Title

Plant Reproduction Under A Changing Climate: Effects Of Drought Stress On The Pollen Quality And Quantity Of The Insect-pollinated Species Phacelia Campanularia

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PLANT REPRODUCTION UNDER A CHANGING CLIMATE: EFFECTS OF DROUGHT STRESS ON THE POLLEN QUALITY AND QUANTITY OF THE INSECT-POLLINATED SPECIES *PHACELIA CAMPANULARIA*

By

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A capstone project submitted for Graduation with University Honors

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University Honors
University of California, Riverside

APPROVED

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ABSTRACT

Climate change-induced drought stress impacts species and their mutualistic interactions. Drought can affect plant-pollinator mutualisms by altering plant physiology and floral traits, such as pollen production. Importantly, pollen development is required for pollination and, ultimately, seed production in most flowering plants. Hence, understanding the effects of drought on pollinator-mediated plant reproduction is necessary to predict ecosystem-level responses to drought. Despite the integral role of pollination in plant reproduction, researchers have primarily studied the effects of drought on floral inflorescence and plant morphological traits, and few studies have focused on the impacts on pollen. Research suggests that coastal sage scrub habitats are adapted to climatic extremes. Anthropogenic warming, however, may significantly affect Southern California's native ecosystems, and the communities in that region may have varying responses to severe drought and long-term climate change. To understand how plants will respond to shifts in climate, we examined the quality and quantity of pollen under water stress using *Phacelia campanularia*, an annual forb. After subjecting plants to drought and ample watering treatments, we weighed and stained pollen grains to determine pollen mass and viability. We found that water deficit leads to decreased pollen mass and reduced pollen viability which may result in a lack of viable male gametes required for plant reproduction. Further, we conclude that reduced pollen quality and quantity may alter resource exchange between plants and pollinators, resulting in cascading effects on plant and pollinator reproductive success and plant-pollinator interactions.

Keywords: drought, pollination, flowering plants, pollen mass, pollen viability, pollinators, native plants

ACKNOWLEDGEMENTS

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INTRODUCTION

Plants and their pollinators form mutualistic relationships that are necessary for ecosystem function. As well as maintaining wild plant biodiversity (Ollerton et al. 2011), both managed and wild pollinators contribute to crop pollination (Garibaldi et al. 2013) and together are responsible for approximately 75% of global food production (Klein et al. 2007). Pollination occurs when pollen is transferred from the anthers, the male part of a flower, to the stigma, the female part of a flower. Pollination is an essential ecosystem service of both economic and ecological value. The economic value of pollinator-dependent crops is an estimated $50 billion per year in the United States (Bauer & Sue Wing, 2016). In most terrestrial ecosystems, pollination provides benefits beyond agriculture, including food, habitat, and resources for many animal species. Further, pollination leads to some outcrossing and thus, promotes genetic diversity in plant populations, allowing species to adapt to changing environments (Olleteron et al. 2011). Thus, mutualistic interactions such as pollination provide indispensable ecosystem functions that support plant and pollinator biodiversity and maintenance (Katumo et al., 2022).

Nearly ninety percent of flowering plants depend, in part, on animal pollination for reproduction and the maintenance of their populations (Ollerton et al., 2011). Further, about 25% of all birds and mammals consume fruits and seeds that require insect pollination (Hopwood). Many animals act as pollinators, including insects such as bees, flies, wasps, butterflies, and moths. As wild plants provide a wide range of resources such as food and habitat for many mammals, birds, and other taxa, pollinators play a critical role in the stability and functioning of terrestrial food webs by pollinating plants (*Pollinators, Pollination, and Food Production* 2016). Bees also play an important role in agricultural pollination: over ninety percent of agricultural crops are pollinated by bees (Patel et al., 2020). Although a few bee species are managed, over 20,077 bee species are wild and unmanaged (*Pollinators, Pollination, and Food Production* 2016). However, a variety of factors influence this mutualism by altering plant-pollinator interactions.

Floral signals and rewards influence plant reproduction by influencing the behavior of pollinators (van der Kooi et al., 2018, Christopher et al., 2019). Floral traits, such as floral size, color, shape, nectar volume, and fragrance, impact pollinator attraction and influence the quality and frequency of pollinator visits (van der Kooi et al., 2018). Flower shape, for instance, influences the orientation of a pollinator's body as it forages on flowers, influencing the quantity of pollen picked up by the pollinator (Minnaar et al., 2019). Kulbaba and Worley (2012) found that larger flowers attracted more pollinators and had higher reproductive success. Further, Kulbaba et al. (2012) found greater reproductive success in plants with narrower corolla tubes that allow extensive contact with the proboscis of hawkmoth pollinators. Changes to plant characteristics such as vegetative growth, floral display, and floral resources like pollen and nectar may impact bee foraging behaviors and influence plant and pollinator reproductive success (Fig 1).

