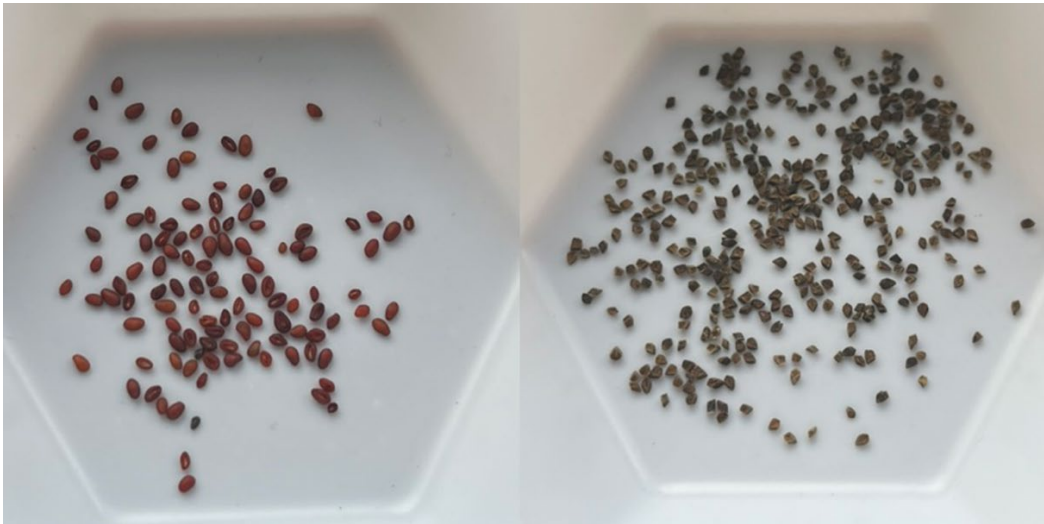


Figure 5: *Collinsia grandiflora* (left) and *Clarkia purpurea* (right) seeds.

Germination Protocol

In late October of 2023, up to 100 seeds from each envelope were placed in petri dishes lined with moistened filter paper. Dishes were organized on tables in a greenhouse (Fig. 6) at the University of Oregon in Eugene, Oregon, USA, and every two days dishes were checked for newly germinated seeds followed by a rotation of the trays. Germination, defined as the seed coat breaking with a visible (typically white) radicle beginning to emerge, was scored every two days by surveying each petri dish for new germinants and re-moistened with a spray bottle. Germinated seeds were removed from the dishes and transferred to seedling trays.

The environmental conditions of the greenhouse chamber were controlled manually by opening and closing windows to mimic similar temperature fluctuations as outside, with no

artificial heating or cooling and window kept open for the majority of the experiment. In nature, these species typically germinate in the fall, therefore the photoperiod in the greenhouse is assumed to be similar to what these species would experience in the wild. A Govee temperature and humidity sensor was placed alongside the petri dish trays to capture the temperature and humidity inside the greenhouse during the period of germination.



Figure 6: Image of petri dishes filled with seeds and ready to begin the germination protocol in the greenhouse.

Seedling Protocol

The first 20 seeds that germinated from each petri dishes were transferred to a 3.8cm x 3.8cm cell in a tray filled with nutrient-poor river sand acquired from Lane Forest Products. Only

one germinated seed was placed in each cell and trays were misted daily for 30 seconds. The seedling trays were placed on a table in the same greenhouse detailed in the previous section and rotated periodically (Fig. 7).



Figure 7: Photo of seedling trays filled with sand and new germinants in the greenhouse.

After 10-11 weeks of growth, seedlings were carefully removed from the sand and submerged in water to remove any non-plant particles attached. Cleaned seedlings were cut at the junction between the epicotyl and hypocotyl to separate the above-ground (shoots) and below-ground parts (roots) (Fig. 8). Seedlings parts were placed in either small plastic vials or small coin envelopes and set to dry in a 70°C oven for at least 24 hours. Following drying, seedling parts

were weighed on a microbalance to determine the mass ratios between the root and shoot parts as well as the seedlings' total dry masses.

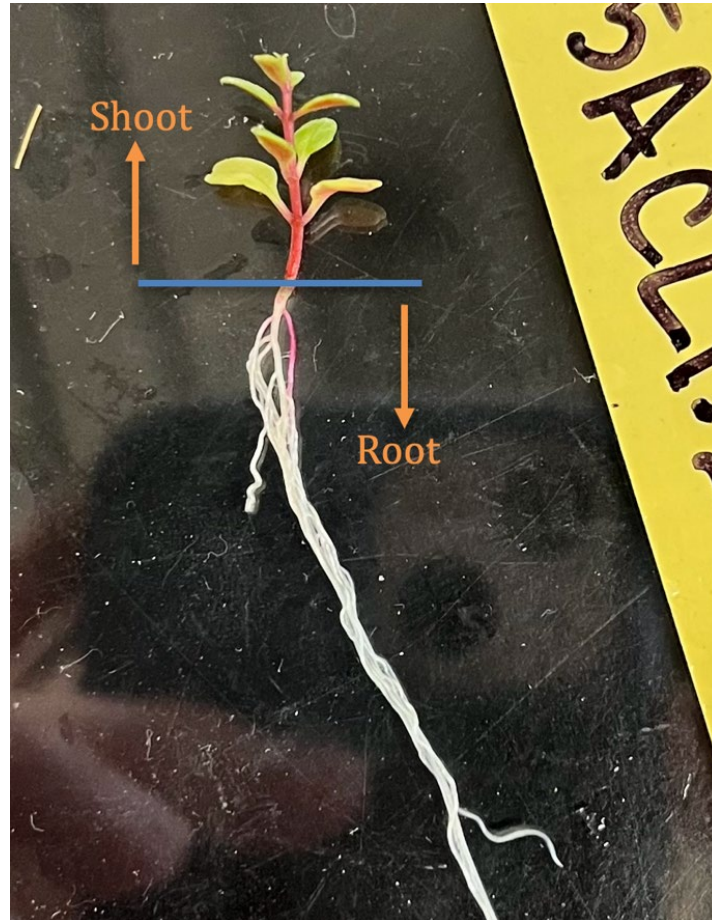


Figure 8: Photo of a *Clarkia purpurea* seedling about to be split into root and shoot parts.

