DIETARY EFFECTS OF *PROSOPIS PALLIDA* ON *APIS MELLIFERA* HEALTH IN HAWAIʻI

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INTRODUCTION

In the late 1890s, as the cattle industry began to grow in Hawaiʻi, common kiawe (*Prosopis pallida*) trees were propagated as a food source for cattle, and honey bee colonies were established nearby to stimulate its pollination (Hall, 1904). As a result, invasive kiawe forests expanded rapidly across the islands and eventually established as the dominant forest type in Hawaiʻi's lowland dry ecosystems. Kiawe finds success in these regions due to long taproots allowing it to access otherwise inaccessible groundwater sources, and, as a nitrogen-fixing tree species, can acquire nitrogen, cycle nutrients, and continue blooming even during periods of little rainfall (Dudley et al., 2014). Today, kiawe remains a primary food source for honey bees in Hawaiʻi's arid, leeward regions (Roddy and Arita-Tsutsumi, 1997; Gallaher and Merlin, 2010).

Despite state laws restricting the movement of used beekeeping equipment and honey bees into Hawaiʻi and between islands, the parasitic mite, *Varroa destructor*, was accidently introduced to Oʻahu in 2007 and Hawaiʻi island in 2008. All other Hawaiian Islands remain varroa-negative, allowing for a unique comparison between varroa-positive and -negative honey bee populations (Grindod et al., 2021). Varroa parasitism results in honey bees with weakened immune systems and they also transmit a number of harmful viruses, including Deformed wing virus (Posada-Florez et al., 2019).

The reliance of honey bees on adequate pollen nutrition, the current varroa-negative status of Maui island, and the feedbacks between nutrition and disease (Dolezal and Toth, 2018) prompted this study. The goal of this thesis is to determine kiawe macronutrient content and evaluate the interaction of nutrition with varroa-positive and varroa-negative apiaries located near kiawe. The overall hypothesis is that if the macronutrient and monthly composition of kiawe pollen varies, then honey bee colony and adult worker bee health will be directly impacted

by this variation. This is addressed through three study objectives: 1) Identify and evaluate kiawe pollen dietary components and nutritional quality (total proteins and lipids); 2) Assess how temporal variability in kiawe pollen macronutrient quality affects hive health and influences hive pest levels, and; 3) Evaluate kiawe pollen's dietary effect on hive bee health from the Wai‘anae, O‘ahu apiary.

Chapter 1: Kiawe Pollen Impacts on Honey Bee Colony Strength in Hawaiʻi

Abstract: Pollen is a honey bee's primary protein and lipid source and the availability, quality, quantity, and diversity of a bee's pollen diet determine its individual health and, therefore, the overall health of the entire colony. Common kiawe (*Prosopis pallida,* Fabaceae) grows in the arid valleys of the leeward regions of all the Hawaiian islands and is a widely available food source for honey bees. This study aimed to determine the impact of kiawe pollen nutrition on colony health. To address this, I identified bee-collected pollen and analyzed its nutritive quality individually from apiaries on Oʻahu and Maui over a period of 14 months. Pollen was collected monthly, sorted by color as a proxy for species, and total proteins and lipid content quantified. Colony health metrics, including brood quality, frame occupancy of hive bees, and varroa and small hive beetle numbers, were also assessed monthly. Results showed there is spatial and monthly variation in kiawe pollen's total protein and lipids, as well as a variation in hive health metrics and bee-collected pollen diversity. On Maui, SHB levels never significantly differed from each other, and were highest in June (10.12 ± 1.78). SHB levels on Oʻahu were highest in April (48.3 ± 7.61) while percent varroa mite infestation on Oʻahu was highest in January (5.67 ± 1.32). Mean brood quality was significantly higher on Maui (81.41 ± 0.43) compared to Oʻahu (78.33 ± 0.49). Also, mean frames of bees were significantly higher on Maui (14.73 ± 0.20) compared to Oʻahu (5.35 ± 0.11). Lipids levels were highest on Maui in June (14.28 ± 0.22 mg/g pollen) and highest on Oʻahu in September (15.48 ± 2.07 mg/g pollen), while protein levels on Maui were highest in January (326.28 ± 11.55 mg/g pollen) and highest on Oʻahu in February (361.53 ± 23.09 mg/g pollen). Additionally, the proportion of kiawe pollen in the homogenate (average percent total from six, 25 mL subsamples) increased during the kiawe blooming seasons (percentage was highest in March with 46.47 ± 6.34%) but these months did not display any

change in hive health. Kiawe macronutrient quality did not significantly impact hive health metrics and hive health seemed more influenced by the absence or presence of varroa mites, showing that hives on O‘ahu are much more stressed than hives on Maui.

1. Introduction

The survival and overall health of a honey bee colony is directly related to the availability of adequate nutritional food sources. Honey bees benefit from plants by obtaining nectar and pollen, which is essential for their health and development. As generalist foragers, honey bees collect pollen and nectar from a variety of flowering crop plants, invasive weeds, and native and naturalized plants (Clare et al., 2016; Wood et al., 2018). In addition to availability, the quality of their forage impacts the overall health and lifespan of individual bees (Brodschneider and Crailsheim, 2010). Pollen supplies the necessary amino acids, lipids, vitamins, and minerals (Thakur et al., 2020; Liolios et al., 2016; Di Pasquale et al., 2013; Yang et al., 2013), while nectar is the main source for carbohydrates (Venjakob et al., 2022; Erler and Moritz, 2016).

Pollen varies in nutritional quality, and pollen quality is typically expressed as total protein and total lipid content. The quality of pollen can vary greatly and is impacted by a variety of factors, such as time of year, geographic region, and plant species (Di Pasquale et al., 2013, 2016; Simanonok et al., 2020). Depending on the plant species, bees are provided with only a select portion of their necessary nutrition requirements (Egan et al., 2018; Knapp et al., 2019). Pollen protein content can range from 2.5 to 61.0% and lipid between 2 and 20% (Liolios et al., 2016; Hanley et al., 2008; Roulston and Cane, 2000), and a single pollen species is unlikely to meet the total nutritional requirement for honey bees adequately. St. Clair (2020) found that honey bee colonies pollinating mostly soybean monocultures were linked to diminished lipid content that negatively impacted their overall colony growth compared to a more diverse farm of fruits and vegetables. Therefore, honey bees not only require an adequate quality of forage but may also require a suitable diversity of pollen types to ensure they receive adequate nutrition (Antunez et al., 2015; Filipiak at al., 2017).

Pollen is necessary for maintaining overall colony health. A strong colony with healthy bees can better handle many stressors, such as hive pests (*Aethina tumida*, Nitidulidae, small hive beetle and parasites (*Varroa destructor*, Varroidae, varroa) (Brodschneider and Crailsheim, 2010; Schmehl et al., 2014; Dolezal et al., 2019). Studies have shown that a diverse pollen diet may lead to a boosted baseline immunity over a monofloral diet (Alaux et al., 2010). A colony with insufficient pollen diversity may therefore be more vulnerable to pests and pathogens (Kang et al., 2015) resulting in fewer, weaker bees.

Moerman et al. (2017) observed that a monofloral pollen diet of common broom (*Cytisus scoparius*) showed better bumblebee colony development compared to a mixed pollen diet of mountain ash (*Sorbus americana*, Rosaceae) and heather (*Erica* sp., Ericaceae), suggesting that the availability of a higher quality pollen source may be more beneficial to colony health than the availability of multiple pollen species. In a cage study by Omar et al. (2017), honey bees fed a pollen mixture or a monofloral asparagus (*Asparagus* sp., Asparagaceae) or chestnut (*Castanea* sp., Fagaceae) pollen diet had comparable development of the hypopharyngeal glands, but a monofloral diet of *Helianthus* sp. (Asteraceaae) and *Sinapis* sp. (Brassicaceae) pollen resulted in poorer development. This could be due to either greater diversity (polyfloral diet performing better) or low pollen quality (nutrient deficient monofloral option) being more impactful when bees are stressed. It is however clear that not only is the quantity of pollen important, but the quality, diversity, and species richness of pollen all impact colony health and resistance to biotic and abiotic stressors.

In Hawaiʻi's leeward, lowland dry ecosystems, invasive common kiawe (*Prosopis pallida,* Fabaceae) stands are commonly established as the dominant vegetation type. Kiawe can flower any time of year, however there are two distinct blooming seasons in Hawaiʻi: January to

March and September to October. Kiawe pollen is a primary food source for honey bees situated in these regions, but its nutritional quality and seasonal variations in composition is unknown. This study aims to determine if the quality or seasonality of kiawe pollen significantly impacts colony health. I tested the null hypothesis that kiawe pollen quality does not differ across sample sites in Hawaiʻi or across growing seasons. I also hypothesized that periods with higher kiawe pollen macronutrient quality would result in colonies with improved health metrics.

2. Materials and Methods

2.1 Study Site

This research was performed from May 2020 to August 2021 in commercial, conventionally managed apiaries located in Wai‘anae, O‘ahu (21° 27' 24" N; 158° 10' 25" W; elevation 32 m; mean annual temperature: 22.6° C; mean annual precipitation: 533 mm, soil type: mollisol) and Kīhei, Maui (20° 47' 16" N; 156° 27' 50" W; elevation 6 m; mean annual temperature: 24.3° C; mean annual precipitation: 381 mm, soil type: aridisol) (Deenik and McClellan, 2007).

2.2 Colony Strength Assessments

A total of 36 colonies were used with 18 hives on O‘ahu and 18 on Maui. Every month, small hive beetle (*A. tumida*, SHB) counts, varroa mite (*V. destructor*) counts, brood quality, and total frames of adult bees were measured as indicators of colony health (Delaplane et al., 2013). SHB were trapped using Beetle Blasters® (Mann Lake, Hackensack, MN) containing vegetable oil (one trap per hive) and replaced monthly. Varroa mite levels were estimated by sampling approximately 300 bees per hive. Varroa mites were dislodged from bees using an alcohol wash method (Dietemann et al., 2013), and colony infestation was expressed as a percentage of mites per 100 bees. Varroa mite treatments using Apivar® strips (Mann Lake, Hackensack, MN) were done October 2020 and May 2021 at the Wa‘ianae, O‘ahu apiary.

Brood solidness was measured 10 times per colony at each sampling, as a proxy for brood quality (Delaplane et al., 2013). Total frames of adult hive bees in the brood chamber were determined visually by estimating total coverage of bees on both sides of all ten frames. Colony strength assessments were collected for 15 months on O‘ahu (June 2020 – August 2021) while

assessments on Maui were collected for ten months (May 2020 – March 2021 with November 2020 omitted due to data not being received from the collaborating beekeeper).

2.3 Pollen Macronutrient Analysis

At both apiaries, six hives were equipped with bottom mount Sundance® pollen traps deployed monthly for a period of 48 hours (n = 15 collection events on O‘ahu from June 2020 through August 2021, and n = 11 collection events on Maui from June 2020 through June 2021, November omitted). A 25 mL subsample of the bee-collected pollen was sorted by color as a proxy for species (Leonhardt and Blüthgen, 2012). Putative color species were standardized between collection months and islands, with kiawe pollen as a target species (kiawe's color was validated with palynology and a reference library). Samples were weighed to determine their relative composition for each collection period.

Total pollen protein concentrations were measured using a BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's instructions for microplate analysis. Approximately 0.025 g of each pollen color group was homogenized in 500 µl ultrapure water. A 10 µl subsample was diluted 10x using ultrapure water. Each sample was then analyzed against a bovine serum albumin standard protein curve (0, 125, 250, 500, 750, 1000, 1500, and 2000 µg/mL) with absorbance measured in duplicate at 562 nm using a Biotek Synergy plate reader (BioTek Instruments, Winooski, VT).

Total lipids for each pollen sample were measured using a phospho-vanillin assay (Vaudo et al., 2020; Van Handel and Day, 1988). A 25 µl subsample of the pollen homogenate was added to a 300 µl 2:1 methanol:chloroform solution and centrifuged for 4 minutes at 15,000 rpm. The supernatant was transferred to a 10x75 mm glass tube and the solvent evaporated on a heat block at 90ºC. 40 µl sulfuric acid was added to each tube and incubated at 90ºC for 2 minutes.