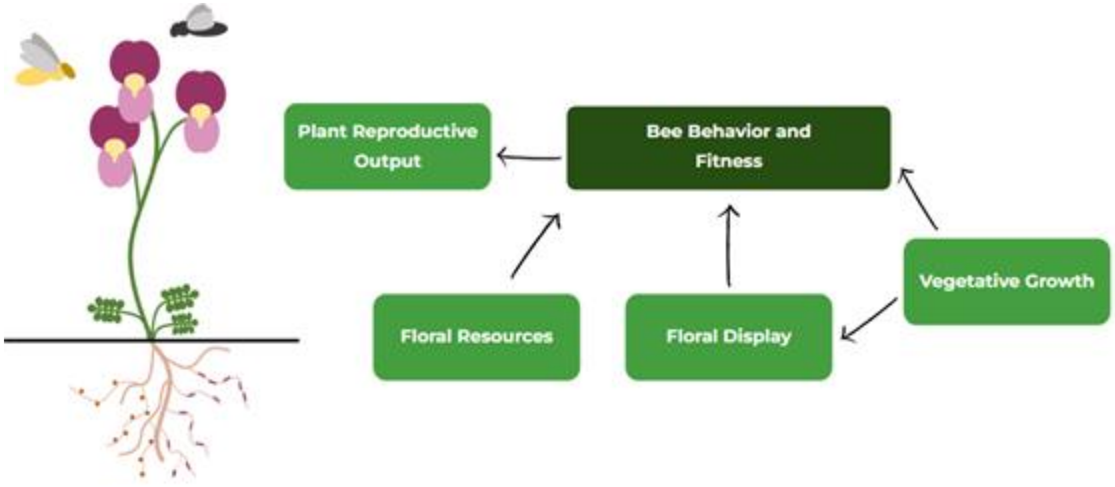

Figure 1: Factors influencing pollination likelihood that may impact plant reproductive output and pollinator fitness. Examples of vegetative growth include plant biomass and the number of flowering stems; floral display: number of flowers and flower size; floral resources: pollen mass,

nectar volume, and nutrients; bee behavior and fitness: floral preferences, foraging energetics, visitation rates, reproductive output, development, and lifespan; plant reproductive output: number of fruits and seeds and seed mass (Modified from Keeler et al., 2021).

As a floral resource, pollen is not only critical to the reproduction of some and outcrossing of all plant species but also provides resources for pollinators such as wild bees and avian species (Russell et al., 2015). Pollinators visit flowers to collect or feed on nectar, oils, perfumes, and resins (*Pollinators, Pollination, and Food Production* 2016). Most importantly, they consume and carry pollen. Pollinating insects travel from plant to plant, allowing the transfer of male gametes necessary for reproduction in flowering plants. However, abiotic factors can directly affect plant-pollinator mutualisms by modifying the availability of mutualistic partners and the traits that plants use to attract and reward pollinators (Keeler et al., 2021).

Anthropogenic global climate change is impacting plant-pollinator interactions by altering abiotic regimes, such as rates of precipitation. These alterations have led to unprecedented drought in California (Cook et al., 2018). Further, models predict that climate change-driven drought will increase in severity over time (Cook et al., 2018). Moreover, an area is more likely to experience drought when precipitation deficits co-occur with warmer temperatures (Diffenbaugh et al., 2015). Climate change has increased the probability of warm and dry conditions co-occurring (Diffenbaugh et al., 2015). Unlike animals, plants are sessile and must endure the various biotic and abiotic stressors present in their environments. Drought can limit the productivity of plants by decreasing photosynthesis, limiting nutrient uptake, and causing premature senescence in plants (Imadi et al., 2016). Studies have shown that drought stress reduces floral abundance, flower size, and nectar volume, but little is known about the effects of drought on pollen quality and quantity (Rering et al., 2020).

While many aspects of plant growth and reproduction can be impacted by drought, we focus here on the impacts of drought on plant pollen production in terms of both pollen quality and quantity. Floral resources like pollen can be modified by drought, causing shifts in the geographic distribution of plants and their pollinators, or by creating spatial and temporal mismatches in plant-pollinator interactions (Byers, 2017). A temporary lack of water at a juvenile stage can inhibit plant development, but it may not affect plant biomass if plants are provided with more water at later stages of growth. However, if plants face water stress during the reproductive stage, impacts on their reproduction and growth may be irreversible (Saini & Lalonde, 1997). Anther and pollen developmental stages can be particularly susceptible to drought (Guo et al., 2016). Pollen grains, formed inside the anther, have high water content (approximately 60% by weight). Thus, parent plants that experience stressful environmental conditions may produce fewer and lower-quality offspring (Firon et al., 2012). Declines in seed set, attributed to increased pollen sterility, were found to affect wheat, rice, maize, and sorghum (Bheemanahalli et al., 2022). Several studies reported that water stress around anthesis induced decreased pollen viability, resulting in crop yield loss (Bheemanahalli et al., 2021; Liu et al., 2022; Wang et al., 2021). Nevertheless, the effect of drought on pollen mass and viability remains understudied in non-agricultural systems.

Recart et al. (2019) investigated the effects of drought and pollen performance on seed production in *Phacelia parryi* (Recart et al., 2019). On average, *P. parryi* produce forty to ninety seeds per flower (Recart et al., 2019). Although *P. parryi* is self-compatible, *P. parryi* produced more seeds when insect-pollinated than when hand-pollinated (Recart et al., 2019). In this experiment, Recart et al. (2019) subjected plants to ample and drought watering treatments. Then, pollen load sizes, seed production, and seed mass were determined for each plant. Their results showed that seed production was 21% higher in non-droughted plants compared to droughtstressed plants. Further, they found that seed production increased with greater pollen deposition on stigmas (Recart et al., 2019). This pollen-seed production relationship indicates that viable pollen can strongly influence plant reproductive success.