Data Analysis

All statistical analyses were conducted in R v4.3.1 (R Core Team, 2024). We analyzed the seed size versus number trade-off with a linear regression model with mean seed mass as the response variable. We analyzed the overall effects and interactions of warming, time of fruit collection (fruit phenology), and site on 6 different measures: (i) mean seed mass, (ii) mean number of seeds per fruit, (iii) percent germination, (iv) time to 50% germination, (v) seedling dry root:shoot ratio, and (vi) seedling dry mass.

All measures, except (iii), were analyzed using linear regression models with site as a fixed effect and a gaussian distribution. (iii) was analyzed using a generalized linear regression model with site as a fixed effect and assuming a binomial distribution.

Results

Seed Traits

For *C. grandiflora*, fruit phenology most strongly impacted seed mass ($p < 0.0001$), with late seeds being lighter. While warming alone did not affect seed mass, its interaction with site did ($p = 0.0122$), and overall site variability affected seed mass as well ($p = 0.0002$). Fruit phenology was the only factor significantly affecting seed number in *C. grandiflora*, with fewer seeds in late-season fruits ($p = 0.0012$).

For *C. purpurea*, neither warming nor site had a standalone effect on mean seed mass, but fruit phenology did ($p < 0.0001$). Significant interactions between fruit phenology and site were also observed ($p = 0.003$). Warming, site, and fruit phenology all significantly affected the mean number of seeds per fruit ($p = 0.0002$; $p < 0.0001$; $p < 0.0001$, respectively), with a notable interaction between warming and fruit phenology ($p < 0.0001$).

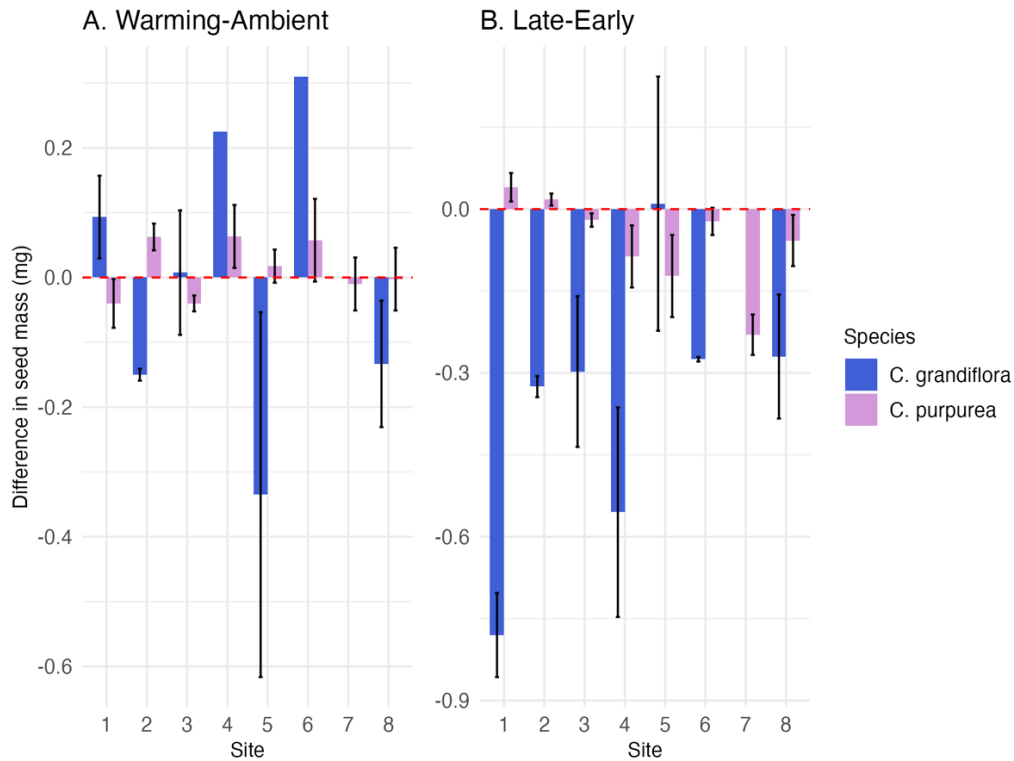


Figure 9: Mean difference in seed mass between treatments for both species across sites.

A. Bar plot showing the mean difference (\pm standard error) in seed mass between warming and ambient conditions. A positive bar indicates increased seed mass under warming and a negative bar indicates decreased seed mass under warming. B) Bar plot showing the mean difference (\pm standard error) in seed mass between late phenology and early phenology seeds. A positive bar indicates increased seed mass when collected late and a negative bar indicates decreased seed mass when collected late.