Samples were then cooled on ice for 1 minute. 960 µl of phospho-vanillin reagent (600 mg vanillin dissolved in 100 mL ultrapure water, diluted to 500 mL total volume with 85% phosphoric acid) was added, tubes vortexed briefly, and the solution incubated at room temperature for 25 minutes. Absorbance was measured in duplicate at 525 nm and compared to a standard lipid curve (0, 1, 5, 10, 25, 50, 75, 100 µg) (from 54 µl olive oil in 50 mL of chloroform) (Vaudo et al., 2020, supplementary materials).

2.4 Statistical analysis

For diversity analyses, the Shannon-Weiner Diversity Index (Shannon, 1948) was calculated using

$$H' = - \sum_{i=1}^{s} p_i \ln p_i$$

where H' = the diversity index, s = the number of species (richness), and p_i = the proportion of individuals of each species to the total number of individuals. Pielou's Evenness Index (Pielou, 1966) was then calculated using

$$J' = H'/\log S$$

where J' = the evenness measure, H' = the Shannon-Weiner diversity index, and S = is the number of species in the sample. Pielou's evenness ranges from 0 (no evenness) to 1 (complete evenness). Both indices provide distinct information about the sampled community: the species diversity in an area depends on both the number of unique species observed (species richness) and the total number of each species, and evenness refers to how evenly diversity is distributed within a community . Evenness is high if all species have similar population densities (Gauthier at al., 2021). Hutcheson's t-test (Hutcheson, 1970) was then used to compare diversity indices:

$$t = \frac{H_a - H_b}{\sqrt{s^2_{H_a} + s^2_{H_b}}}$$

where H_x = the Shannon-Weiner Diversity Index for each of the two samples (subscripted a and b) and s^2 = the variance of each of the samples. The variance was calculated using

$$s^2_H = \frac{\sum p.(\ln p)^2 - \left(\sum p.\ln p\right)^2}{N} + \frac{S-1}{2N^2}$$

where $s^2{}_H$ = variance, S = the number of species, N = the total abundance, and p = the proportion of each species to the total number of individuals.

For colony strength assessment variables that were not normally distributed (SHB, varroa mites, and frames of bees), a Box-Cox transformation was performed to meet the assumptions of normality and homoscedasticity. A two-way ANOVA was used to test the significance of the interaction effect and main effects of month and island (Oʻahu vs Maui), followed by Tukey post-hoc tests. Pollen species evenness and Shannon-Weiner diversity index were compared between islands. All statistical analyses were performed using the statistical software R (version 3.6.2) except for diversity analyses which were calculated in Microsoft Excel (version 16.58) and Pearson’s correlation analyses performed using the statistical software JMP (version 16.1, SAS Institute 2020).

3. Results

3.1 SHB and Varroa mite levels

Analysis by two-way ANOVA of SHB counts indicated a significant interaction between the effects of month and location (F = 23.816, d.f. = 8, 512, $p < 0.001$) as well as variation in SHB counts between months (F = 16.98, df = 9, 512, $p < 0.001$) but not between location (F = 0.191, df = 1, 512, p = 0.66) (Fig 1).

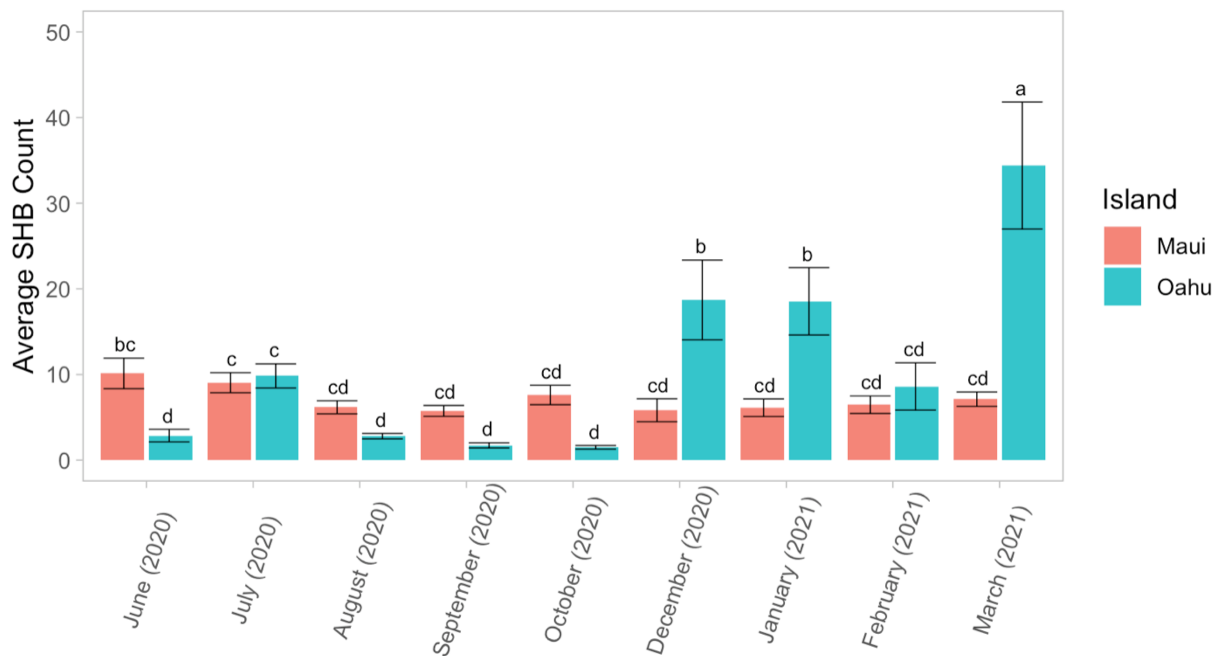


Figure 1. Mean small hive beetle counts (±SE) per hive by month at Maui and O'ahu commercial apiaries. Means sharing a common letter are not significantly different according to the Tukey-test with 95% confidence level ($p < 0.05$). Figure displays untransformed data.

SHB counts were collected on O‘ahu from June 2020 through August 2021. Analysis by one-way ANOVA indicated significant variation in average SHB count by month on O‘ahu ($F = 28.52$, d.f. = 14, 300, $p < 0.001$) (Fig 2).

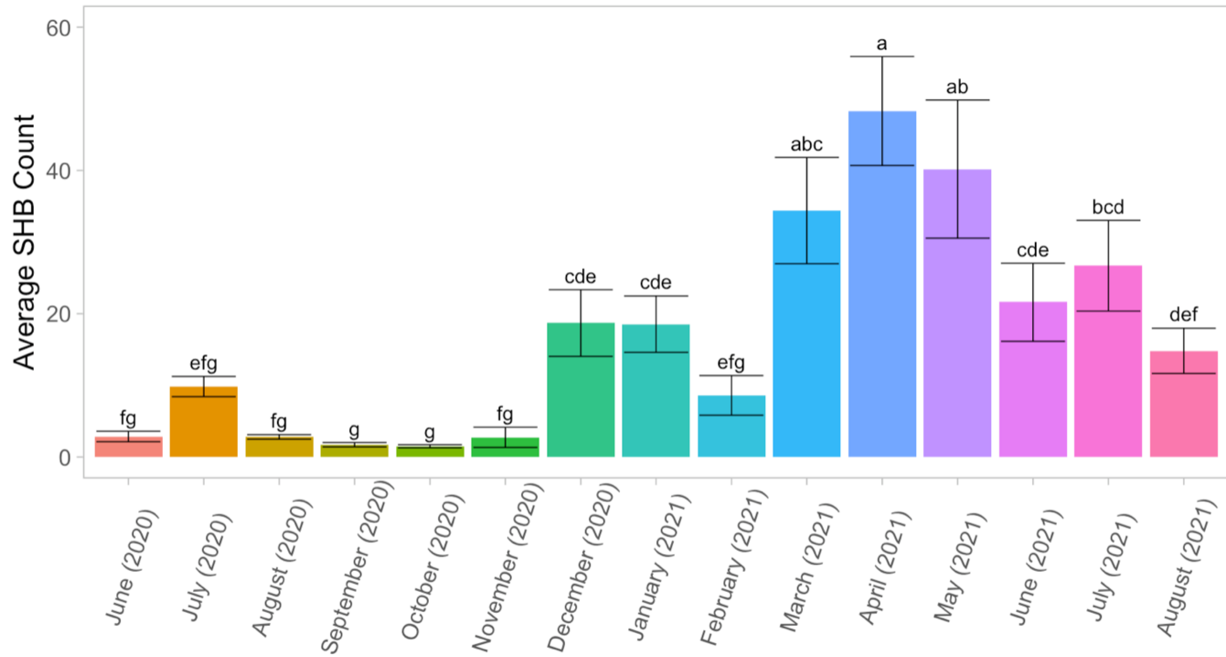


Figure 2. Mean SHB count (±SE) by month at the O‘ahu commercial apiary. Means sharing a common letter are not significantly different according to the Tukey-test with 95% confidence level ($p < 0.05$).

Analysis by one-way ANOVA indicated significant variation in percent varroa mite parasitism by month ($F = 10.09$, d.f. =15, 230, $p < 0.001$). Percent parasitism peaked in January 2021 (5.67 % ± 1.32) (Fig. 3). The Maui apiary was confirmed to be varroa negative.

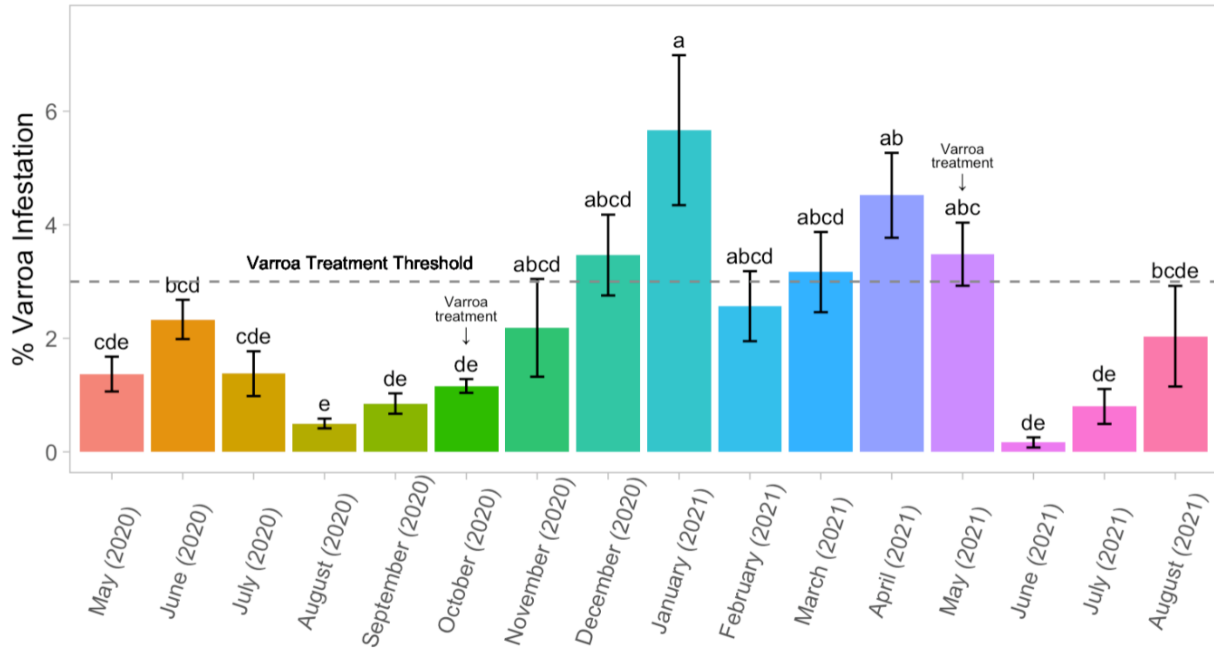


Figure 3. Mean percent bees parasitized with varroa mite (±SE) by month at the O'ahu commercial apiary. Means sharing a common letter are not significantly different according to the Tukey-test with 95% confidence level ($p < 0.05$). The recommended varroa treatment threshold of 3% (Delaplane and Hood, 1997, 1999) is indicated by the dashed line. Figure displays untransformed data.

3.2 Brood Quality and Frames of Bees

An analysis by two-way ANOVA indicated a significant interaction between the effects of time and location in brood quality as measured by the percent of brood coverage ($F = 3.44$, d.f. = 9, 531, $p < 0.001$), as well as variation in average percent brood solidness between islands ($F = 22.1$, df = 1, 531, $p < 0.001$) and between months ($F = 3.1$, df = 15, 531, $p < 0.001$) (Fig 4).