The effects of drought on pollen are likely to impact pollinators as well as plant fitness. Research suggests that the indirect effects of water stress on pollinators (e.g., reduced floral rewards or signals due to drought) are more severe than direct effects on pollinators (Descamps et al., 2021). Given that many plants and pollinators have evolved to have a mutualistic dependency, drought may affect the reproductive success of both plants and pollinators (Descamps et al., 2021).

To determine the effects of drought on plant reproduction, both pollen quality and quantity must be assessed. Pollen quality and quantity can be used to assess plant fertility, monitor pollen viability during storage, predict seed set, and understand pollen and stigma interactions (Sulusoglu et. al, 2014). Since pollen must be viable at the time of pollination for seed (or fruit) production, pollen viability is important in agriculture and for plant breeders (Sulusoglu et. al, 2014). Additionally, viable pollen is necessary for plant fitness, dispersal, and survival (Impe et al., 2020). A wide range of methods for determining the quality of pollen has been developed to better understand crop breeding, pollen storage, pollen genetics, and pollen fertility (Shivanna & Rangaswamy, 1992). Pollen quality is determined based on pollen tube growth and intact central cytoplasm, the source of nuclei responsible for fertilization. (Ottaviano & Mulcahy, 1989). Thus, in vitro pollen viability and germination tests are used to measure pollen performance. Similarly, pollen quality influences pollen germination and tube growth rates (Hildesheim et al., 2019). Although pollen loads that are too large may lead to clogged stigmas and lowered seed production, on average, seed quality and quantity increase with larger conspecific pollen loads (Hildesheim et al., 2019). Thus, increases in both pollen quality and quantity may lead to increases in plant reproductive fitness.

In this study, we explored the impact of drought on pollen quality and quantity in a Southern California forb. Using a greenhouse experiment, we compared plant reproductive outcomes under both ample and drought watering conditions. We asked how drought impacts not only the mass of pollen produced but also its viability. Our examination of these questions may lead to future research investigating whether the effects of climate change-driven drought on plant-pollinator interactions have the potential to affect plants and pollinators at the community level. Understanding the underlying effects of environmental stresses at the community level can allow for further research regarding the conservation of native plants and pollinating insect communities.

MATERIALS AND METHODS

Study site

In this experiment, study plants were grown in greenhouse 7D at the University of California, Riverside (UCR; 33.97294402507459, -117.324078796202). The ambient temperature was set between 71-82°F to represent approximate spring temperatures of a Mediterranean climate, specifically that of Riverside, California. Before the initiation of the experiment, each potted plant was hooked up to an irrigation system, which dispensed 118 mL in non-droughted plants and 17 mL in droughted plants every two days at 6:00 AM.

Focal plant species

We used the annual spring-blooming plant, *Phacelia campanularia* (Boraginaceae), in this study. *Phacelia campanularia* is endemic to California and occurs primarily in coastal sage scrub and chaparral communities (California Native Plant Society). This plant species is an ideal candidate for this study because it is relatively drought tolerant and because recent work at UCR by Annika Rose-Person et al. (unpublished) has shown that drought impacts other floral traits, such as nectar volume and sugar concentration, of *P. campanularia* (Petanidou, 2003; Rose-Person et al., unpub.).

We purchased seeds from Theodore Payne Foundation, a local native plant nursery. In January 2023, we planted 720 seeds across 170 peat pellets at a seeding rate of four seeds per pellet. After two weeks, 170 seedlings were picked and transferred to 1.9-L pots containing UC soil mix 3 (plaster sand, peat moss, and nutrients). For six weeks, plants were checked every three days and received water *ad libitum*.

Experimental design

Approximately six weeks after planting and two weeks before plants began to flower, we divided the plants into two groups: a non-droughted control group to be given ample water and a droughted group to be grown under drought watering conditions. Plants under drought conditions were given 17 mL of water when their soil moisture reached below 5% volumetric water content (VWC), and plants under ample water conditions were given 118 mL of water when their soil reached below 15% VWC on average. This specific treatment reflects the ample and drought watering conditions used in Rose-Person et al. (unpublished). A sample size of 170 plants was used, with 75 plants in each treatment.

Quantifying pollen quantity

To measure pollen mass, we developed a method to measure pollen mass per flower. In early spring (April 2023), we collected *P. campanularia* flowers at the balloon stage (Fig 2). One flower was collected per flowering plant per treatment. Each flower bud was stored on moistened filter paper in a Petri dish overnight. The next day, dehisced anthers from flowers that underwent anthesis were collected and placed in a 1.5-mL pre-weighed microcentrifuge tube (Fig 2). After adding 0.75 mL of DI water, anthers were shaken in a centrifuge. The anthers, filaments, and excess water were discarded, and tubes containing the pollen pellet were left open and placed in a closed container with silica gel packets for drying. Finally, tubes with dried pollen were weighed using an analytical scale. This process was repeated for a total of 150 flowers, and 75 flowers per treatment.

To calculate the total quantity of pollen produced by each plant, we also counted the number of flowers produced by plants. We counted the number of flowers on a subset of 20 plants per watering treatment 12 weeks after applying the drought treatment.

Figure 2: a) Flower at the balloon stage (collected when petals are uniformly dark purple and the top of the bud is flat) (left), b) post-anthesis flower with un-dehisced anthers (middle), c) post-anthesis flower with dehisced anthers (dehisced overnight to this stage) (right) (*Google Images*).