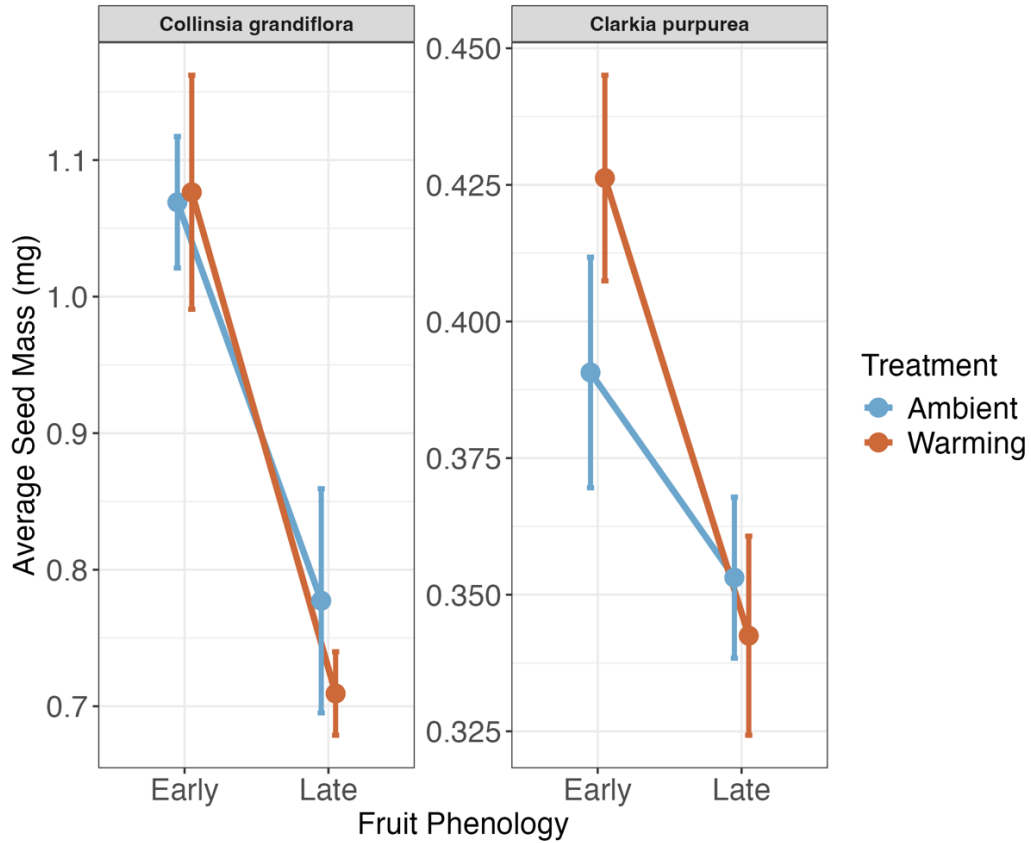


Figure 10: Interaction effect of fruit phenology and warming on seed count for both species, averaged across sites.

We found no evidence of a trade-off between seed mass and number. Instead, seed mass positively correlated with seed count for both *C. grandiflora* and *C. purpurea* ($p < 0.0001$, $R^2 = 0.22$; $p < 0.0001$, $R^2 = 0.29$, respectively). Neither warming nor fruit phenology impacted the positive correlation between seed mass and number.

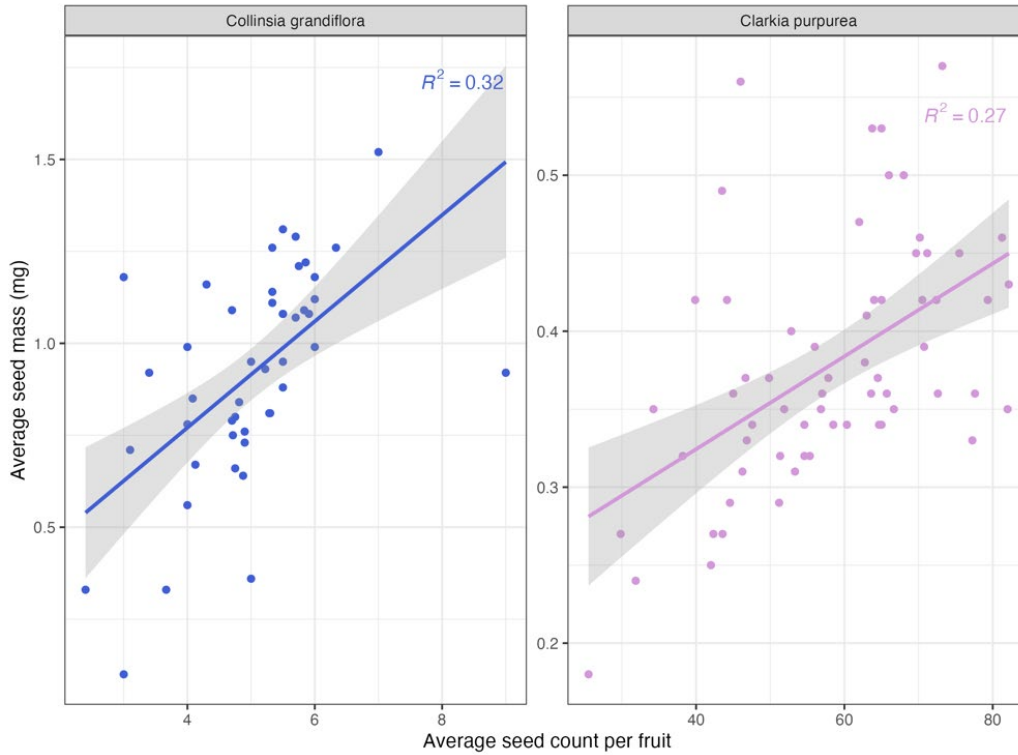


Figure 11: Relationship between mean seed mass (mg) and mean number of seeds per fruit for *Collinsia grandiflora* and *Clarkia purpurea*

Linear regression lines with 95% confidence intervals (shaded areas) are shown for each species. The coefficient of determination (R^2) values is 0.32 for *C. grandiflora* and 0.27 for *C. purpurea*.

