Drought-induced impacts on honey bee nutrition and productivity --

Final Report

Prepared by: Erin E. Wilson Rankin

University of California, Riverside

Prepared for: National Honey Board

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I. PROJECT BACKGROUND

This project aimed to assess how reduced water availability affects the nutrition provided by flowers and to determine the subsequent effects on bee fitness and productivity. Bees are some of the most economically important beneficial insects in the U.S. (James and Pitts-Singer 2008). Due to their vital ecological role and economic value as pollinators in agricultural ecosystems and producers of honey, maximizing bee health is of paramount importance. Stressors on the ecosystem-level can affect both plant and pollinator health (Cayan et al. 2008). One such emerging threat is climate change, which includes multiple stressors, such as increasing temperatures and incidences of drought (Intergovernmental Panel on Climate Change 2002). Changes in temperature or water availability are known to significantly affect plant physiology and phenology (Penuelas et al. 2004; Andresen et al. 2010). Because bees are obligate florivores and acquire most of their nutrients from nectar and pollen (Jordano et al. 2006; Winfree 2010), any altered availability and quality of bee forage may affect bee health, with cascading effects on honey production and pollination services. **This project addresses key priority areas of the National Honey Board: (1) effects of climate and environmental variables on bee forage (the quality and quantity of nectar and pollen), and (2) effects of nutrition on bee health and production.** Our research utilized a combination of experiments to develop recommendations regarding maximizing pollination services and nutrient availability under drought conditions. This work investigated the following objectives:

> **Objective 1 [bee forage]:** We will measure the quantity and quality of nutrient availability in pollen and nectar from bee forage (e.g., buckwheat) grown in (a) optimum or (b) low water conditions.
>
> **Objective 2 [bee health]:** We will quantify the health and development of larval honey bees fed diets based on nutritive quality and quantity of forage under normal or drought conditions as determined in Obj 1.

II. DROUGHT IMPACTS ON NUTRITIVE VALUE OF BEE FORAGE

Pollinators play a critical role in plant reproduction, contributing to population propagation for many plant taxa. Therefore, pollinator management and identifying factors influencing plant-pollinator relationships is necessary for maintaining a healthy ecosystem. Healthy bee hives require substantial amounts of carbohydrates (nectar) and protein (pollen) from forage plants. Therefore, the first step to investigate how drought modifies the pollination mutualism is to assess how the resources offered by the plant changes under drought.

Summary of Experimental Design

We measured the quantity and quality of nectar and pollen under optimum and low water conditions using a series of established analysis methods. For the laboratory manipulation of pollen and nectar, we focused on a common forage plant, clover. While we originally grew both buckwheat and clover in the greenhouse, it was not feasible to reliably extract all the nectar from buckwheat florets. Therefore, we switched to exclusively working on a native clover, *Trifolium willdenovii*.

For each experiment, we grew four sets of 40 plants in Deepots (D40, Stuewe & Sons) in a temperature controlled greenhouse: two sets experienced optimal water conditions and two sets experienced reduced water conditions (drought). Optimal water conditions were assessed by determining how much water was required for the soil to become thoroughly moistened. The reduced water treatment (drought) experienced 30% less water than the optimal treatment, in line with required water use reductions for the state of California (California Water Boards 2016). We noted the date of bloom, as well as the number and size of all flowers. Pollen and nectar were also collected from each flower during bloom period to measure pollen and nectar quantity and quality. To determine if reduced water had impacts on plant fitness, we grew an additional set of 40 plants each under the two watering conditions, hand pollinated the inflorescences and quantified the resulting seed set. All means are reported ± standard error.