Quantifying pollen quality

The iodine staining method is widely used to determine pollen viability. In this method, pollen grains are stained dark if nuclear materials in the pollen grain are intact and if pollen grains contain starch, which signifies a functional cytoplasm (Sulusoglu et. al, 2014). The advantage of this method is that it is cost-effective, allows for easy identification of pollen viability, and is useful for screening many samples.

Pollen grains collected from dehisced anthers were spread onto a microscope slide. Then, one drop of laboratory-grade 0.5% iodine potassium iodide solution was placed over the pollen. Using a compound microscope, we counted at least 100 grains to quantify the percent mean staining value. Percent staining values were determined by dividing the number of darkly stained pollen grains by the total number of pollen grains counted and expressed as a percent. A stained pollen grain showed developed cytoplasmic and nuclear materials (Yeamans et al., 2014). Grains darkly stained were considered viable, whereas lightly stained or unstained grains were unviable. Further, pollen grains that were non-spherical or ovular were sterile (Yeamans et al., 2014). (Fig 5). Black-stained pollen was considered viable.

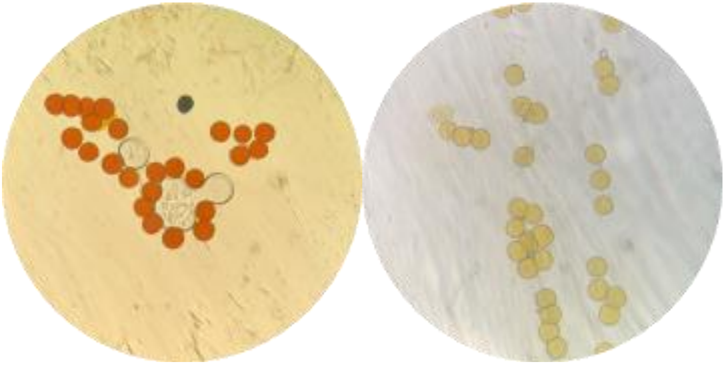

Figure 3: a) Viable pollen grains in dark orange and a single black pollen grain, which is also viable (left); b) Non-viable grains in yellow, indicating non-functional cytoplasms (right).

Statistical analyses

All analyses were performed in R version 2023.03.0+386 (R Core Team 2023). We first checked the normality of our data for pollen mass by watering treatment using a Shapiro-Wilks test. The p-values for drought and ample watering treatments were 2.165E-08 and 0.3026, respectively. Assuming non-normal distribution, we performed a Wilcoxon signed-rank test. Similarly, the Shapiro-Wilks test for the floral abundance by watering treatment and pollen viability by watering treatment data sets gave p-values below 0.05, indicating a non-normal distribution. Thus, a Wilcoxon signed-rank test was performed for all data sets.

RESULTS

Pollen quantity by watering treatment

We recorded pollen mass for 150 flowers, 75 flowers per treatment. Results from the pollen mass by watering treatment compared watering treatment against pollen mass per flower in milligrams (Fig. 4). Non-droughted plants produced flowers with more pollen than droughted plants (p<.0.0001). On average, non-droughted flowers had 1.54 mg of pollen per flower as compared to 0.97 mg of pollen for drought-modified flowers. Thus, we found that non-droughted flowers had almost two times more pollen per flower than droughted flowers.

Previous research by Rose-Person et al. (unpublished) and observation suggested a difference in floral abundance between drought and non-droughted plants. To explore this pattern, we measured floral abundance to calculate the overall pollen quantity per plant by watering treatment. We counted flowers from a subset of 20 plants (each plant visually representing the average plant from the entire set) per watering treatment twelve weeks after applying the drought treatment. Non-droughted plants produced more flowers than droughted plants (Fig. 5, p<0.0001). The average number of flowers per plant for drought and non-droughted plants were found to be 48.6 and 18.4, respectively. Non-droughted plants had more than twice as many flowers per plant than droughted plants. To determine the total average pollen quantity per plant, we multiplied the average pollen mass by the average number of flowers for each treatment. The average pollen quantity per plant was 74.8 mg for non-droughted plants and 17.8 mg for drought plants. Using this calculation, we found that non-droughted plants had over four times more pollen mass per plant than drought plants. This suggests that water stress negatively impacts pollen quantity.