Germination Traits

We found that *C. grandiflora* fruits collected late in the season produced seeds with increased germination success ($p < 0.0001$), with significant site variability. Conversely, *C. purpurea* showed decreased germination success from late-season fruits ($p < 0.0001$), again with significant site variability. *C. purpurea* also showed a strong interaction effect of warming and late fruit phenology, with warming lessening the negative impacts of late fruit phenology on germination success ($p < 0.001$). Both species' germination successes were not affected by warming overall.

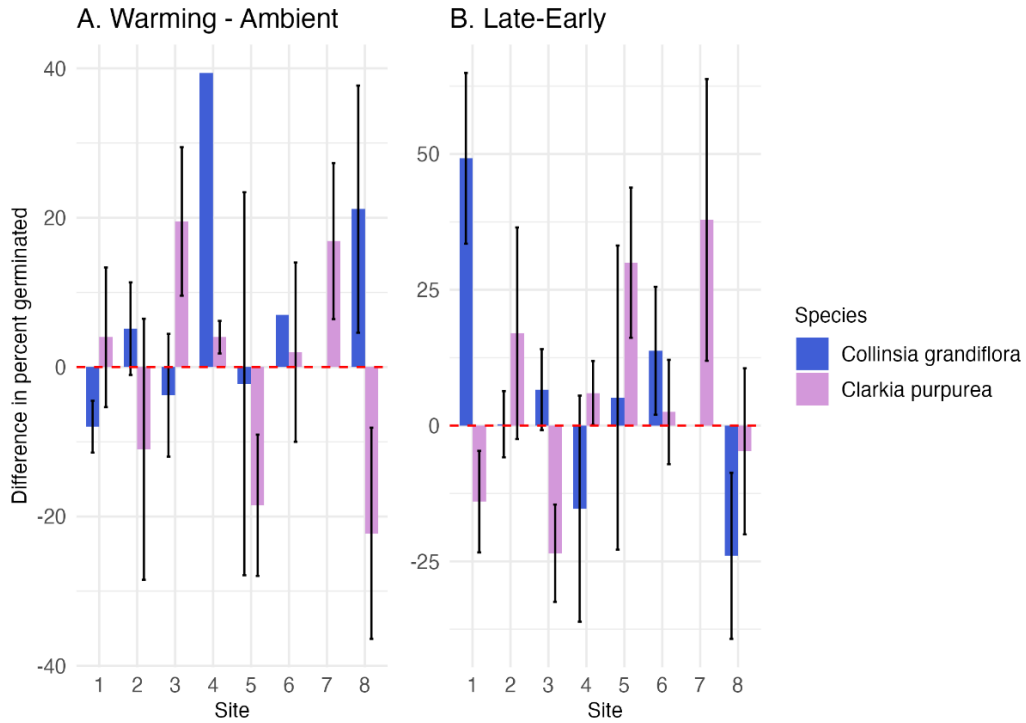


Figure 12: Difference in percent germination between treatments for both species across sites.

A. Bar plot showing the mean difference (\pm standard error) in percent germination between warming and ambient conditions. A positive bar indicates increased germination under warming and a negative bar indicates decreased germination under warming. B. Bar plot showing the mean difference (\pm standard error) in percent germination between late phenology and early phenology seeds. A positive bar indicates increased germination when collected late and a negative bar indicates decreased germination when collected late.

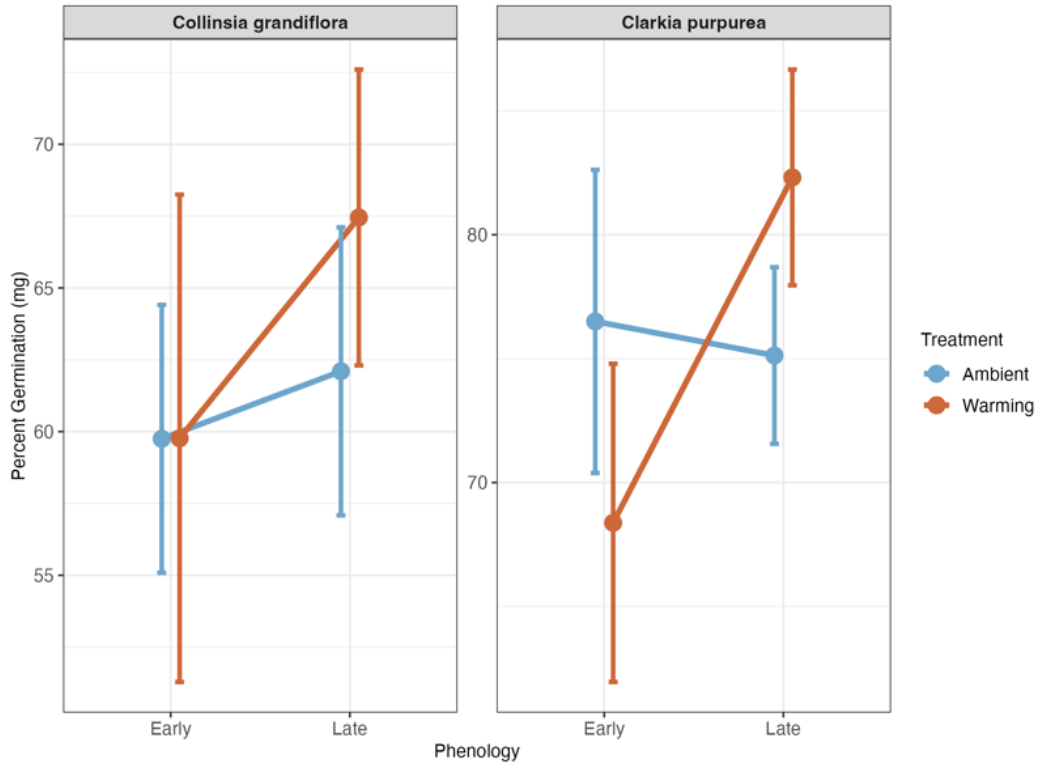


Figure 13: Interaction effect between warming and fruit phenology on percent germination for both species averaged across sites.