Phenology

Watering regimes did alter the phenology of clover in the greenhouse. Time from seed scarification until first floret produced was 5 days longer for reduced water (drought) plants than optimal water plants ($F_{1,56}$= 6.76, p=0.01). Furthermore, the total number of inflorescences produced per plant was highly significantly different between treatments where reduced water plants produced an average of 2.75 ± 0.5 inflorescences and optimal plants had an average of 7.9 ± 0.7 inflorescences (Figure 1: $F_{1,56}$=31.2, p<0.0001) over the collecting period. Correspondingly, plants in the optimal watering treatment had 71% more biomass than plants in the reduced water treatment ($F_{1,56}$ = 138.06, p < 0.0001).

Seed set

Plants in the optimal treatment produced significantly more florets per inflorescence (16.9 ± 1.2 florets) than those in the reduced water treatment (11.9 ± 1.2 florets) ($F_{1,33}$ = 8.18, p = 0.0007). Moreover, reduced water (drought) plants had an average of 80.5 ± 8.2 total florets and optimal produced an average of 101.1 ± 11.3 total florets. However, there was no difference in the percentage of florets that set seed (reduced water: 48.6% and optimal: 50.9%, NS). Furthermore, the total number of seeds produced per floret did not differ among treatments ($F_{1,33}$ = 1.35, p = 0.25). Many plants are still in flower, however analysis to date shows that the number of seeds produced per plant were two-fold higher in the optimal treatment as compared to the reduced water treatment (Figure 1: 57.3 ± 12.5 seeds vs 28.8 ± 11.4 seeds, respectively; X^2 = 34.2, p < 0.0001).

Nectar quantity and quality

Effect of treatment on nectar volume: Clover nectar was collected and pooled for each plant over 20 days in pre-weighed tubes. Using micro-capillary tubes, the total volume of collected nectar was measured and divided by the sum of collected florets, yielding a volume of nectar produced per floret. Optimal plants produced inflorescences with an average of 0.106 ± 0.03 µL per floret while drought treated plants exhibited an average of 0.004 ± 0.01 µL per floret (F_1=6.1, p=0.017). In addition to volume measurements, all collected nectar was weighed, yielding the mass of nectar produced per floret. Water treatments also had a highly significant effect on nectar weight (optimal: 0.077 ± 0.018 mg vs drought: 0.016 ± 0.003 mg; $F_{1,56}$ = 109.3, p < 0.0001). Nectar mass per floret correlated tightly with nectar volume per floret ($F_{1,56}$ = 738.2, p < 0.0001). Similarly, plants under the optimal watering treatment produced more nectar overall (Figure2: 2.67 ± 0.59 mg and 3.8 ±1.02 uL nectar) than did the reduced water plants (0.26 ± 0.07 mg and 0.25 ± 0.2 uL nectar) (nectar mass (mg): $F_{1,58}$ = 10.97, p = 0.0016; nectar volume (uL): $F_{1,58}$ = 7.76, p = 0.007).

Figure 1: *Reduced water impacts plant phenology and fitness. Top: Number of inflorescences per plant when grown optimal and 30% reduced water conditions. Bottom: Number of seeds per plant produced under optimal and 30% reduced water conditions.*