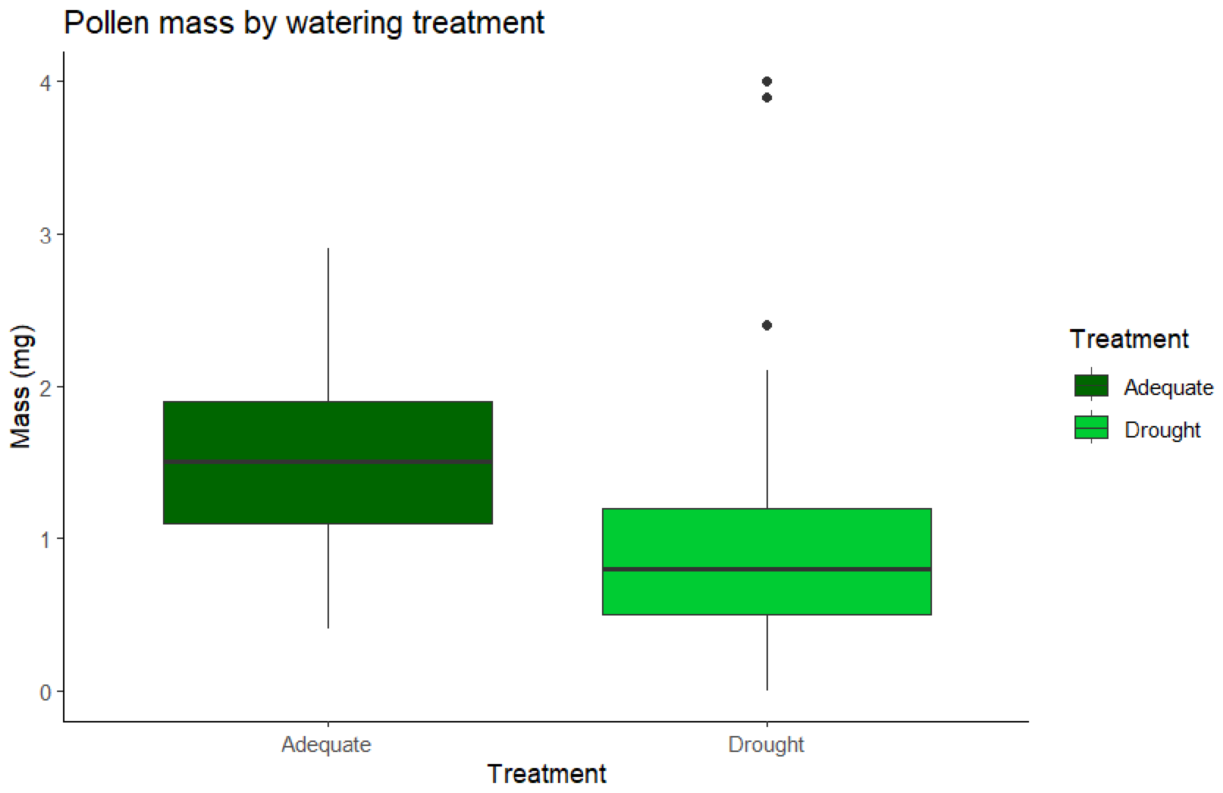

Figure 4: Box-whisker plot for pollen mass (in milligrams) per flower by watering treatment

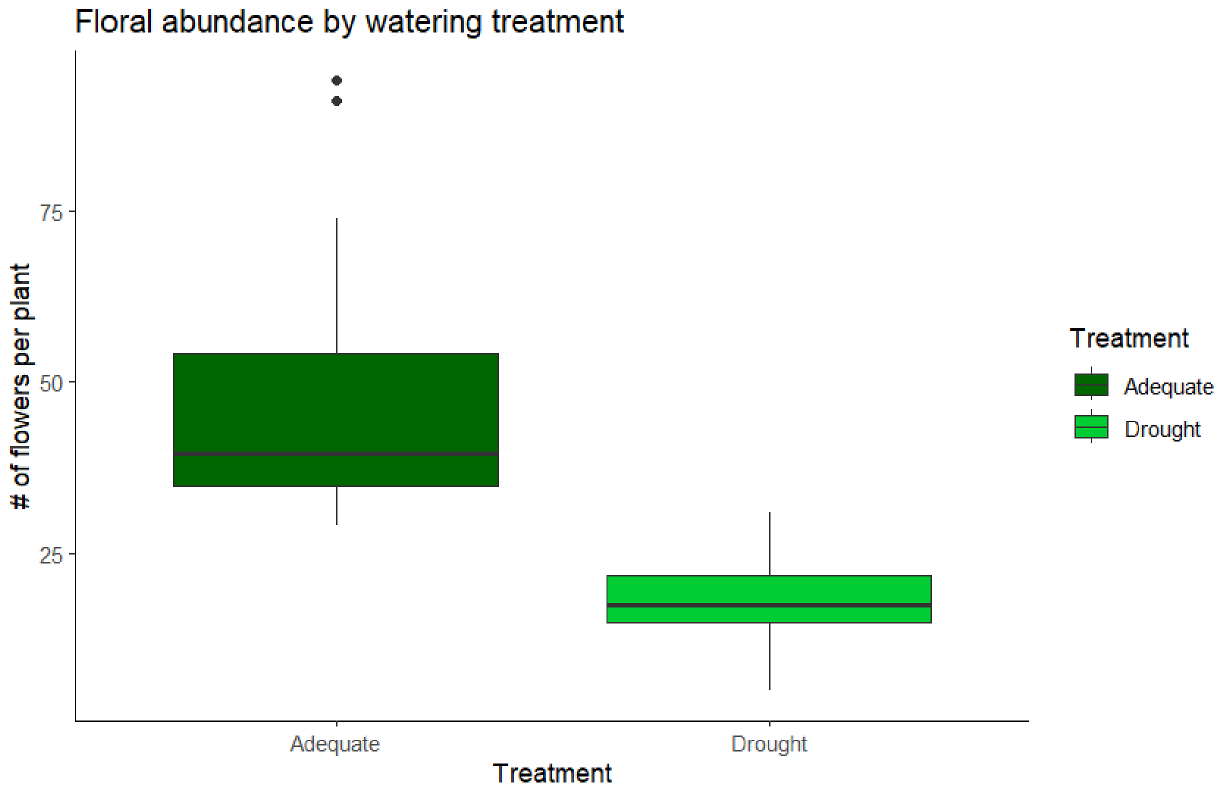

Figure 5: Box-whisker plot for floral abundance (number of flowers per plant) by watering treatment

Pollen quality by watering treatment

We measured pollen viability of 150 samples, for 75 per watering treatment. Pollen viability was significantly higher in non-droughted plants (Fig. 6, $p<0.0001$). Pollen viability samples for non-droughted treatment were most concentrated between the 75-100% pollen viability regions on the graph, while drought samples were equally distributed between 0-100% pollen viability. On average non-droughted plants had a pollen viability of 80.5%, while drought plants had a pollen viability of 54.1%.