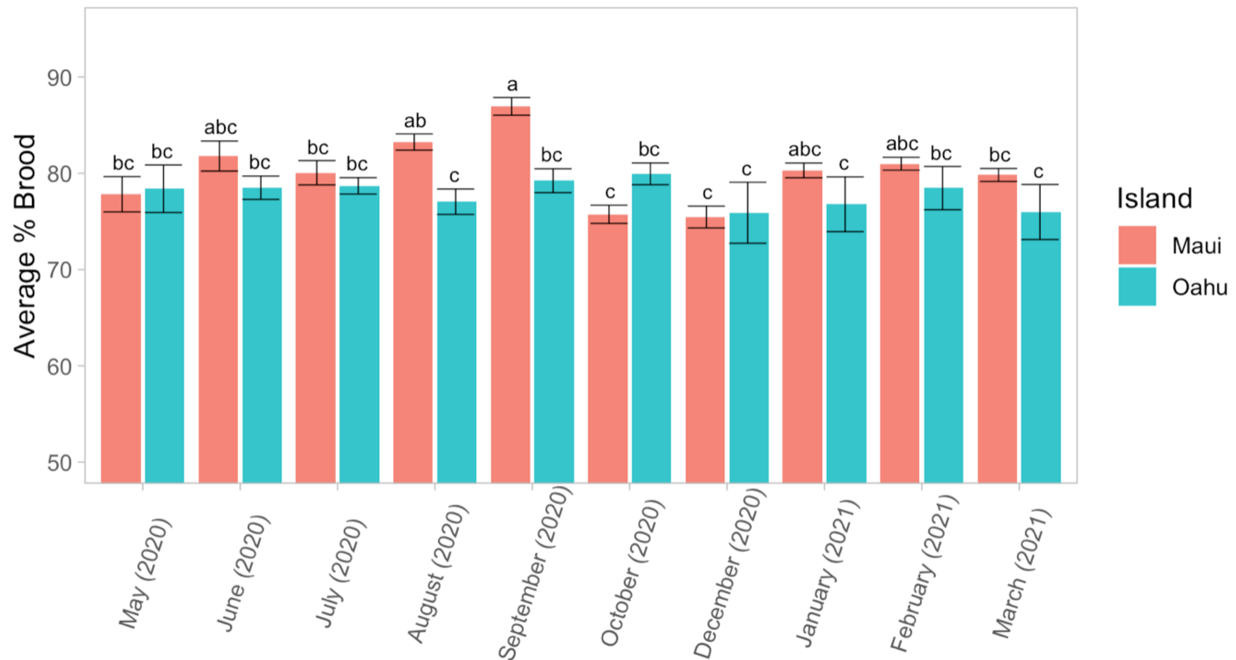


Figure 4. Percent brood quality (±SE) by month on Maui and O‘ahu commercial apiaries. Means sharing a common letter are not significantly different according to the Tukey-test with 95% confidence level ($p < 0.05$).

Two-way ANOVA indicated a significant interaction between the effects of time and location on the frames of adult bees ($F = 4.66$, d.f. = 9, 561, $p < 0.001$) as well as variation in frames of adult bees between island ($F= 20.18$, d.f.= 1, 561, $p < 0.001$) and between months ($F= 19.03$, d.f.= 9, 561, $p < 0.001$) (Fig 5).

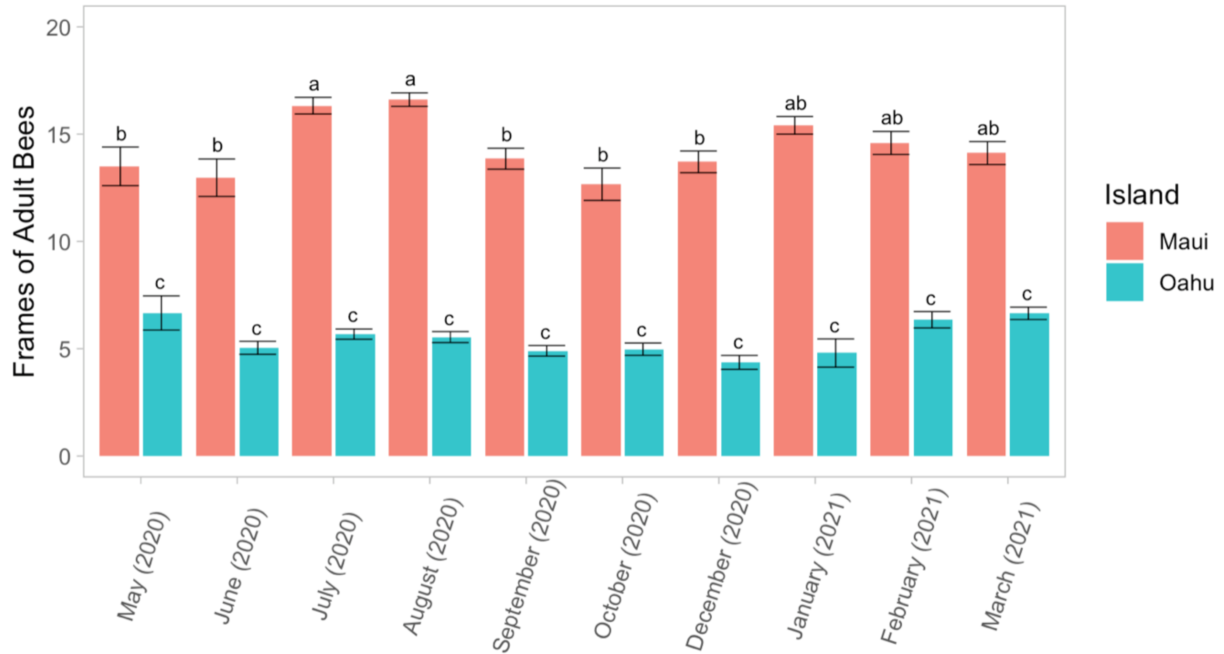


Figure 5. Mean frames of adult bees (±SE) by month at Maui and O'ahu apiaries. Means sharing a common letter are not significantly different according to the Tukey-test with 95% confidence level ($p < 0.05$).

3.3 Kiawe Nutrition and Dietary Composition

The results of the average total protein and lipid assays of the target pollen species, kiawe, are shown in Figures 6 and 7, respectively. An analysis by two-way ANOVA indicated significant variation in average protein content by location ($F = 7.377$ d.f. = 1, 100, $p = 0.008$) and by time ($F = 5.14$, d.f. = 11, 100, $p < 0.001$) (Fig. 6), but no significant interaction between the effects of time and location ($F = 1.28$, d.f. = 11, 100, $p = 0.248$).

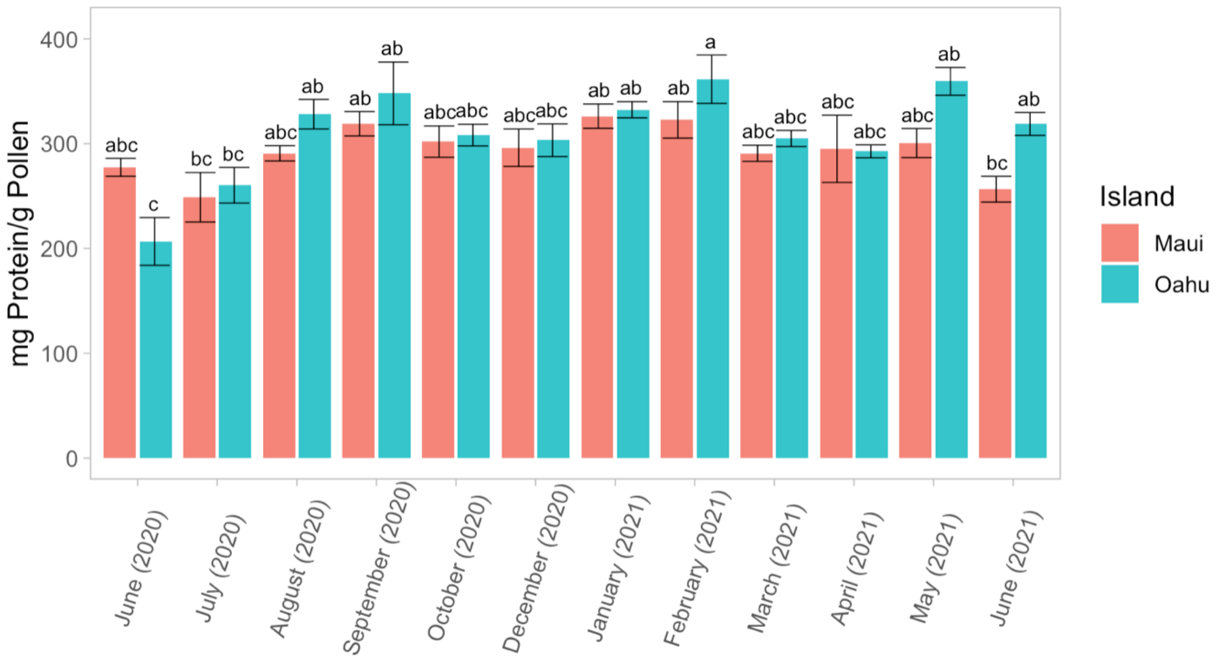


Figure 6. Mean protein (±SE) of kiawe pollen by month. Means sharing a common letter are not significantly different according to the Tukey-test with 95% confidence level ($p < 0.05$).

For lipids, analysis by two-way ANOVA indicated a significant interaction between location and time ($F = 3.22$, d.f. = 11, 100, $p < 0.001$) and significant variation in average lipid content by time ($F = 4.87$ d.f. = 11, 100, $p < 0.001$) but not by location ($F = 1.64$, d.f. = 1, 100, $p = 0.204$) (Fig 7).

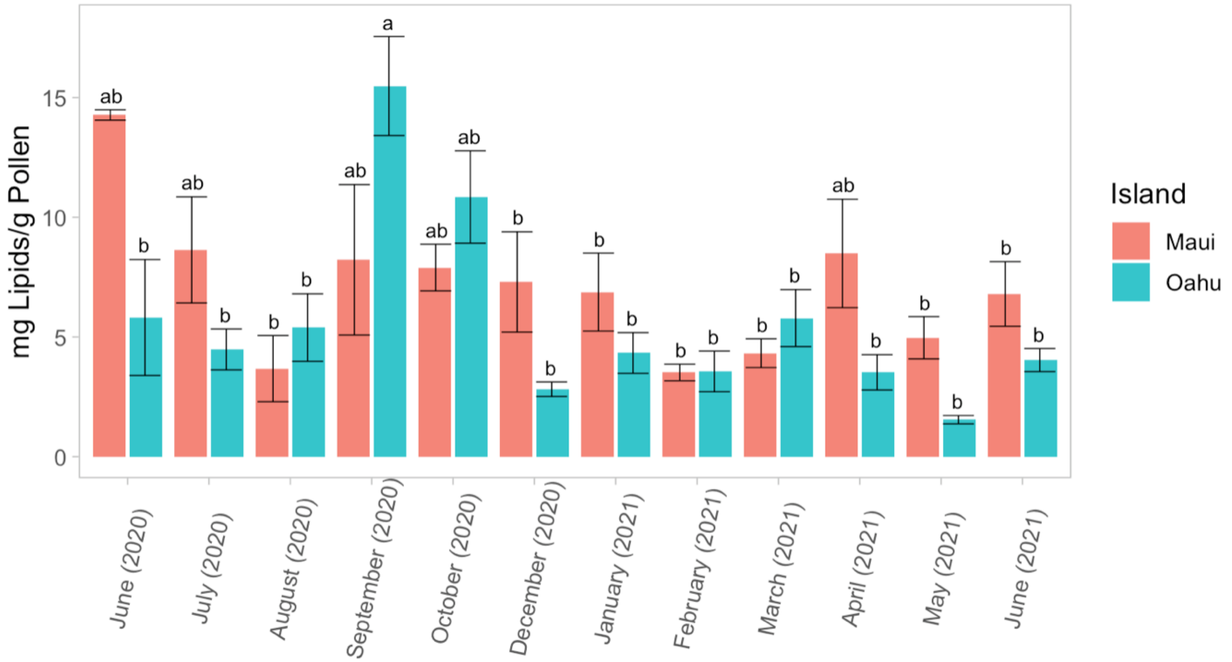


Figure 7. Mean lipid content (±SE) of kiawe pollen by month at Maui and O'ahu experimental apiaries. Means sharing a common letter are not significantly different according to the Tukey-test with 95% confidence level ($p < 0.05$).