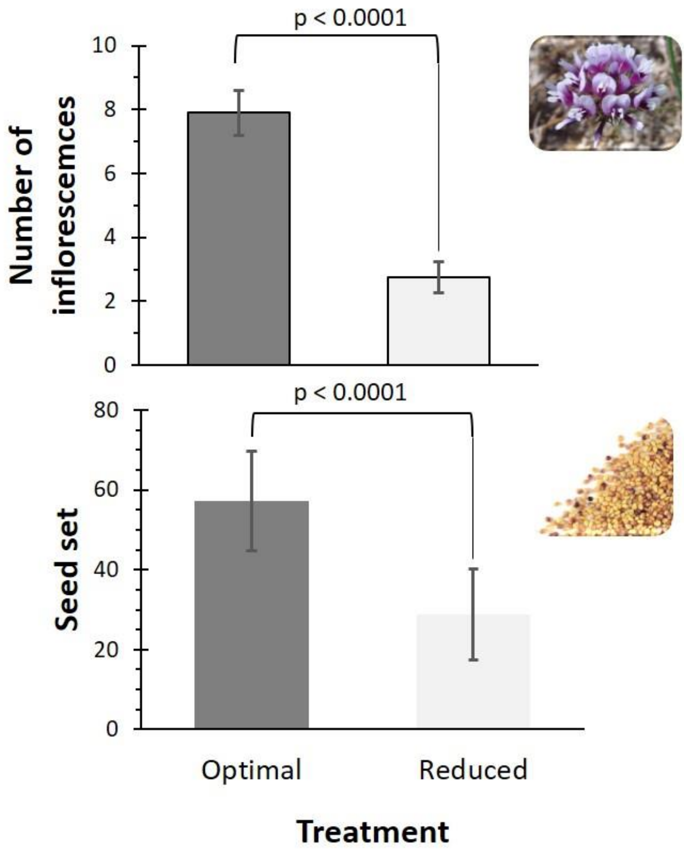
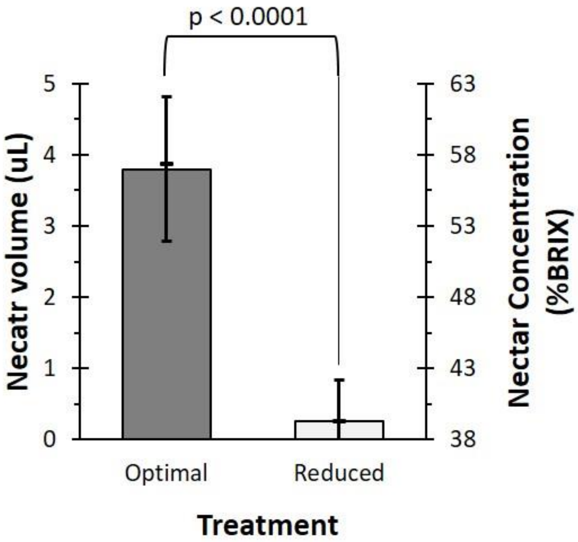


Figure 2: *Effect of water treatment on nectar volume produced and nectar concentration in percent BRIX.*



Effect of treatment on nectar quality: To date, only 27 drought-treated plants (out of 40) and 36 optimal treatment plants yielded sufficient amounts of nectar for accurate BRIX measurement. Additional 80 plants are being cultivated and nectar collected to increase this sample size. However, analyses to date show that nectar from optimal plants appears to have a 46% higher sugar concentration than nectar from reduced water plants (Figure 2: $F_{1,59} = 7.39$, $p = 0.0086$). Preliminary tests indicate that water reduction make have an effect on the proportion of sugars in the nectar. Sucrose is the dominant sugar in nectar from optimal plants, whereas glucose and fructose are found in higher proportions in nectar from plants in the reduced water treatment. Due to limited sample volume from drought plants for the sugar analysis, we are in the process of raising additional plants to replicate these experiments before conducting statistical tests and drawing conclusions about the response of sugar composition in nectar to water reduction.

Pollen quantity and quality

Effect of treatment on pollen quantity: While harvesting nectar from each plant over 20 days, the pollen was also collected and pooled by plant in a pre-weighed tube. The total mass of pollen collected per plant was then divided by the number of florets collected yielding the average mg of pollen per floret for each plant. The average mass of pollen per floret for drought-treated plants was 0.076 ± 0.006 mg and 0.062 ± 0.009 mg for optimal plants, and this trend was not quite significant ($F_{1,20}=3.1$, $p=0.09$). Similarly, there was no effect of water treatment on the total amount of pollen produced per plant ($F_{1,20} = 0.40$, $p = 0.53$: 5.99 ± 0.63 mg for optimal treatment vs. 5.52 ± 0.41 mg for reduced water treatment).