For *C. grandiflora*, warming overall increased the time to 50% germination (t_{50}) ($p = 0.033$), and had a strong interaction effect with site ($p < 0.001$). For *C. purpurea*, late fruit phenology overall significantly increased t_{50} ($p = 0.011$) and both warming and fruit phenology had significant interaction effects with site ($p = 0.006$, $p = 0.002$, respectively).

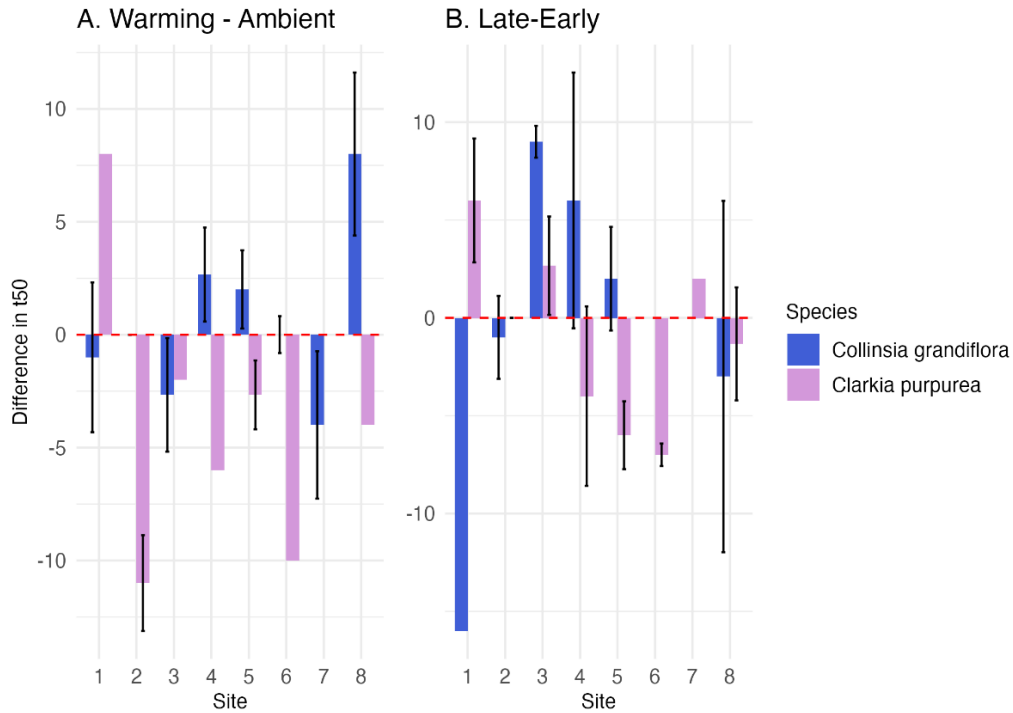


Figure 14: Difference in time to 50% germination (t50) between treatments for both species across sites.

- A. Bar plot showing the mean difference (\pm standard error) in t50 between warming and ambient conditions. A positive bar indicates increased t50 under warming and a negative bar indicates decreased t50 under warming. B. Bar plot showing the mean difference (\pm standard error) in t50 between late phenology and early phenology seeds. A positive bar indicates increased t50 when collected late and a negative bar indicates decreased t50 when collected late.

Seedling Traits

C. grandiflora seeds from warming plots grew into seedlings that had lower root:shoot ratios (RSRs) ($p = 0.001$). Additionally, late phenology seeds also grew into seedlings with lower RSRs ($p = 0.047$) and there was large variability in RSRs across sites ($p < 0.001$).

On the other hand, *C. purpurea* late phenology seeds grew into seedlings with higher RSRs ($p = 0.009$). Although we found no overall effect of warming, there was a significant interaction between warming and site ($p = 0.011$) as well as an overall site effect ($p < 0.001$).