A two-way ANOVA of pollen composition indicated significant variation in the average proportion of bee-collected kiawe pollen by location ($F = 19.09$, d.f. = 1, 92, $p < 0.001$) and by month ($F = 5.322$, d.f. = 11, 92, $p < 0.001$) (Fig 8), with no significant interaction between time and location ($F = 1.756$, d.f. = 11, 92, $p = 0.073$).

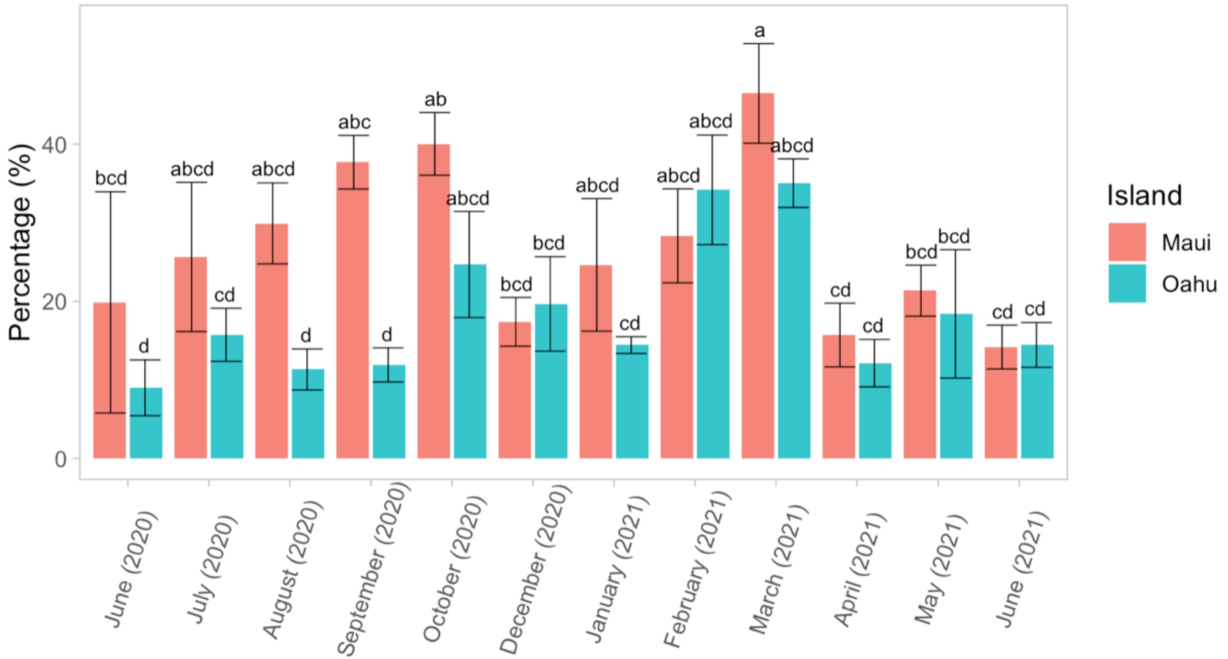


Figure 8. Average percent bee-collected kiawe pollen (±SE) by month from Maui and O‘ahu commercial apiaries. Means sharing a common letter are not significantly different according to the Tukey-test with 95% confidence level ($p < 0.05$).

3.4 Kiawe Macronutrient Correlation to Pest Levels

A correlation analysis comparing monthly varroa mite and SHB pest levels to total kiawe protein and lipid macronutrient measurements was conducted. On Maui, a significant positive correlation was indicated between SHB and kiawe lipids ($r = 0.254$, $p < 0.05$) where SHB increased as kiawe lipid content increased, as well as a negative correlation between SHB and kiawe protein ($r = -0.185$, $p < 0.05$) where SHB levels decreased as kiawe protein content increased, and between kiawe protein and kiawe lipids ($r = -0.351$, $p < 0.05$) where kiawe protein and lipid content decreased together (Appx. 1).

On O‘ahu, a significant positive correlation was found between SHB and varroa mite numbers ($r = 0.415$, $p < 0.05$) where SHB increased as varroa mites increased, as well as a negative correlation between SHB and kiawe lipids ($r = -0.352$, $p < 0.05$) where SHB decreased as kiawe lipids increased, and between varroa mites and kiawe lipids ($r = -0.325$, $p < 0.05$) where varroa mite levels decreased as kiawe lipids increased (Appx. 2).

3.5 Pollen Diversity

A diversity analysis was completed to compare both the Shannon-Weiner Diversity Index (SDI) and Pielou’s Evenness Index (PEI) for pollen species on O‘ahu and Maui. From observed bee-collected pollen, O‘ahu had a species richness (S) of 84 compared to Maui’s 37. Analysis by Hutcheson’s t-test showed a significant difference in SDI between O‘ahu ($H' = 3.81 \pm 0.13$) and Maui ($H' = 3.29 \pm 0.18$); $t_{214} = 4.61$, $p < 0.001$. PEI was not significantly lower on O‘ahu ($J' = 0.86$) compared to Maui ($J' = 0.91$).

4. Discussion

The data collections in this study came from two specific locations on the islands of Maui and Oʻahu, so while there was replication within a specific apiary, the island-wide (and thus varroa-effect) replication was missing. Additionally, only the nutritional content of kiawe pollen is reported when other pollen types may have also been important, and so the focus on kiawe in the objectives may have missed other important species.

Comparisons of pest levels showed a significant variation in SHB counts between Kīhei and Waiʻanae bee apiaries. Waiʻanae colonies experienced an increase in beetle numbers beginning in March 2021, reached a peak in April 2021, and began trending downwards until the end of the study period. Varroa mite levels had a strong correlative relationship with SHB numbers (Appx. 1) and this may explain why SHB levels in Kīhei remained low throughout the sample period, as the presence of varroa is often associated with increased susceptibility to other stressors, including SHB. A varroa infested hive may be less capable of resisting SHB, due to the feedback interaction of these stressors (Ellis and Hepburn, 2006). The SHB lifecycle includes pupation in the soil, and their population growth is limited by the suitability of the environment to sustain this life stage (Ellis et al., 2004; Meikle and Diaz, 2012). Other factors such as colony placement, beekeeper control methods, or amount of stored pollen influence SHB infestation rates. Varroa mite infestation levels also showed significant variation across time in Waiʻanae hives. Varroa mite infestations began to increase in November 2020, plateaued in January 2021, and did not significantly decrease until a treatment was administered in May 2021 in the Waiʻanae hives, a curve that closely aligns with SHB pressure.

The significant interactions detected between main effects (e.g. location, date) are not easily explained with the current data. As only a single location was sampled on each island, it is

not possible to say unequivocally whether there was true effect attributable to some factor associated with each location, or whether the observed interactions were simply the results of sampling variance within apiaries. To better elucidate possible location effects and their differences among islands, it would be necessary to conduct trials replicated by island.

Brood quality measurements showed significant variation between hives at the Kīhei and Wai‘anae study sites. Frame occupancy of adult bees also significantly varied by study site with Kīhei colony average measurements much greater than those in Wai‘anae. The disparity of frames of adult bees is possibly linked to the presence of varroa mites in Wai‘anae. As varroa invade an apiary and infestation and subsequent virus infection increases, bees that survive to adulthood are increasingly weakened, have shorter life spans, and are more susceptible to external stressors (Posada-Florez et al., 2019; Flores et al., 2021). Kīhei hives did not face this stressor and had, on average, more adult bees in the hives (Fig. 5). Average brood quality was nearly equal between locations, which is more a reflection on queen health, brood health, or adequate removal of diseased larvae than on overall colony health (Harbo and Harris, 2009). A healthy queen will move through an empty brood frame and oviposit eggs with a high degree of solidness. And as bee larva develop, so do varroa. To reproduce, a female mite will dislodge from their adult host and enter a brood cell before it is capped. The female mite then lays her eggs within the capped pupa’s cell, the eggs then hatch and feed on the developing bee pupa. When the adult bee emerges, the varroa disperse and infect more brood (Roth et al., 2020). To combat this, nurse bees will perform varroa sensitive hygiene and remove bees from mite-infested patches of brood (Kim at al., 2018). Overall, pest levels may directly influence brood health, but are likely also influenced by a number of covariates not included in this study, such as disease load (Dolezal and Toth, 2018).

Significant variation was observed in total protein content in kiawe pollen between Waiʻanae and Kīhei, and Waiʻanae collected pollen displayed significant variation over time, peaking in July and August 2021. This variation is consistent with previous studies where the protein content of bee-collected pollen varied both seasonally and geographically (Nascimento et al., 2019; Al-Kahtani et al., 2020; Simanonok et al., 2020). July and August 2020 being significantly lower than 2021 suggests that the time of year alone is not the explanatory reason for the increase in kiawe protein content observed in 2021. This is further supported by the fact that Kīhei samples did not show any significant variation in average protein content across time.

Similarly, kiawe total lipid content varied significantly over time within islands but not spatially. Similar to pollen protein, temporal variation in pollen lipids does occur (Nascimento et al., 2019) and is also more multifaceted than time of year alone (land use, precipitation, soil type, etc.). The results show that the months in Waiʻanae with peak lipid values also align with peak kiawe blooming seasons (September – October, Feb – March) (Gallaher and Merlin, 2010). The proportion of kiawe pollen in the homogenate was also significantly greater during these months compared to non-blooming months. Pollinator attraction is essential for entomophilic plants, and the evolution of the traits that attract one pollinator over another are determined by complex selective pressures. As a generalist forager, variations in bee-collected kiawe composition are driven by bee foraging preference (Roulston et al., 2000; Ghosh et al., 2020), pollen availability (Urbanowicz et al., 2020), or other influencing factors.

Comparing percentage of kiawe pollen contributing to the total incoming bee-collected pollen per hive, March showed a significant peak in both Kīhei and Waiʻanae. This increased kiawe pollen composition coincides with kiawe winter blooming periods, but do not align with any significant increase or decrease in kiawe macronutrient levels. The kiawe winter blooming

period begins in January and occurs through March, however the compositional increase was only in March on Maui and observed in February and March in Waiʻanae. However, a lower proportion of bee-collected kiawe pollen does not directly equate to a lack of kiawe availability. Lau at al. (2019) found that when abundances of Asteraceae bee-collected pollen were high during one season (summer), and decreased into the next season (fall), that foragers likely favored collecting pollen from other floral resources, rather than the availability of Asteraceae decreasing. The further ties into the diversity analyses, which showed that pollen species diversity is significantly greater in Waiʻanae (84) compared to Kīhei (37). Alpha diversity is directly influenced by evenness. Despite a higher pollen species richness in Waiʻanae, both study sites had similar evenness. Higher species richness in Waiʻanae reflects a greater availability of unique pollen species present near the study's commercial apiary, inflating the SDI, but a similar PEI indicates that these unique species were in relatively equal abundance, and Waiʻanae hives collected a similar proportion of other pollen species compared to Kīhei hives. Overall, Kīhei hives had a significantly greater percent composition of kiawe pollen, but the lower species richness contributed to a significantly lower SDI. Therefore, kiawe pollen represents a greater average proportion of a hive's pollen diet in Kīhei compared to Waiʻanae, but is more even in composition to other pollen species in Kīhei compared to Waiʻanae. It is shown that pollen nutrient content can vary by species (Roulston and Cane, 2000), and a certain level of floristic diversity can guarantee adequate nutrition for honey bees (Di Pasquale, 2016), but these concerns usually are raised when hives are in a more managed landscape (Topitzhofer et al., 2019). It seems that both the Kīhei and Waiʻanae locations had more than sufficient floral diversity and landscape heterogeneity to provide adequate colony health.

The results of this study do not support the hypothesis that periods with higher kiawe pollen macronutrient quality result in hives with improved health metrics. Hive health seemed more influenced by the presence of varroa, with hives in Waiʻanae much more stressed than hives in Kīhei. Kiawe pollen macronutrient levels did fluctuate over time for both locations, but neither protein nor lipid content seemed to be correlated with improved overall hive health, as measured by brood quality, frame occupancy, and pest levels. When total lipid levels in kiawe pollen at the Kīhei study site were highest in June 2020, neither brood quality nor frame occupancy measurements showed any significant increase in the following months. The highest kiawe lipid levels from the Kīhei samples coincided with the highest average SHB count there, both occurring in June 2020. This suggests that increased lipids in kiawe pollen increased SHB pressure, as they are scavengers, and their larval stage will tunnel through comb and eat stored honey and pollen. These relationships do not support the hypothesis that higher quality kiawe pollen improves hive health metrics. With high pollen diversity present in Kīhei, Maui and Waiʻanae, Oʻahu, isolating the fluctuations of a single pollen species, in this case kiawe, did not have a significant impact on overall hive health. When bee-collected pollen diversity is high, it is likely that factors other than pollen quality play a larger role in the complex system of overall colony health.