Effect of treatment on pollen quality: Pollen collected and weighed in previous section was then analyzed for its protein composition. First we extracted total protein and then used a BCA assay. While we have additional assays pending, analyses to date indicate that there was no significant effect of water treatment in percent protein for the water treatments ($F_{1,20} = 0.47$, $p = 0.50$). Optimal plants were found to produce pollen consisting of 3.4% protein (w/w) whereas reduced water plants produced 3.0% protein (w/w). The percent protein was instead highly correlated with the amount of pollen extracted (in mg), even after accounting for this in the statistical analysis. Additional replicates are underway to confirm these findings.

III. DROUGHT IMPACTS ON BEE HEALTH AND FITNESS

Diet affects bee health, particularly immunocompetency (Alaux et al. 2010), and bees require a rich and diverse diet for the best health (Di Pasquale et al. 2013). Therefore, the second step to investigate how drought modifies the pollination mutualism is to assess how the resources offered by the plant under drought conditions affect bee health and productivity.

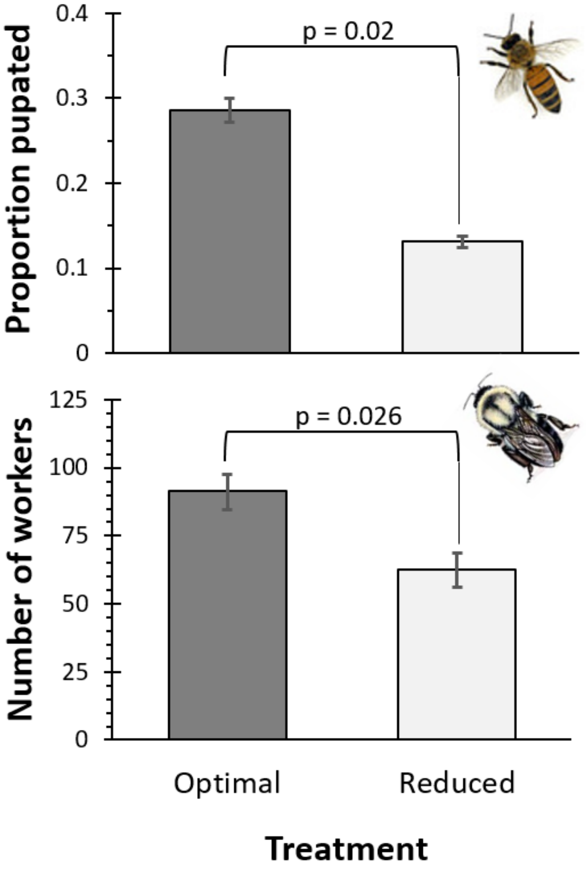
Summary of Experimental Design

Because worker bees can adjust their foraging to compensate for differences in resource volume, we focused on resource quality. This may be especially important for pollen as bees are known to have difficulty assessing the quality of undigested pollen. Due to logistical complications with obtaining honey bee hives early in this project, we added a component examining the impacts on bumblebees as well. Diets based on the nutritional composition determined above were created from basic ingredients (sucrose, fructose, and glucose, pollen or royal jelly) and were provided *ad libitum* to developing larval bees (*Apis mellifera*) or colonies (*Bombus impatiens*).

Effect of nutrition on developing bees: Apis mellifera

To examine the effect of differences in nutrition, we used a combination of techniques. First, we grafted 96 one-day old honeybee larvae onto 250 uL of diet based on the nutritional differences of our experimental plants. Mortality rates did not differ between the treatments (optimal: 19% vs 30% water reduction: 15%). Growth rates were slightly higher for the optimal diet versus reduced water diet, with both more larvae growing past first instar and growing to fifth instar faster (X^2 = 14.46, p = 0.0001). Pupae were first observed on Day 9 for the optimal diet and Day 11 for the reduced water diet. More than two-fold more larval bees grafted on diet based on optimal clover plants' nutritional make-up pupated than those grafted on diet based on drought nutritional quality (t_4 = 3.45, p = 0.026). Nutrition also had a significant impact on total development time (t_4 = 18.03, p = 0.035). Individuals raised on the optimal diet emerged as adults on average 7 days after pupating, whereas those from the reduced water diet emerged as adults nearly nine days after beginning pupation (t_4 = 139.6, p = 0.0004). While adult bees from the optimal diet had higher body mass than those from the drought treatment (103.6 ± 5.6 mg vs 87.1 ± 12.5 mg), this difference was not significant (t_4 = 2.18, p = 0.27).