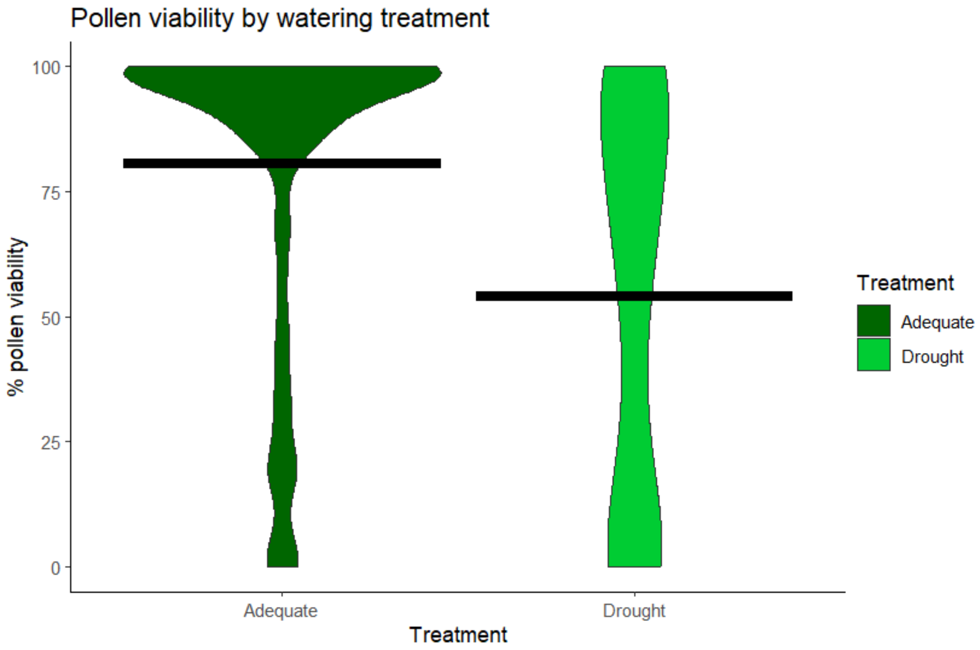

Figure 6: Violin plot for pollen viability by watering treatment in percent viability

DISCUSSION

This study focused on the impacts of water stress on pollen quality and quantity in *P. campanularia.* Our results show that drought negatively affects plant pollen production by reducing pollen mass and viability. On average, non-droughted plants had four times the mass of pollen than droughted plants. Furthermore, the pollen viability of non-droughted plants was 26.4% less than that of droughted plants.

Similar studies have corroborated that drought decreases pollen quantity and viability (Yu et al., 2019). This lack of viable pollen may prevent ovule fertilization, thereby reducing plant reproduction. Further, water limitation may reduce plant fitness by reducing floral abundance, as shown in our study, decrease seed production, and may increase flower abortion rates (Jorgensen & Arathi, 2013). These impacts on plant physiology could result in a lack of viable male gametes necessary for plant progeny. Even if seed numbers were unaffected by drought, seed viability may be reduced, impacting plant reproductive success (Akhalkatsi & Lösch, 2005). Drought is likely to also have indirect, pollinator-mediated effects on plant reproduction.

Results from recent work at UCR by Rose-Person et al. (unpublished), researching the impacts of drought on pollinator choice in *P. campanularia* are represented in Fig. 7. The box-whisker plot compares the percent time foraging on non-droughted plants against the time of day, either morning or afternoon. Any points below the fifty percent line indicate a preference for droughted plants, and anything above fifty percent shows a preference for non-droughted plants. Rose-Person et al. (unpubl.) found that pollinators preferred non-droughted flowers in the morning, at a foraging rate of around 60%. Even more so, pollinators preferred non-droughted plants in the afternoon, spending around 85% of their time on non-droughted flowers. These findings suggest that drought-modified floral resources disrupt plant-pollinator interactions by altering insect foraging behaviors, which may reduce plant reproductive success and rates of outcrossing (Fig 7; Rose-Person et al., unpub.).