Chapter 2: Effects of Kiawe Pollen on Individual Honey Bee Health

Abstract: Pollen is the primary protein and lipid source for honey bee development. In Hawaiʻi, common kiawe (*Prosopis pallida,* Fabaceae) grows in the arid, leeward regions and is a widely available pollen food source for honey bees. However, kiawe pollen macronutrient quality and its effect on individual bee health is yet to be established. This study aimed to determine the impact of kiawe pollen nutrition on individual bee health in laboratory feeding trials. Pollen was collected from hives in Waiʻanae, Oʻahu. Newly emerged adult bees maintained for eleven days in a laboratory incubator were fed one of four diet treatments: Bee-Pro®, a homogenate of bee-collected pollen, kiawe pollen, and a negative control of sugar water only. Pollen consumption, sucrose consumption, and bee mortality were measured. Total bee protein, lipids, and vitellogenin were measured on days zero and eleven and compared between diet treatments as markers of honey bee health. Results showed variation in pollen and sucrose consumption across diet treatments trials (homogenate pollen was the most consumed pollen diet (2.69 ±0.12 g) across all replicates, while the BeePro diet treatment consumed the most sucrose (11.67 ±0.21 g)), and increase was observed in individual bee physiology across treatments and particularly in the August replicate. Bee mortality differed significantly between treatments and replicates, with BeePro having the highest mean percent surviving (92.03 ± 0.86) and the January replicate having the highest mean percent survival (94.99 ± 0.39) . Trials during May and January had the lowest overall mortality rate and the May trial had increased protein and lipid levels, as well as decreased C_t values, an inverse measure of the concentration of target in the PCR reaction.

1. Introduction

Pollinators, including honey bees, provide a pollination service that is essential to the reproduction of many plants and the health of an ecosystem. Pollinators receive floral rewards for this service as pollen and nectar, which are necessary food sources for their health and survival. As the primary protein source for honey bee development, the availability, quality, and quantity of a bee's pollen diet determine both its individual health and the overall health of the entire colony (Frias et al., 2016). Pollen is also the major source of lipids for honey bees, necessary for their reproduction, development, and nutrition (Manning, 2001). An additional component of honey bee health established through pollen consumption is vitellogenin (Vg), a specific egg-yolk precursor protein. Created in the fat bodies, Vg provides a number of immunological functions that protects and maintains the bee's cells from pathogenic bacteria and fungi (Harwood and Amdam, 2021). Therefore, the survival of honey bees is tethered to the availability of these floral resources. Honey bees are generalist foragers and will collect pollen and nectar from a wide range of flowering crop plants, native flora, and invasive weeds. However, pollen nutritional levels (measured as protein and lipid levels) differ among floral species (Roulston and Cane, 2000), and depending on the species, pollen protein levels can range anywhere from 2.5 to 61% (Liolios et al., 2016; Hanley et al., 2008; Roulston and Cane, 2000). Several studies found that honey bees deal with this variability in available resources by balancing their nutritional uptake using both taste and smell characteristics of forage plants (Linander et al., 2012; Hendriksma and Shafir, 2016; Ruedenauer et al., 2020; Vaudo et al., 2020). When bees feed on low quality pollen, and a low diversity of pollen it reduces their growth and development (Schmidt et al., 1987; Frias et al., 2016) and increases their sensitivity to diseases (Naug, 2009) and pesticides (St. Clair et al., 2020). Foraging within low diversity

systems may be linked to insufficient protein and lipid intake and limits honey bee growth and fitness as a result (Filipiak et al., 2017). Additionally, as species vary by system, so do their nutritive qualities, and some can be worse for bees than others. Moerman et al. (2017) found that bumble bees may receive sufficient nutrition from some monoculture diets such as *Cytisus scoparius* (Fabaceae, common broom) and *Sorbus aucuparia* (Rosaceae, mountain-ash) compared to a diet of *Erica* sp. (Ericaceae, heather family) pollen which produced smaller larvae. Depending on the plant species, bees may be provided with only a select portion of their necessary nutrition requirements if depending on a single species (Egan et al., 2018; Knapp et al., 2019). Therefore, diversity of the pollen is important when quality is lacking.

In Hawaiʻi, the non-native, common kiawe (*Prosopis pallida*) stands are commonly established as the dominant vegetation type in the islands' leeward, rain-shadowed regions due to its ability to outcompete other tree species (Dudley et al., 2014). Kiawe flowers year round but has two distinct blooming seasons in Hawaiʻi: January to March and September to October. As shown in Chapter 1 results, there are months during the year where kiawe is more than 40% of a leeward apiary's total pollen supply. Despite being a significant food source for honey bees situated in these regions, the impact of kiawe's nutritional quality on individual bee health is unknown. This study aimed to determine the effect of an unvaried kiawe pollen diet on worker honey bee physiological health and survivability when compared to a more diverse pollen diet. I hypothesized that honey bees fed a monofloral kiawe pollen diet will have lower physiological health (protein, lipid, and Vg levels) compared to bees fed multifloral pollen or a commercial substitute.

2. **Materials and Methods**

2.1 Experimental Apiary

Twelve colonies from a commercial apiary in Wai‘anae, O‘ahu were used to supply the pollen and adult bees used to conduct this research. Six hives were equipped with bottom mount Sundance pollen traps (Ross Rounds, Canandaigua, NY) and deployed each month for a period of 48 hours, collecting approximately 70% of pollen foraged by bees for that period. Brood frames with upright eggs (indicating same-day laying) from similar strength, queen-right colonies were marked. After twenty days (worker bee development from egg to adult emergence is 21 days (Human et al., 2013)), marked frames with capped pupae were removed and transported to a laboratory incubator and maintained at 34ºC and 70% relative humidity, where adults emerged overnight (Human et al., 2013; Williams et al., 2013).

2.2 Laboratory Feeding Trials

Newly emerged hive bees were randomly assigned to cages (50 bees/cage) and maintained in an incubator at 32.5ºC and 70% relative humidity. Cages consisted of 12 oz cardboard cups (Eco-Products, Boulder, CO) with a cheesecloth cover held in place with a rubber band. The workers were fed a 50% (vol/vol) sugar solution from two drip feeders that were weighed and refilled daily. Some drip feeders were faulty and spilled their entire contents over the first day and were replaced with functioning feeders. The caged workers were supplied with one of the following diet treatments: a homogenate of bee-collected pollen, kiawe pollen, Bee-Pro® pollen substitute (Mann Lake, Hackensack, MN), or a negative control with sugar water only. Homogenate pollen was gathered from bottom mount Sundance pollen traps deployed monthly at the apiary and stored frozen at -20ºC until use. Kiawe pollen was hand-sorted from excess homogenate pollen based on color (validated with palynology and a reference

library). Bee-Pro was chosen as the protein supplement to compare the data to similar studies of honey bee feeding trials that used this product as a comparison to bee-collected pollen (Watkins de Jong et al., 2019; Fleming et al., 2015; Azzouz-Olden et al., 2018). The nutritional value of pollen has been reported to not degrade within the first year of frozen storage and does not negatively affect the lifespan of caged workers (DeGrandi-Hoffman et al., 2010). All three diet treatments were mixed at a ratio of 90% (weight/weight) pollen with water to form a paste and provided to the bees in a modified 1.5mL tube (ThermoFisher Scientific, Waltham, MA) with both ends open. These pollen tubes were weighed on days four, eight, and eleven to assess change in mass to quantity pollen consumption, and refilled on days four and eight of the trials. Each treatment and control consisted of five replicate cages for a total of 20 cages per trial (five cages x four feeding treatments). Trials were conducted every ten weeks in October 2020, and January, March, May, and August 2021. The pollen used in each trial was collected within two months of the start of each respective trial. Bee mortality was recorded throughout the trial and dead bees removed daily.

2.3 Vitellogenin analysis

Ten bees were removed at t=0 (initial) from the pool of bees to establish a baseline concentration of protein, lipid, and vitellogenin (measured in cycle threshold (C_t), a relative inverse concentration of target in the PCR reaction). Each feeding trial lasted 11 days, corresponding to a worker bee's division of labor periods of cell cleaning (ages 0 – 4 days) and nursing (ages 4 – 12 days). Five live bees per cage were removed for analysis from all cages after days 4 and 11 (Johnson, 2010). Samples were frozen at -20ºC until dissection.

The abdomen was separated from each bee and the alimentary canal and stinger apparatus removed. Dissected samples were stored at -80ºC until analysis. Within each feeding

trial, a single dissected bee from each of the five treatment replications for days 4 and 11 was randomly selected and pooled for analysis, for a total of 45 samples of 5 bees per sample (4 feeding treatments x 2 sampling time points x 5 replicate trials, plus one t=0 sample per replicate trial). Samples were homogenized in 1 mL RNase-free water using a Bead Mill 4 Homogenizer (ThermoFisher Scientific, Waltham, MA) at 5 m/s for 3 minutes. 750 µl of the homogenate was transferred to a 1.5 mL tube for vitellogenin (Vg) analysis, and 250 µl reserved for protein and lipid analyses.

For Vg analysis, RNA was extracted from samples using TRIzol reagent (ThermoFisher Scientific) following the manufacturer's instructions. RNA quantity and quality were determined using a NanoDrop 2000 (ThermoFisher Scientific). Prepared RNA was stored at -80ºC unless immediately analyzed.

Following RNA extraction and quantification, gene transcript levels of vitellogenin were analyzed as an indicator of general health (Simone et al., 2009). Actin was also analyzed for target gene normalization (Chen et al., 2019), acting as a stable reference to analyze the relative expression levels of the target gene, Vg. cDNA was synthesized with total RNA using SuperScript IV VILO Master Mix (ThermoFisher Scientific) following the manufacturer's instructions. Reverse transcriptase-quantitative PCR (RT-qPCR) for Vg and actin was then conducted on all homogenized samples (n = 45) using GoTaq Probe qPCR Master Mix (Promega, Madison, WI) and CXR Reference Dye (Promega) with a QuantStudio3 thermocycler (ThermoFisher Scientific). Primers for Vg and actin (Table 1) amplification were added and each sample was run in duplicate using the following protocol: 95 ºC for 1 min; and then 40 cycles of 95 ºC for 15 s and 60 ºC for 1 min.

Primer name	Sequence 5’ to 3’
Actin-F	TTGTATGCCAACACTGTCCTTT
Actin-R	TGGCGCGATGATCTTAATTT
VgMC-F	AGTTCCGACCGACGACGA
VgMC-R	TTCCCTCCCACGGAGTCC

Table 1: Primers for Actin and Vitellogenin (from Simone et al. 2009)

2.4 Protein and lipid analysis

Total bee protein was measured using a BCA Protein Assay Kit (ThermoFisher Scientific), following the manufacturer’s instructions for a 10x dilution of the sample homogenate. Samples were compared to a standard protein curve of 0, 125, 250, 500, 750, 1000, 1500, and 2000 µg/mL. Absorbance was measured in duplicate at 595 nm using a Biotek Synergy plate reader (BioTek Instruments, Winooski, VT).

Total lipids for each sample were measured using a phosphoro-vanillin assay adapted from Vaudo et al. (2020) and originally described by Van Handel and Day (1988). A 25 µl subsample of the pollen homogenate was added to 300µl 2:1 methanol:chloroform and centrifuged for 4 minutes at 15,000 rpm. The supernatant was transferred to a 10x75 mm glass tube and the solvent evaporated on a heat block at 90ºC. Forty µl of sulfuric acid was added to each tube and incubated at 90ºC for 2 minutes. Samples were then cooled on ice for 1 minute, and then 960 µl of phosphoro-vanillin reagent (600 mg vanillin dissolved in 100 mL ultrapure water, diluted to 500 mL total volume with 85% phosphoric acid) was added, vortexed briefly, and the solution incubated at room temperature for 25 minutes. Absorbance was measured in duplicate at 525 nm and compared to a standard lipid curve of 0, 1, 5, 10, 25, 50, 75, 100 µg (from 54 µl olive oil in 50 mL of chloroform) (Vaudo et al., 2020, supplementary materials).