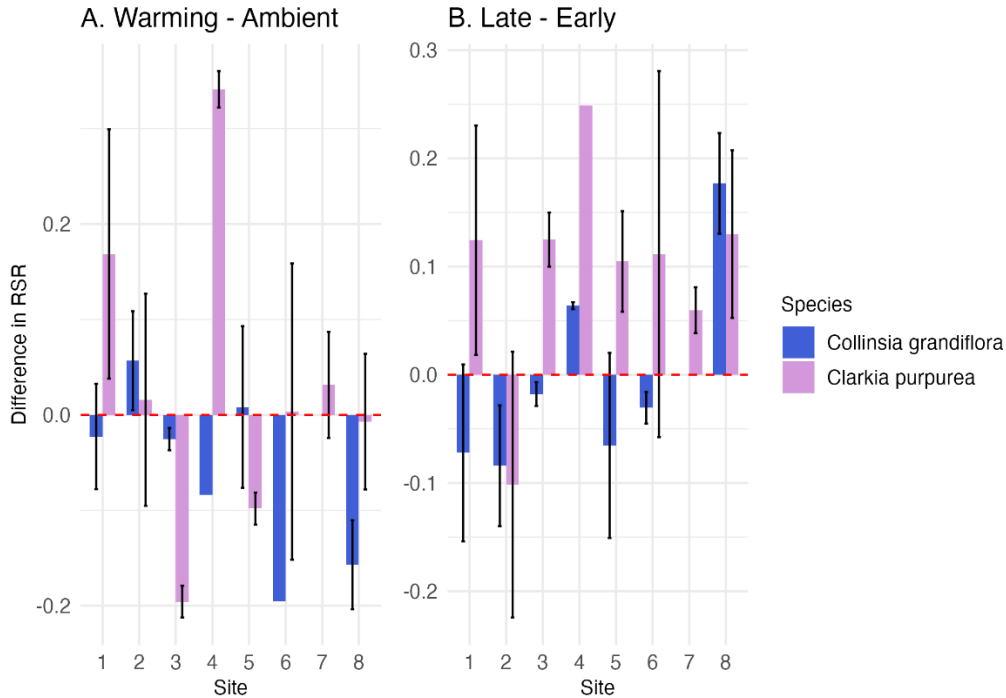


Figure 15: Difference in dry seedling root:shoot ratio (RSR) between treatments for both species across sites.

A. Bar plot showing the mean difference (\pm standard error) in seedling mass between warming and ambient conditions. A positive bar indicates increased seedling mass under warming and a negative bar indicates decreased seedling mass under warming. B. Bar plot showing the mean difference (\pm standard error) in seedling mass between late phenology and early phenology seeds. A positive bar indicates increased seedling mass when collected late and a negative bar indicates decreased seedling mass when collected late.

While the two species showed contrasting effects of phenology on RSR, late phenology seeds of *C. grandiflora* and *C. purpurea* grew into seedlings with decreased mass ($p = 0.015$, $p < 0.001$, respectively) and had significant variation across sites ($p < 0.001$). Moreover, both had strong significant interaction effects between warming and site ($p = 0.005$, $p < 0.001$, respectively), and site and fruit phenology ($p = 0.007$, $p < 0.001$, respectively).

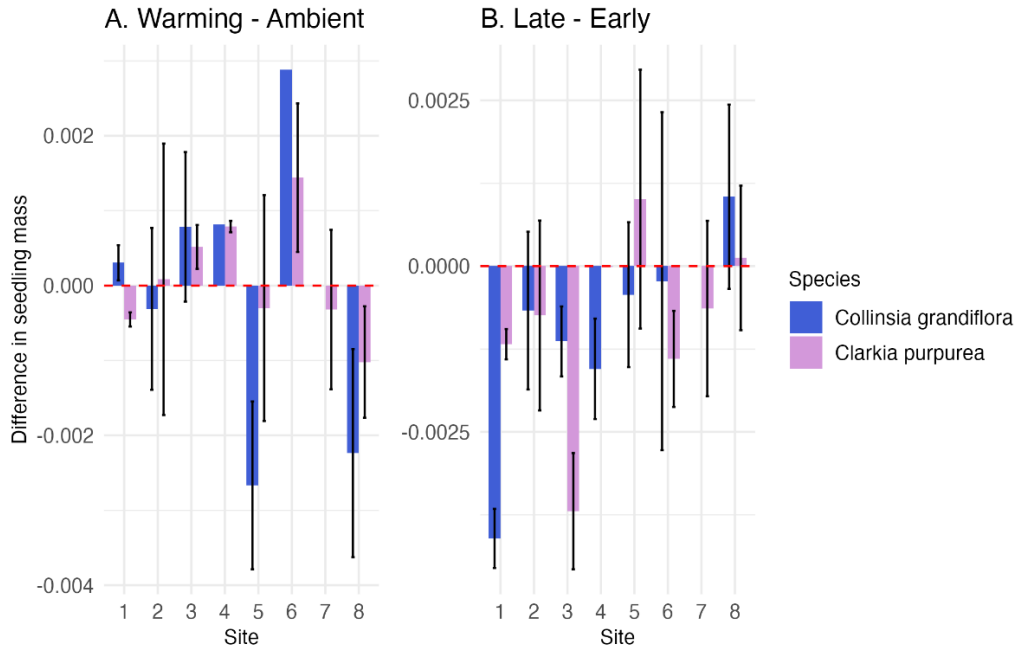


Figure 16: Difference in total dry seedling mass between treatments for both species across sites.

A. Bar plot showing the mean difference (\pm standard error) in seedling mass between warming and ambient conditions. A positive bar indicates increased seedling mass under warming and a negative bar indicates decreased seedling mass under warming. B. Bar plot showing the mean difference (\pm standard error) in seedling mass between late phenology and early phenology seeds. A positive bar indicates increased seedling mass when collected late and a negative bar indicates decreased seedling mass when collected late.

Discussion

With continued climate change, plants will be exposed to increasingly warmer average conditions which will impact plant populations, including changes in reproduction. In the present study, we used *C. grandiflora* and *C. purpurea* as a case study to better understand the effects of climate change on Willamette Valley prairie annuals by attempting to determine how warming and the timing of fruit maturation impacts seed traits, germination traits, and early growth strategies. Overall, we found that fruit phenology had the largest effect on seed and seedling traits, followed by local abiotic conditions, and with warming showing little to no direct effects. However, several significant interactions between warming, fruit phenology, and site indicate the complexity of the mechanisms involved and how warming may affect plants through indirect interactions.

Seed Traits

Although there was no overall effect of warming on seed mass in either species, the significant interaction between warming and site suggests different impacts of warming based on environmental conditions that varied across sites, like slope, elevation, and soil characteristics. For instance, a more intense incline can allow for water to run-off more quickly, resulting in decreased water availability (Owuor et al., 2016), thus, it is possible that more inclined sites result in drier conditions which impacts seed mass independently of warming. For *C. grandiflora*, the sites with the lowest incline (1 & 6) showed an increase in seed mass under warming while the most inclined site 5 showed a decrease concurrent with the water availability hypothesis. However, this is only seen in *C. grandiflora*, and we would expect both species to be impacted similarly by less water availability, which is not the case for *C. purpurea*, complicating the interpretations of the results.