Finally, our results demonstrate that drought will decrease the quantity, and possibly quality, of pollen available to insect pollinators. Pollen is an important resource for insect pollinators: it provides lipids and proteins critical to pollinator reproduction (Vaudo et al., 2020). Insects at the larval stage must consume pollen for proper development and are required by adult females for reproduction (Vaudo et al., 2020). In turn, they influence the stability, diversity, and function of ecosystems (Saunders, 2017). Wild bees are known to increase seed set and the quality of many crops (Saunders, 2017). Research suggests that almost eight percent of global crop production would be lost without bee pollinators, requiring changes to human and animal diets to resolve the shortfalls in crop availability (Khalifa et al., 2021). Thus, mutualisms between plants and insect pollinators not only sustain plant diversity but also maintain over 350,000 animal species (Ollerton et al,. 2017). Here, we focused on the effects of water stress on plant reproduction at the individual level. However, these effects may also have consequences at the community level (Fig 12). Drought may affect plant species' geographic distributions and phenologies, which may lead to temporal or spatial mismatches between plant species and insect pollinators if pollinators are not able to locate these plants in their environments (Descamps et al., 2021). At the individual scale, our study and others have shown that drought can alter floral resources such as pollen quantity and quality, which may lead to recognition mismatches between plants and pollinators if pollinators need to change foraging behaviors (Descamps et al., 2021). These mismatches lead not only to disruptions in plant-pollinator interactions but can also to changes in the abundances of insect-pollinated species and pollinators (Waser & Price, 2016). In addition, the cascading effects of drought stress on pollinator populations due to reduced floral resources can cause declines in pollinator populations over time, leading to reduced visitation numbers (Waser & Price, 2016; Rering et al., 2020). This, in turn, decreases plant reproduction due to low levels of pollen transfer (Waser & Price, 2016; Rering et al., 2020). Therefore, we expect that both direct (plant physiology-mediated) and indirect (pollinator-mediated) effects of drought stress on *P. campanularia* would lead to declines in plant-pollinator populations due to reduced reproductive output and reduced seed set.

Climate models predict climate change in the form of more severe drought in Southern California (Lynam & Piechota, 2021). Our research brings insight into the impact of environmental change on native plant communities. With the potential for increased drought in the coming century, the need for conservation and restoration of native habitats increases. Results from this study could be used to guide decisions about protecting native plants and pollinators, given the devastating impacts of water stress on ecosystem services provided by plants and their pollinators.

Although much research has shown how drought negatively affects plants, pollinators, and plant-pollinator interactions, future research that explores environmental stressors on plants and pollinators in an uncontrolled environment using non-manipulative approaches will be valuable. It will be especially important to explore these patterns over longer periods of time and in perennial species to determine the role of phenotypic plasticity in mediating the effects of drought on plant fitness. Further, longer-term studies could provide information on whether plant and pollinator communities are affected when environments experience severe drought followed by wetter periods. By using these long-term data sets and climate modeling, we can predict future changes in plant-pollinator interactions on a global scale. Such data can be used to better understand threats to ecosystem conservation and food security.

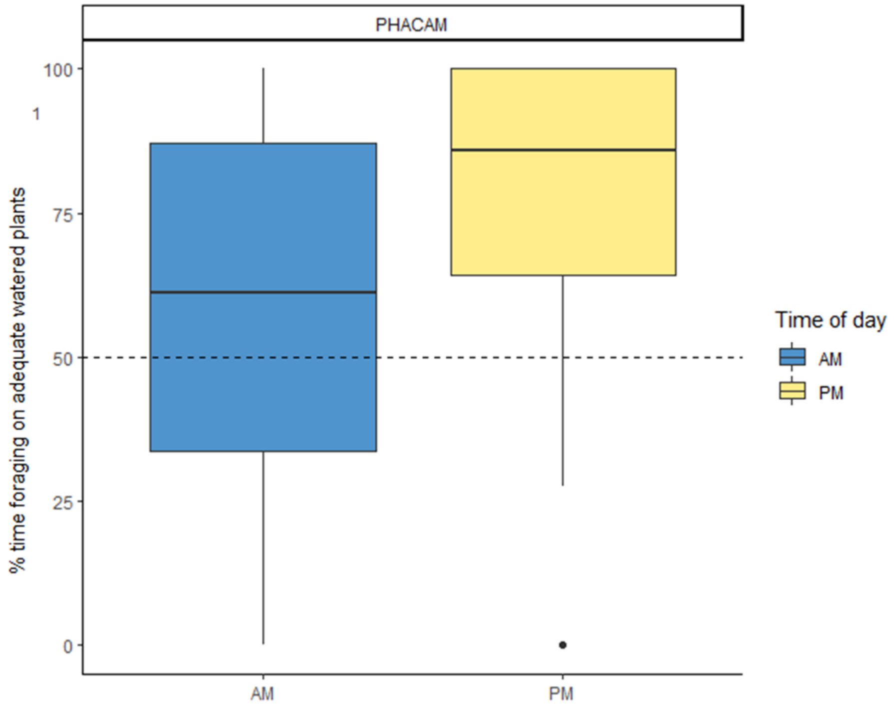

Figure 7: *Osmia lignaria* (blue orchard bee) percent time spent foraging by watering treatment in *P. campanularia* (Rose-Person et al., unpub.)