2.5 Statistical analysis

Protein feeding treatment and sugar water consumption were tested for normality and homoscedasticity and analyzed with two-way ANOVA with treatment factors of diet and trial replicate, followed with a post-hoc Tukey-test . Survival data for the laboratory feeding trials were analyzed using mixed-model ANOVA. Protein, lipid, and Vg levels were evaluated as pooled samples of five bees each fed different diets of pollen blends or a control diet. All analyses were performed using the statistical software R (version 3.6.2).

3. **Results**

3.1 Caged bee diet consumption

Analysis by two-way ANOVA indicated significant interaction between diet treatments and trial replicates (F = 3.69, d.f. = 8, 57, p = 0.002), as well as significant difference in pollen consumption between trials (F = 47.36, d.f. = 4, 57, p < 0.001) and between treatments (F = 51.34, d.f. = 2, 57, p = <.001). Pollen consumption was highest during August (3.07 ± 0.09 g) and lowest during January (1.89 ± 0.04 g). Pollen consumption by treatment was highest among homogenate diets (2.69 ± 0.12 g), followed by kiawe (2.33 ± 0.09 g), with Bee-Pro (1.95 ± 0.09 g) diets consuming the lowest amount (Fig. 1).

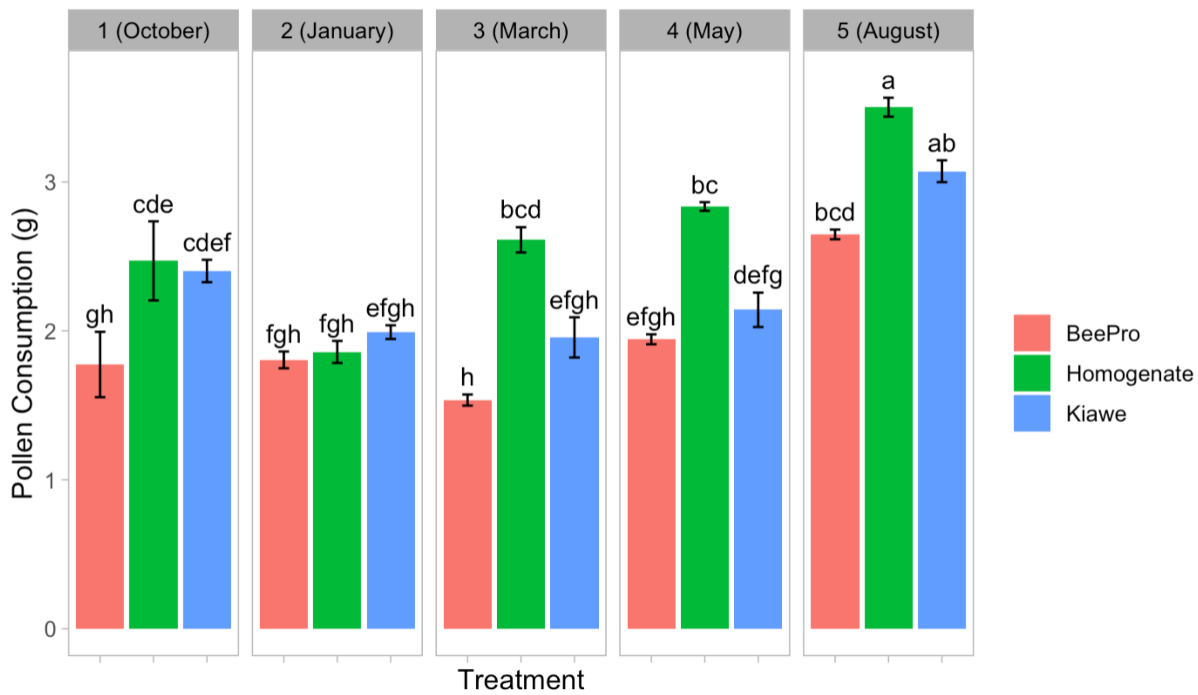


Figure 1. Mean consumption of pollen in grams (± SE) of caged *Apis mellifera* over 11 days in the laboratory. Means sharing a common letter are not significantly different according to the Tukey-test with 95% confidence level (p < 0.05).

Analysis by two-way ANOVA indicated significant difference in sucrose consumption between trials (F = 7.06, d.f. = 4, 76, p < 0.001) but not between treatments (F = 1.56, d.f. = 3, 76, p = 0.201) with no significant interaction (F = 1.09, d.f. = 12, 76, p = 0.38). Sucrose consumption was highest during January (12.21 ± 0.36 g) and lowest during March (10.74 ± 0.72 g). Variation in mean consumption by protein feeding treatment was not statistically significant between the four diets (Bee-Pro (11.67 ± 0.22 g), homogenate (11.13 ± 0.23 g), kiawe (11.15 ± 0.21 g), negative control (11.11 ± 0.30 g)) (Fig. 2).

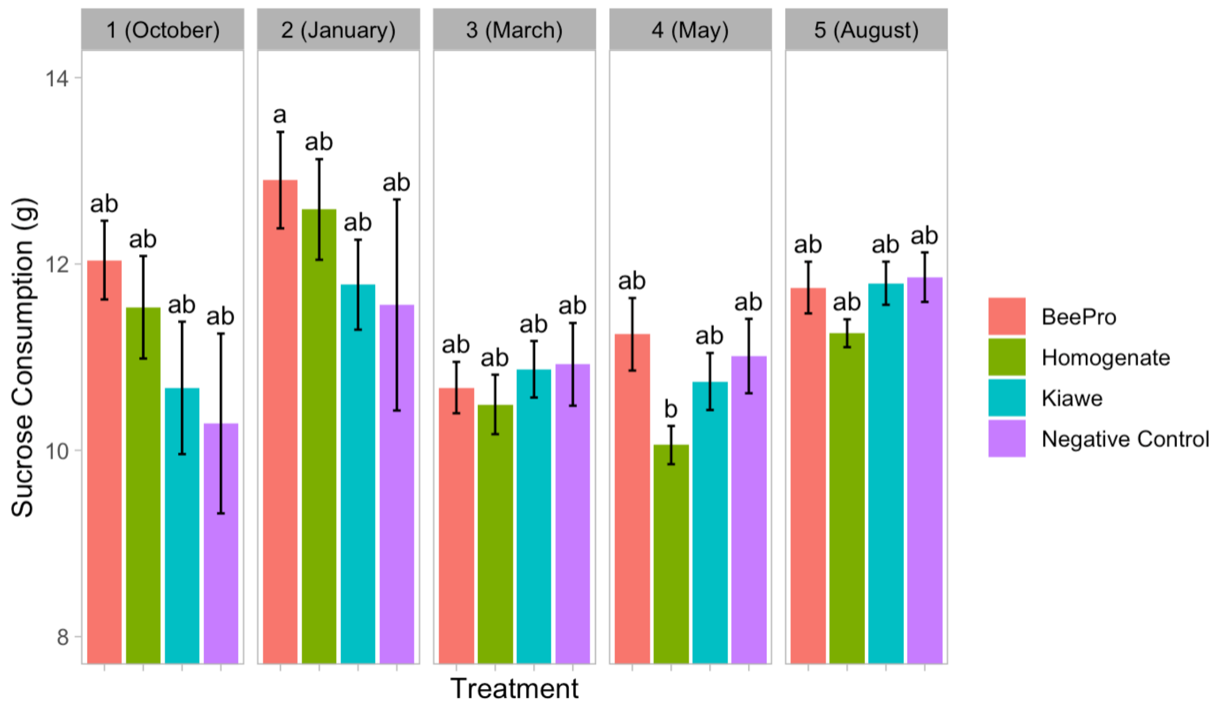


Figure 2. Mean consumption of sucrose in grams (±SE) of caged *Apis mellifera* over 11 days in the laboratory. Means sharing a common letter are not significantly different according to the Tukey-test with 95% confidence level (p < 0.05). Overall treatment comparisons are indicated by capital letters and within treatment comparisons with lowercase letters.

3.2 Caged bee survival

Analysis by mixed-model ANOVA indicated a significant interaction between trials and treatment ($F = 11.076$, d.f. = 3,240, $p < 0.001$) and significance for all main effects of treatment, trial (month), and day (Fig. 3).

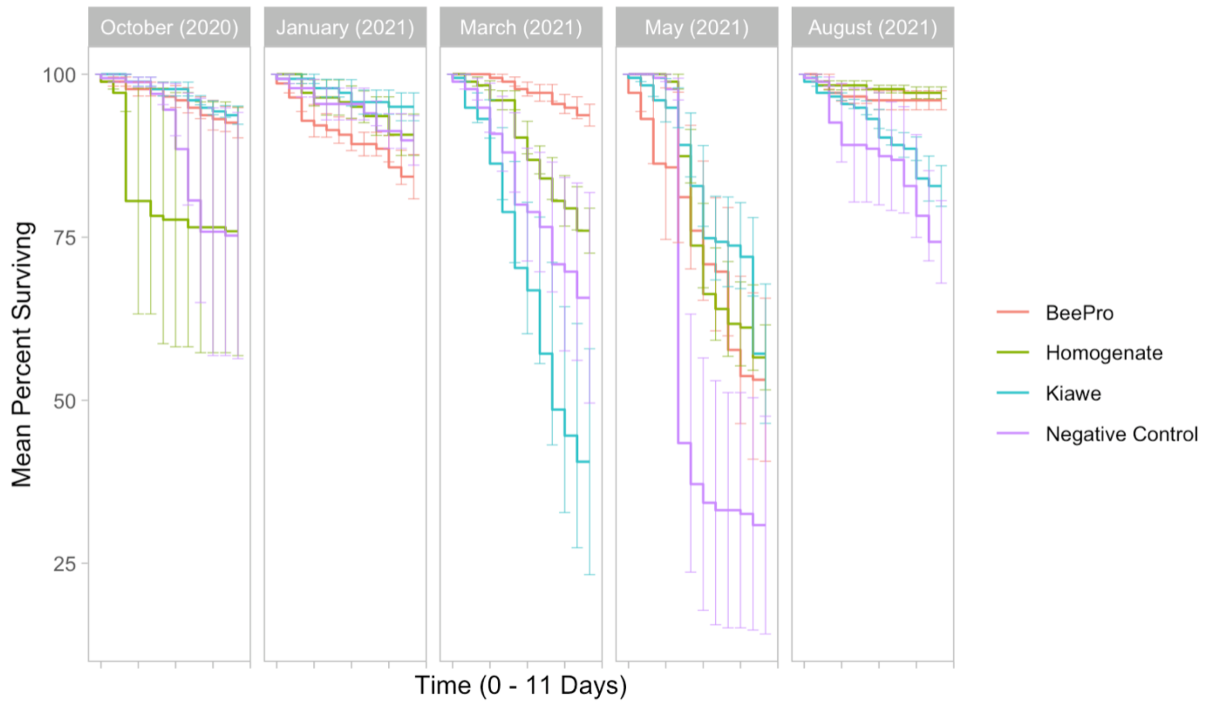


Figure 3. Survival curves of mean daily survival (±SE) of caged *Apis mellifera* over 11 days in the laboratory.

3.3 Protein and Lipids

Protein levels in µg x 1000 / bee by trial and diet treatment are shown in Fig. 4 and lipid levels in µg / bee by trial and diet treatment presented in Fig. 5.