Our lack of an overall response to warming on seed mass diverges from the results from Zi et. al's meta-analysis (2023), where the authors found an overall increase in seed mass under warming for the studies analyzed, as well as Notarnicola et al's 2023 warming experiment where the authors found a decrease in seed mass under warming. This suggests there is no consensus on how seed mass will be impacted by warming for plants as a whole, and researching the responses of different phylogenetic groups to warming may be needed to better understand the overall patterns of if and how populations will shift.

Despite no overall effects of warming on seed mass, we did find an effect of warming on the mean number of seeds per fruit for one of the species. *C. purpurea* plants under warming producing fruits with approximately 15 more seeds than in ambient conditions. This contradicts the results from other studies examining the effects of warming on seed count (Notarnicola et al., 2023a; Zi et al., 2023), again, suggesting that there are species-specific responses to warming.

Fruit phenology proved to have the largest effects on seed mass and count for both species and had significant interactions with both site and warming for *C. purpurea*. Our results showed an overall decrease in seed mass and count when fruits were collected late in the season for both species, with warming exacerbating the decline in size and number from early to late fruits. Plants rely on the flow of water through their organs to draw up nutrients from the soil and grow reproductive structures, and as the season progresses more into the summer, less precipitation occurs and therefore less water is available to the plants. With less water, plants are unable to allocate as much resources into making seeds (Recart & Campbell, 2021), which is likely why we saw a decrease in seed mass and count at the end of the season.

Since annual plants do not need to survive until the next year, all available resources can go towards reproduction once the conditions are right, therefore annual plants are encouraged to

produce their seeds as soon as they are able to, as if they wait too long, environmental conditions could become unfavorable again. Thus, in the present study, the slightly warmer conditions in the warming treatment combined with increased precipitation compared to when these plants would typically experience these temperatures, could have provided the plants more ideal conditions in the beginning of the season, leading to the further increased seed mass and count in early fruits under warming. By the end of the season, the plants are no longer experiencing ideal conditions to produce seeds, leading to decreased mass (Roach, 1986). Additionally, the effects of warming are often confounded by reductions in soil moisture which is typical for warming experiments (Rustad et al., 2001) therefore, in the present study, the further decreased water availability under warming conditions may have led to the marginally lower seed mass and count later in the season.

We found no evidence of a seed size versus number trade-off for either of the species and contrarily found a strong positive correlation between the seed mass and size. Although the Smith & Fretwell (1974) model of seed size/number trade-off has been useful in understanding the theoretical mechanisms involved in reproductive output, an often overlooked assumption of the model is that the overall parental investment in offspring remains constant across the population (Notarnicola et al., 2023b). Several studies suggest that the trade-off is not realistic for plants with limited resources (Notarnicola et al., 2023b; Paul-Victor & Turnbull, 2009), and considering the present species are prairie annuals that require the ability to persist through periods of low-rainfall and must compete for resources, the strong correlation observed between seed mass and number is likely an indication that the plants were growing with limited resources, regardless of treatment conditions.

Germination Responses

Similarly to the seed mass and count responses, warming had no overall effect on germination percentage but there were different directions of responses at the different sites, likely contributing to the overall negligible effect. Again, the lowest inclined sites show a strong response in one direction (increased germination under warming) while site 5, with the highest incline, shows a strong response in the other direction (decreased germination under warming).

Interestingly, despite both species having decreased seed mass for late phenology fruits, *C. grandiflora* late phenology seeds exhibited higher germination success while *C. purpurea* late phenology seeds exhibited lower germination success, with both having large variations across sites. It is currently uncertain what prompts this difference between the two species, but it is plausible that they have different germination requirements, and some aspect of late phenology seeds is favorable for the germination of one while unfavorable for the germination of the other species, compared to seeds produced earlier in the season.

C. purpurea also showed a strong interaction effect of warming and late fruit phenology, with warming lessening the negative impacts of late fruit phenology on germination rates. Therefore, despite having reductions in germination potential at the end of the season, warming diminishes this trend and allows for late phenology seeds to have higher germination success, but overall these responses varied in strength across the sites. This highlights the species-specific nature of these response variables and how even small environmental changes can make a big difference in the success of the next generation.

C. grandiflora seeds from warming plots took longer to reach 50% germination than those from ambient plots at almost all sites. Although it is unclear how warming impacts the germination speed of seeds in this study, other studies on seed functional traits have posited that

earlier germinating seeds develop into larger plants, which can have direct consequences on the plant's fecundity and ability to compete with other individuals (Roach, 1986; Ross & Harper, 1972). Therefore, the slower germination speed of seeds produced under warming could be of concern for these species, as they must compete with both native and invasive species like grasses. Thus, more research needs to be conducted to understand the mechanism behind warming's impact on germination speed and if the slower germination under warmer conditions poses a large problem, restoration and conservation practitioner may want to seed prairies with seeds grown in cooler conditions for optimal success. `

While no overall effect of warming was found to be significant for *C. purpurea*, warming appeared to have different effects based on site, with site 2, 5, and 8 having faster germination speeds under warming and sites 3 and 7 having slower germination speeds under warming. This again illustrates how environmental context is important when attempting to predict population outcomes and further research needs to be done on how specific environmental conditions during seed production of different species can impact germination. Additionally, *C. purpurea* late phenology seeds took longer to germinate. We also found a strong interaction effect between warming and fruit phenology for *C. purpurea*, with the differences between early and late fruit phenology being exaggerated by warming. Again, the mechanisms behind these responses are unclear, but as successful germination is key in small annual plant populations, we may need use patterns observed in studies like these to inform best practices for restoration and conservation.