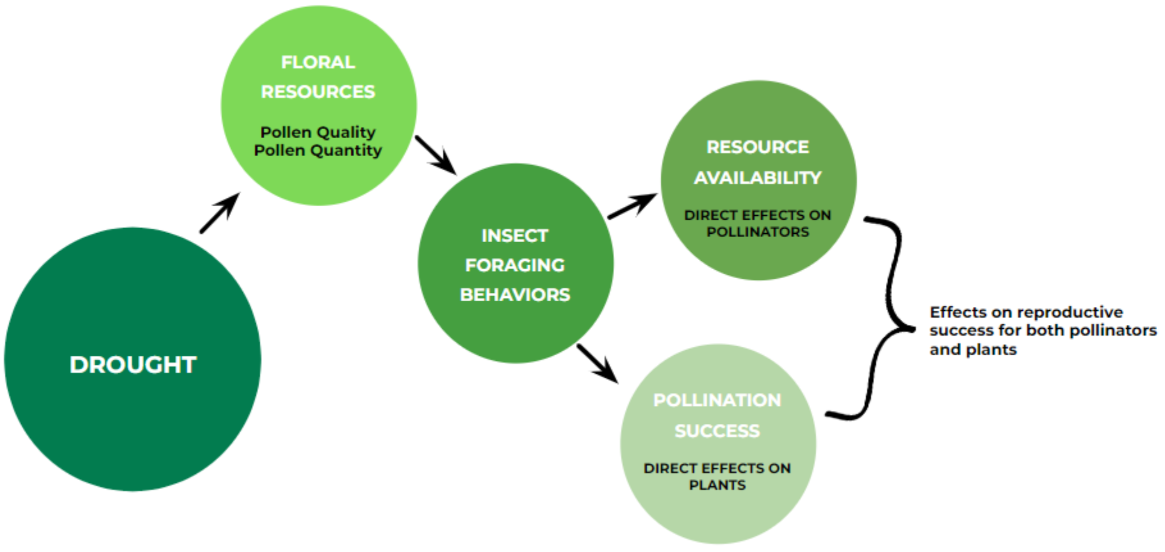

Figure 8: The indirect and direct impacts of drought on plant-pollinator reproductive success. As shown in this study, drought can modify the availability of floral resources (e.g. pollen quality and quantity). This may impact bees and plants indirectly by altering plant-pollinator interactions and

more directly by altering resource availability for pollinators and pollination success for plants (modified using Descamps et al., 2021)

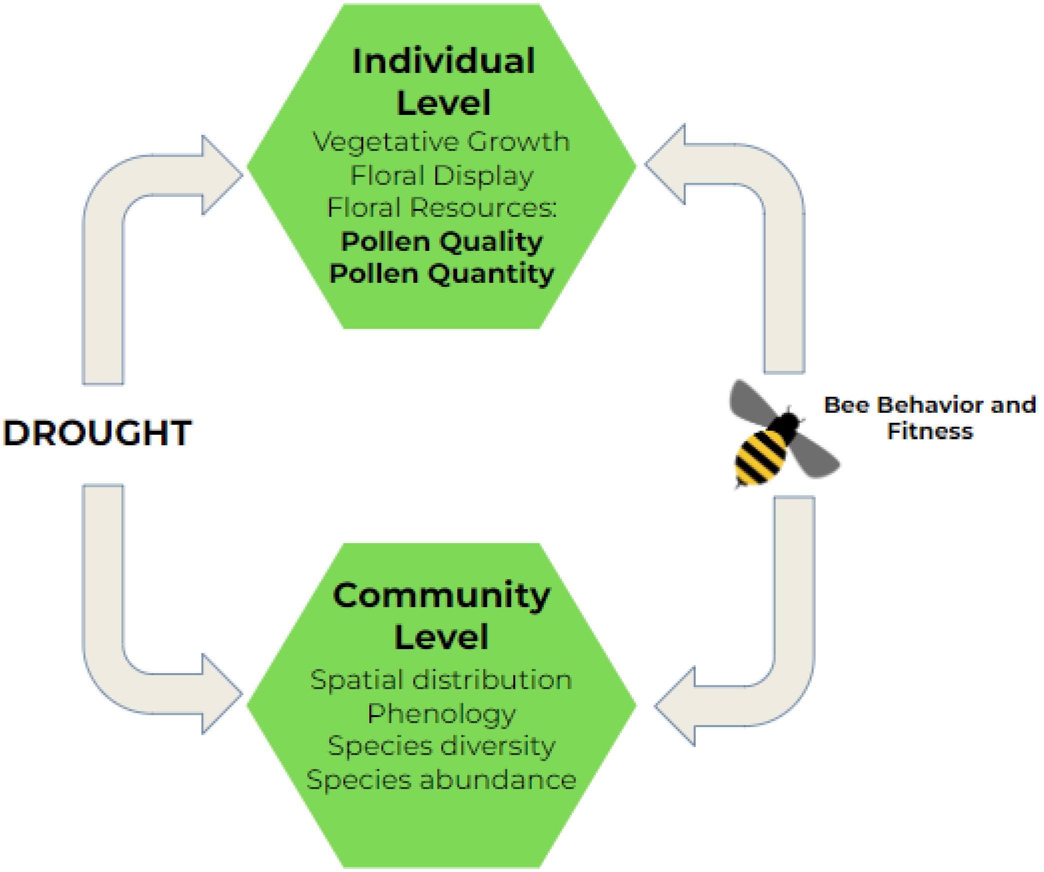

Figure 9: Potential effects of water stress on plants and pollinators at the individual and community level (modified using Descamps et al., 2021). At the individual level, drought can alter vegetation growth, floral display, and floral resources which may alter bee behavior and fitness. At the community level, drought may result in temporal and spatial mismatches between plants and their pollinators resulting in changes to plant-pollinator interactions and community composition.

CONCLUSION

This study demonstrates that drought negatively impacts pollen viability and quantity. Our work implies that drought conditions may decrease seed set via reduced pollen rewards resulting in changes to plant-pollinator interactions and community composition. Similar research has found that drought reduces pollinator visitation, suggesting that drought will have both direct and indirect effects on plant fitness. If drought conditions increase as predicted, plant and insect population declines are likely, diminishing ecosystem services provided by this mutualism.

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