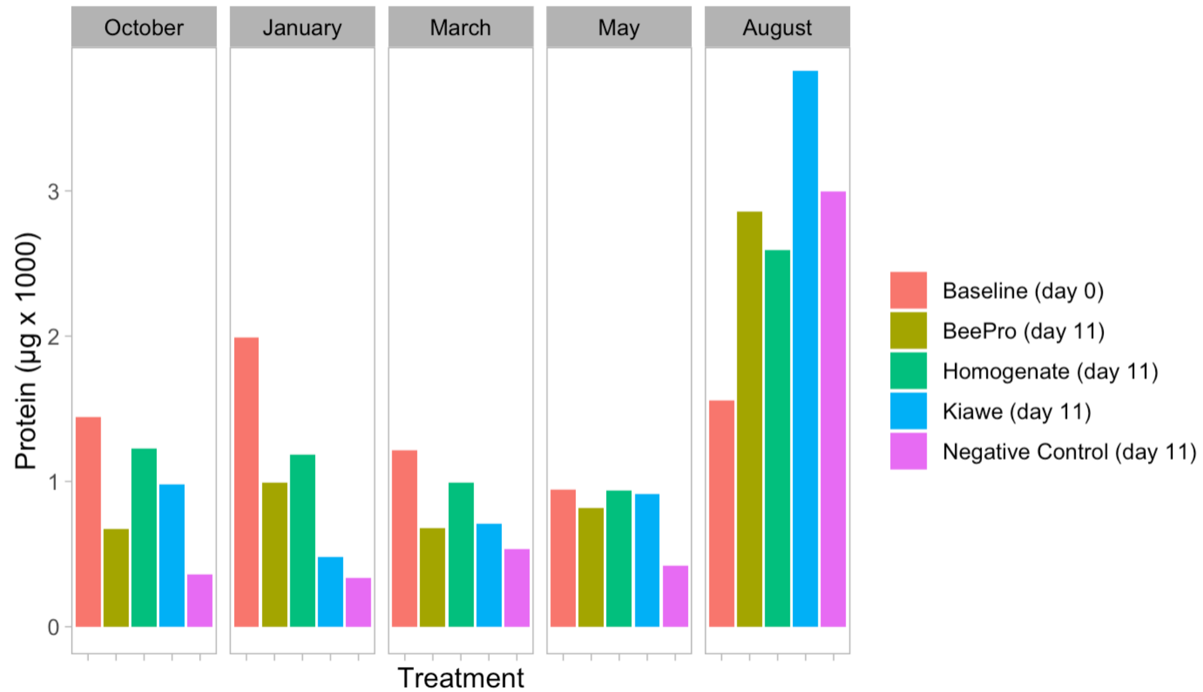


Figure 4. Measured protein levels in µg x 1000 of caged *Apis mellifera* on days 0 (baseline) and 11 in the laboratory for pooled samples of five bees each fed different diets of pollen blends or a control diet.

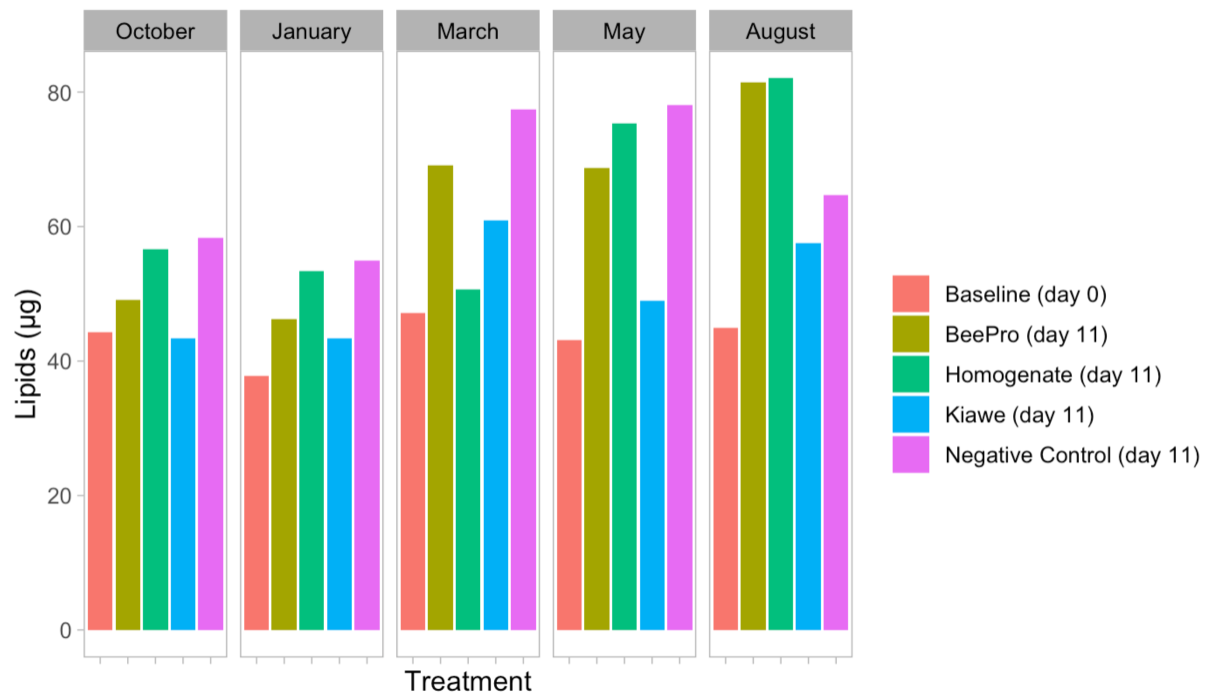


Figure 5. Measured lipid levels in μg of caged *Apis mellifera* on days 0 (baseline) and 11 in the laboratory for pooled samples of five bees each fed different diets of pollen blends or a control diet.

3.4 Vitellogenin

Figure 6 shows the measured C_t value, a proxy for cDNA abundance, by trial and diet treatments.

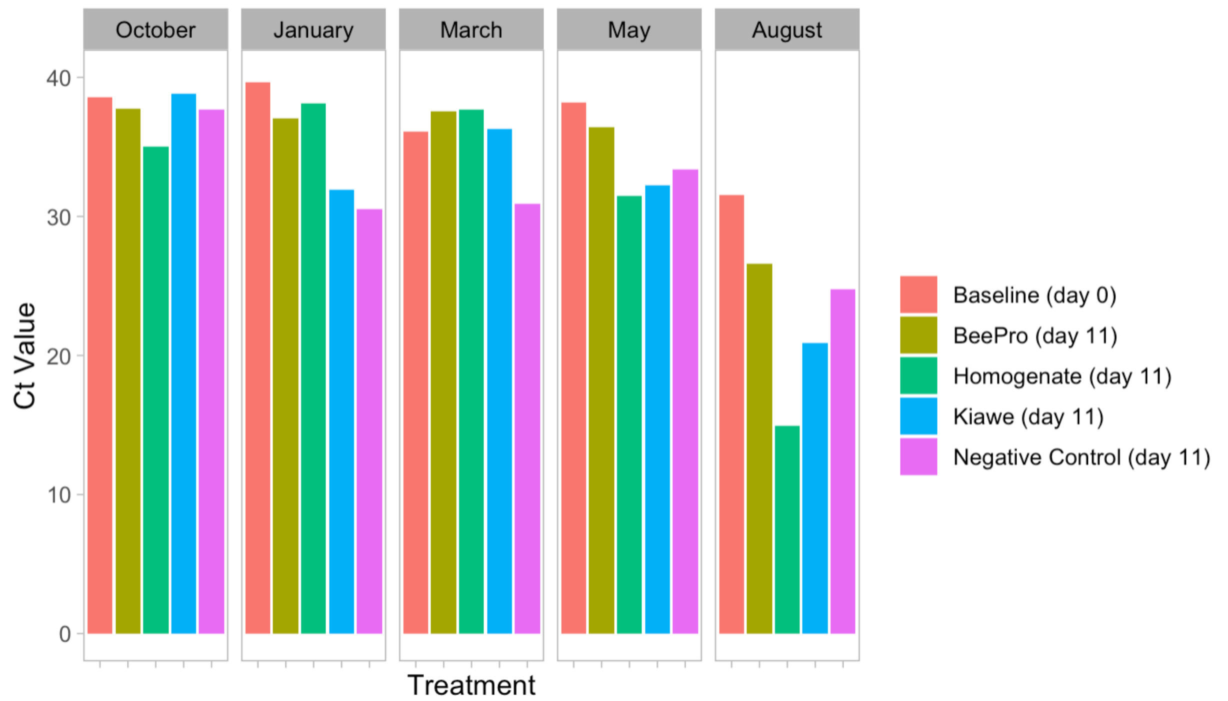


Figure 6. Vitellogenin C_t values of caged *Apis mellifera* (n = 5 per treatment) on days 0 and 11 in the laboratory for pooled samples of five bees each fed different diets of pollen blends or a control diet.

4. **Discussion**

The bees used in this study came from one apiary location in Waiʻanae, Oʻahu, and therefore the results of the study are limited to the condition of those hives in that location at a particular point in time. Additionally, the quality of Bee-Pro and homogenate pollen was not measured across the five feeding trials to compare differences in nutritional content of their diets. With this comparison missing, it is difficult to discuss if observed variations in bee health across trials is from a varying diet quality or from another variable that was unaccounted for. This discussion recognizes these limitations, and it is hoped that this study can encourage further work analyzing specific aspects in greater depth.

The results of this study suggest that seasonality can influence individual bee health and that nutritional composition matters. When comparing different feeding trials of newly emerged hive bees reared in the laboratory incubator, results showed that bees consumed on average more pollen during the May 2021 trial than any other trial and increased carbohydrate consumption during the January 2021 trial. Consumption increases corresponded to specific time of year, and may be better understood when considering an epigenetic effect, where emerged bees behave according to the season they are reared in. Pollen and nectar availability do vary in space and time, and are influenced by abiotic factors (rainfall, sunlight, nutrients) (Cao et al., 2017; Lawson and Rands, 2019) and biotic factors (pollinator visitation and nectar replacement rates) (Edge et al., 2011). However, the observed consumption increases are likely explained by honey bee physiology; increased pollen collection during the spring blooming period (Lau at al., 2019), and increased nectar collection in preparation for the overwintering period (Nürnberger and Steffan-Dewenter, 2019).

The same trials that had increased carbohydrate and pollen consumption also had fewer average daily deaths and higher average surviving bees at the end of the 11-day study. Proteins are known to increase survival (Keller and Imdorf, 2005; Brodschneider and Crailsheim, 2010), and carbohydrates are necessary to meet a honey bee's energy needs (Rodney and Purdy, 2020). Increased consumption of either resource improved survivability in these experiments, including the negative control treatment where pollen was unavailable, suggesting time of year may influence bee's feeding behavior.

Trials where bees had increased pollen consumption also resulted in increased bee macronutrient content. When pollen consumption was highest in trial 5 (Fig. 1), bee protein levels were higher than any other trial, especially seen in kiawe and Bee-Pro treatments. As not all pollens have the same macronutrient quality (Ghosh et al., 2020; Liolios et al., 2016) and quality can vary seasonally (Di Pasquale et al., 2016), this suggests a combination of seasonal change in pollen quality or increased consumption improved bee protein macronutrient levels. Furthermore, homogenate diet treatments did not have as high a measured increase in bee protein levels but did result in an increase on measured bee lipid levels. When pollen and carbohydrate consumption was significantly lower in trials (trials 1 – 4 for pollen; 1 and 3 – 5 for carbohydrates), bee lipid levels were comparable across all trials. This is perhaps explained by their pollen diet not significantly influencing adult bee macronutrient profiles over an 11-day period for these treatments. A study by Nicholls et al. (2021) found that newly emerged adult bees have their nutritional status established by what they ate during their larval stage, as a single larva will consume 25 - 37.5 mg of protein from pollen prior to pupation and colonies will terminate their brood rather than produce malnourished pupae (Brodschneider and Crailsheim, 2010). Despite the caged adult bees not being fed any pollen diet the negative control treatment

in trial 5 had a higher measured level of bee protein, further supporting the findings of these studies. Additionally, the negative control treatment bee lipid levels measured the same as, and sometimes higher (trials 2 and 3) than all other diet treatments, suggesting bee lipid levels may be stabilized by larval stage consumption. This stabilization raises an issue of controlling across trials, however as seen in Di Pasqaule et al. (2016) the pooling and redistributing of same-day emerged adult bees was the best known method for controlling nutritional status within and across trials. A future study that controls the in-hive larval diet and tests their nutritional status across seasons before emerging would be needed to confirm this.

Vitellogenin (Vg) expression showed similar patterns to measured bee protein levels. When pollen consumption was highest during trial 5, Vg C_t levels measured much lower than the other four trials (levels are inversely proportional to amount of Vg nucleic acid; a lower C_t value indicates a greater abundance of Vg in the sample). The initial measurement of Vg for trial 5 was lower than the other 4 trials, which further supports that larval diet influences adult bee health metrics. Di Pasquale et al. (2013) reported that nurse bee Vg expression when feeding on a mixed pollen diet did not significantly differ from any of the pure pollen diets provided (*Cistus*, *Erica*, *Castanea*, *Rubus)*, but all diets had significantly greater expression compared to a negative control diet. As seen in Figure 6, the sugar water negative control was not consistent with their study (Di Pasquale et al. (2013)) at all times of the year. The August trial had increased Vg expression, especially in kiawe and homogenate diet treatments, while all other trials had comparable levels comparable to the initial state. Di Pasquale et al. (2016) did a further study that looked at pollen quantity and Vg expression levels, finding that as oilseed rape pollen quantity increased for caged honey bees, the expression level of Vg was significantly higher. While pollen quantity remained the same in my study, increased consumption showed a

relationship with increased Vg expression level in caged bees. Future studies manipulating the quantity of available pollen diet by season may continue to show similar trends to Di Pasquale (2016).