Seedling Responses

Despite no overall effects of warming on the root:shoot ratios (RSRs) of *C. purpurea* seedlings, *C. grandiflora* was found to have a lower RSRs under warming compared to ambient plots. This translates to seedlings having allocated more resources to their shoots and/or less

resources to their roots, which can indicate more favorable conditions for the plants (Harris, 1992; Lopez et al., 2023). Since all seedlings were grown under the same conditions regardless of parental treatments, there is likely an aspect of seeds that pre-determines initial growing strategies. In addition, RSRs for both species had a large variation across sites, once again indicating a strong environmental component.

Both species were significantly affected by fruit phenology but interestingly, late phenology seeds of *C. grandiflora* grew into seedlings with lower RSRs (more relative allocation towards the shoots), yet the opposite was seen in *C. purpurea*, with late fruits producing seedlings with higher RSRs (more relative allocation towards the roots), showing how these species have different early-growth strategies for seeds produced late in the season. Additionally, when examining the correlation between seed mass and seedling RSR, we also noticed different trends between the two species. For *C. grandiflora*, there was a slight increase in RSR as the mean seed mass increased, while for *C. purpurea*, there was a slight decrease in RSR with increasing seed mass. In the present study it is unclear if the different RSR response was a result of the fruit phenology itself, or a downstream effect of the late fruit having decreased mass, but it is evident that seeds are impacted by the timing of their maturation and could have effects on plant populations over time.

These species- and site-specific responses may be crucial in understanding how these plants will do in the future, as the first months of life are incredibly important for annual plants (Larson et al., 2020). Winter annuals, including the two focal species of this study, germinate in the fall and remain in a vegetative state throughout the winter before flowering and fruiting in spring and summer, thus the early growth of seedlings is crucial in establishing a solid

population. More research needs to be done to better interpret how these early life growth strategies impact the overall success of these populations.

Despite the different results of the RSRs observed in the two species, total dry mass of the late phenology seedling was decreased for both species. We hypothesized that smaller seeds would develop into smaller seedlings, since no extra nutrients were supplied to the seedlings and smaller seeds have less resources stored, but there was no correlation observed between seed size and total seedling mass, meaning there must be some other mechanism for conditions during seed development to impact the initial growth of the seedling.

Additionally, we found a significant overall effect of site on the total dry mass of seedlings, indicating that even small changes in environmental conditions can impact the future success of seedlings produced.

Limitations and Future Directions

Our study, although providing valuable information on these species, is relatively limited in terms of its scope. With only two species within a 2-km² area, our results are not widely generalizable, but considering the small body of literature on these species, our findings can at least inform further research and provide some information to practitioners about these specific species. Additionally, some caveats may have arisen in our results due to some minor limitations of the experimental set-up and methodology. Open-top warming chambers, although widely used in ecology, have been criticized for having confounding factors, as increased temperatures often lead to drier conditions, and it is hard to separate what are the warming effects versus drying effects. However, warmer temperatures across the globe have also correlated with lower soil moisture (Drobinski et al., 2020), therefore even though we cannot distinguish between the two possible effects, the increased temperature and possible decreased soil moisture could be

representative of warming conditions in the future. Moreover, the effects of the physical presence of plastic walls around the plot are unaccounted for and could also present a confounding factor in our warming results. With decreased air movement and a physical barrier to some horizontal growth, plants may have experienced different environmental conditions other than temperature.

With future related experiments, expanding the environmental gradient over a large spatial area would provide us with better information on these species under different conditions. Increasing the number of species studied would also provide us with more information on prairie annual populations as a whole. In addition, to better understand the mechanisms behind the results we found, a greenhouse or common garden experiment would be useful in order to control more environmental variables. With the use of temperature and humidity-controlled growth chambers, we could repeat the experiment and have stronger confidence in the resulting effects of warming on seed and seedling traits. However, the results may be confounded by the artificial conditions of such chamber or greenhouse, promoting the need to combine field and greenhouse data to have the more accurate interpretations.

To expand the study further, examining the long-term effects of warming across multiple generations would allow us to better predict population dynamics in the future. This could be done by continuing to grow the seedlings until maturity and collecting the new seeds for further testing and repetition of the experiment. With data across multiple years, we could improve our knowledge on how these plants may adapt over time with a changing climate.

Conclusion

Overall, we found varying effects of warming on these species' seeds, with the timing of fruit collection having been found to be the strongest predictor of seed mass, seed count, germination success, and seedling traits. We found that seeds from fruits of both species collected late in the season were smaller, fewer, and produced smaller seedlings. Whether these effects are independent or inter-related, these annual plants tended to produce their best seeds at the beginning of the fruiting season when they have the most water availability and resources, resulting in fewer seeds of lesser quality later in the season. Increased warming has shifted the phenology of many plant species and most notably has resulted in earlier flowering due to appropriate conditions for flowering being reached earlier in the year (Parmesan & Yohe, 2003). This, in combination with warming and drying conditions further limiting resources as the season continues, may significantly impact the overall populations in multiple ways and requires further research.

Furthermore, the significant variation in responses across sites shows the extremely nuanced ways in which warming can impact plant populations. Even within a 2-km gradient, opposing responses to warming and fruit phenology were found, highlighting the need to further research how plants in different abiotic conditions respond to warming. Concluding this study, we call for more incorporation of ecological variability into climate change models so that we can better inform restoration and conservation strategies to ensure the persistence of vulnerable prairie annual populations.

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