Figure 3: *Reduced water impacts bee fitness. Top: Proportion of larval honeybees pupating when grafted onto diets based on clover under optimal and 30% reduced water conditions. Bottom: Number of bumblebee workers produced in colonies raised on diet from clover grown under optimal and 30% reduced water conditions.*



Effect of nutrition on developing bees: Bombus impatiens

In addition to honey bees, we raised bumblebees on either diets based off the nutritional values of the clover grown under the optimal or the reduced water conditions. We raised entire *Bombus impatiens* colonies (N = 2 colonies each treatment), as well as creating and maintaining 74 microcolonies each consisting of 3 workers, on these artificial diets.

Colony growth rates: We observed that colonies raised *ad libitum* on the optimal diet had 50% significantly higher number of bees than the colonies raised *ad libitum* on the reduced water diet ($F_{2,25} = 4.64$, $p = 0.02$: 91.5 ± 5.9 bees vs. 62.8 ± 6.8 bees). Colonies raised on the optimal diet produced 63% more callow bees and 38% more queens than colonies raised on the reduced water diet ($X^2 = 8.27$, $p = 0.004$).

Individual biomass and longevity: Notably, there was no difference in forager body mass between the treatments (optimal: 91.4 ± 3.8 mg; reduced water: 93.0 ± 4.0 mg). However, foragers lived nearly 70% longer when raised on the optimal diet: 50.9 ± 2.7 days vs. 30 ± 2.5 days, respectively ($F_{1,246} = 33.42$, $p < 0.0001$).

IV. SUMMARY

Climate change is predicted to have large impacts on agriculture and pollinators, thus the experiments involved in this study can contribute to a proactive approach in managing drought impacts on bee health. We show that nutritional value of nectar for bees is indeed decreased by reduced water availability to plants. Given the current drought in California and the mandatory water restrictions region-wide, this will likely have cascading effects on available nutrition to wild and managed bees in drought-affected areas. Bees raised on diets simulating the nutrition produced by plants under reduced water conditions fared significantly worse than bees raised on diets from optimally watered plants. This effect was observed despite bees being given unrestricted access to the experimental diets.

Currently, additional plants are being raised to increase sample size for nutritional analyses of nectar because the reduced water treatment (drought) led to significant reduction in nectar production. Pollen from these additional plants will also be analyzed to confirm the findings presented above. We anticipate submitting this research for publication this fall.

This research clearly shows that reduced water, either due to natural weather patterns or mandatory water restrictions, will have a significant impact on the phenology, floral display, and nectar resources of the bee forage plant, clover. Subsequent experiments raising two commercially important pollinator species on artificial diets determined from the clover experiments found that both honeybees and bumblebees had significantly lower fitness when raised on the reduced water diet—despite being given *ad libitum* access. Preliminary findings suggest that nectar and not pollen nutrition are most likely to be impacted by shortage or reduction in water. With increased understanding of how bee forage plants respond to decreased water (and not just its absence), we can develop strategies to manage bee health and crop pollination given the threatening and serious environmental stressor of drought and climate change.

V. ACKNOWLEDGMENTS

Sarah K. Barney was instrumental in conducting this research. We are grateful for the assistance of Giselle Lozano in the greenhouse and with the maintenance of *Bombus* colonies. *Bombus* colonies were obtained from Biobest and kept at UC Riverside under CDFA Permit # 3162. We are grateful for the assistance of Ning Di with the grafting of honey bee larvae and for Quinn McFrederick providing honeybee larvae. This project was supported by a grant from the National Honey Board.

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