In conclusion, honey bee physiology in a caged environment do vary seasonally and seem heavily influenced by bee phenology in the hive they originate from. The late summer season produces bees with an increase in pollen consumption, and additionally shows improved bee protein levels, lower mortality, and increased Vg concentration. Similarly, the late fall season/winter season bees increased carbohydrate consumption and had a decrease in mortality across all diet treatments. Kiawe pollen appears to provide enough nutritional value, as measured by impact on adult bee health and longevity. Most importantly, consuming a steady diet of either carbohydrates, pollen (monofloral or homogenate), or a pollen substitute can maintain bee health, and is especially important during seasons when bee colony phenology does not prompt increased consumption. As seen in Chapter 1 results, apiaries situated within a kiawe dominated habitat in Hawaiʻi can have up to 40% composition of kiawe pollen. While many studies show that pollen diversity is better for bee health (Kang et al., 2015; Moerman et al., 2017; Omar et al. 2017), during certain times of the year kiawe alone may be adequate to provide the nutrition needed for increasing hive bee macronutrient levels and maintaining overall survival. A survival study comparing various diet ratios of commercially available pollen substitutes to bee-collected pollen supplementation could be insightful, as Bee-Pro is intended to be a supplement and not the sole source of protein and lipids for honey bees in the field.

APPENDIX

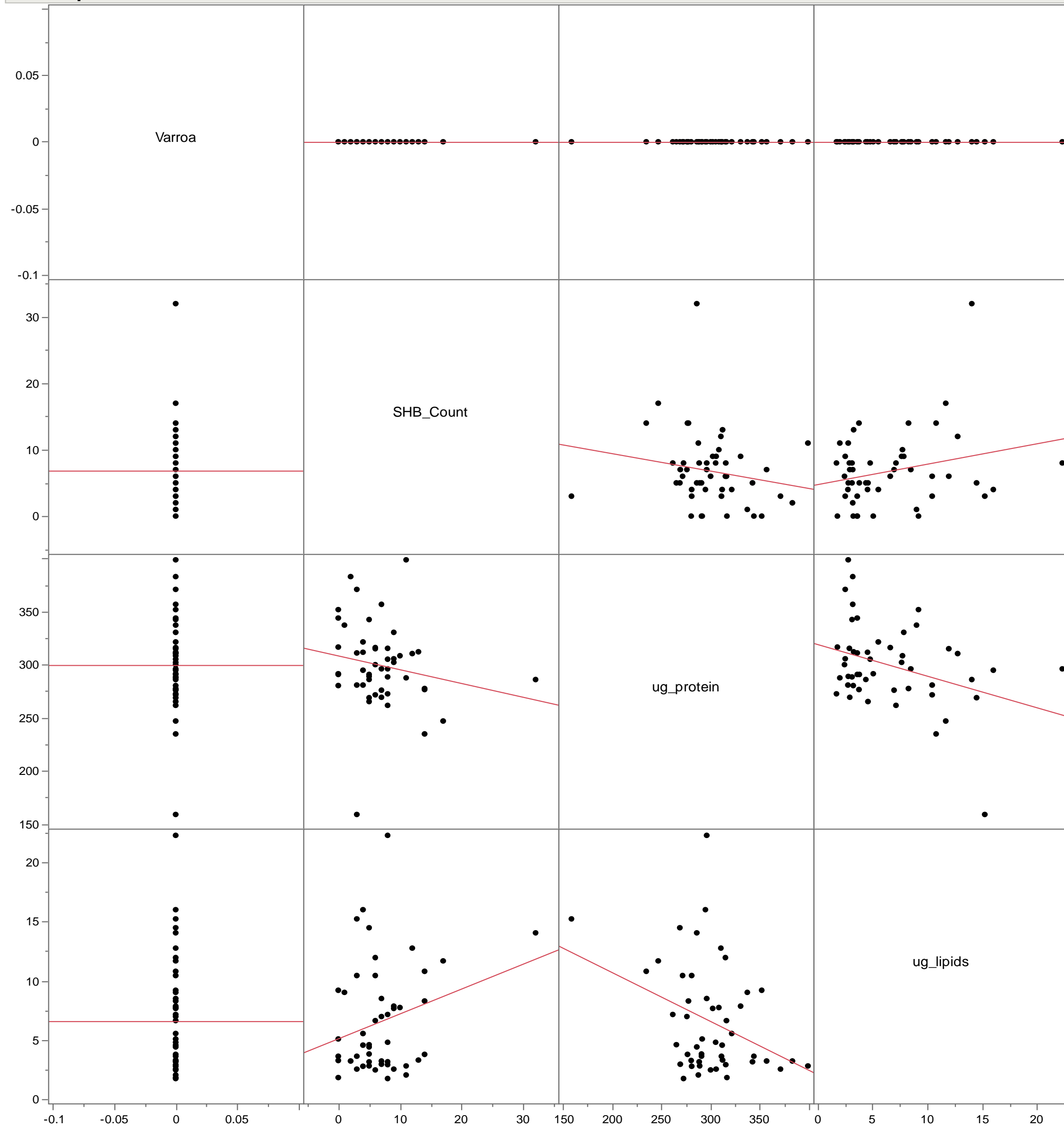
Multivariate Location=Maui, Color_ID=kiawe

Correlations

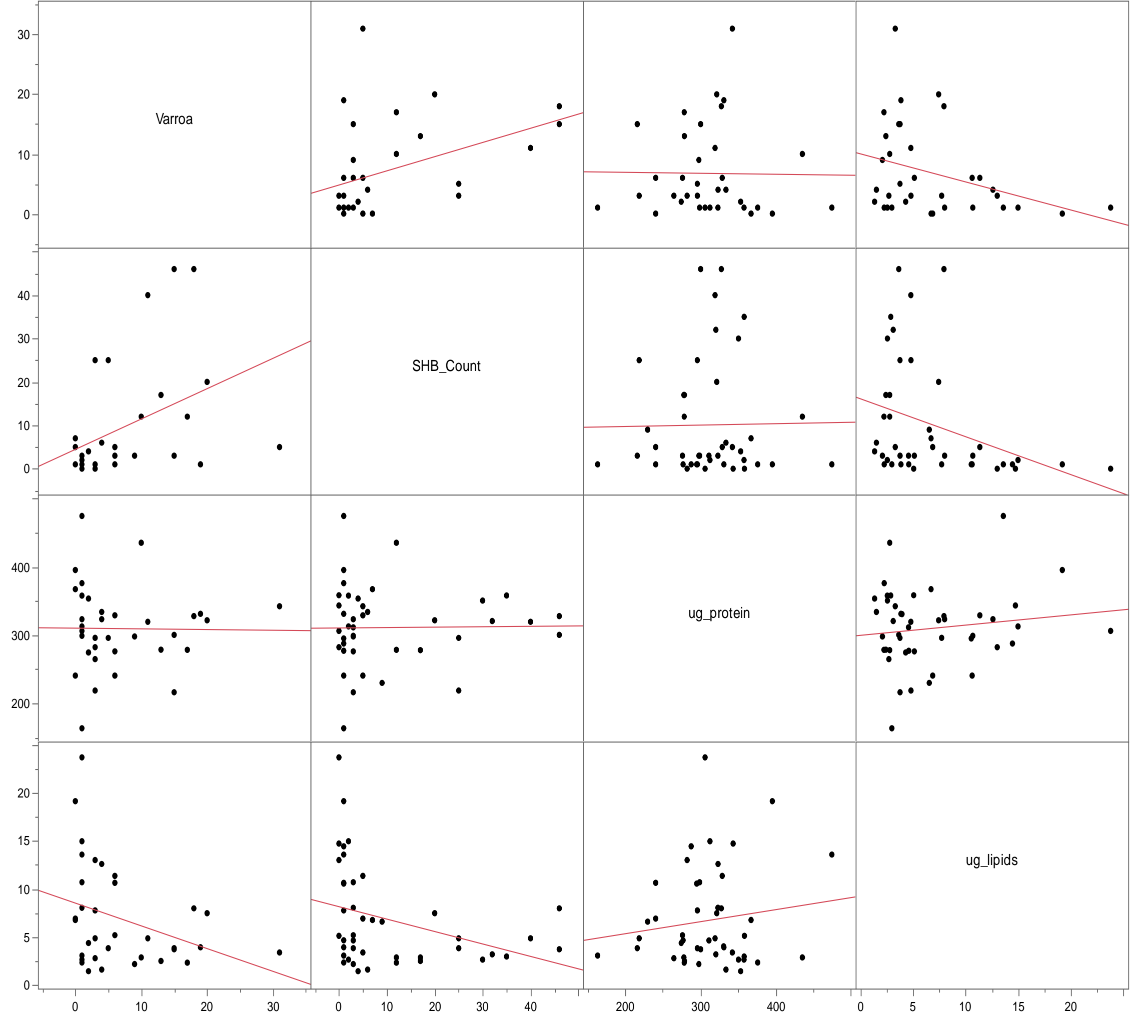
	Varroa	SHB_Count	ug_protein	ug_lipids
Varroa	1.0000	0.0000	0.0000	0.0000
SHB_Count	0.0000	1.0000	-0.1848	0.2544
ug_protein	0.0000	-0.1848	1.0000	-0.3511
ug_lipids	0.0000	0.2544	-0.3511	1.0000

The correlations are estimated by Row-wise method.

Scatterplot Matrix



Appendix 1. Correlation analyses matrix between pest levels and kiawe macronutrients on Maui. A shaded correlation coefficient value indicates significance ($p < 0.05$) (blue = positive correlation, red = negative correlation). Axis labels (x and y labels) are provided in the diagonal cells and should be read above and below the diagonal cells, as well as to the left and right of the diagonal cells.



Multivariate Location=Oahu, Color_ID=kiawe

Correlations

	Varroa	SHB_Count	ug_protein	ug_lipids
Varroa	1.0000	0.4145	0.0207	-0.3522
SHB_Count	0.4145	1.0000	0.0159	-0.3246
ug_protein	0.0207	0.0159	1.0000	0.1377
ug_lipids	-0.3522	-0.3246	0.1377	1.0000

There are 15 missing values. The correlations are estimated by REML method.

Appendix 2. Correlation analyses matrix between pest levels and kiawe macronutrients on O‘ahu. A shaded correlation coefficient value indicates significance ($p < 0.05$) (blue = positive correlation, red = negative correlation). Axis labels (x and y labels) are provided in the diagonal cells and should be read above and below the diagonal cells, as well as to the left and right of the diagonal cells.

CONCLUSION

This research aimed to identify the seasonal macronutrient content of kiawe pollen and assess its impact on overall colony health and individual bee health. Based on the results of this study, it can be concluded that bee-collected kiawe pollen has variability in protein and lipid content that vary temporally and spatially, and this is supported by several studies. Seasonal kiawe pollen macronutrient variations do not appear to play a direct role in improving colony health metrics, which are likely influenced more by other contributing factors such as pest pressure, beekeeper management, or other weather and climate variables.

Individual bee health also varied temporally, with fluctuations in measured protein, lipids, and vitellogenin depending on the time of year the caged trial took place. The greatest increase in individual bee protein and Vg expression aligned with the same season that kiawe pollen composition and total proteins were significantly higher than other months of the year (July and August). During certain times of the year, kiawe alone may be able to provide the nutrition needed for increasing nurse bee macronutrient levels and maintaining overall survival. At the two study sites on Maui and O'ahu, colonies were never faced with a true monoculture of kiawe. Even as pollen composition approached nearly half of a colony's incoming pollen diet, the availability of other flowering options likely stabilized their diet to the point that fluctuations in kiawe pollen quality and composition did not subsequently impact the health metrics that were measured in this study. Kiawe is a floral resource among many available to bees in Hawaii, and despite its dominant presence in Hawaii's dry, leeward regions, it does not drive any drastic seasonal influence on bee health but remains a valuable food source for bees situated in these regions. Ultimately, this project was a study of two specific apiaries in a small region of two islands in Hawaii. In an ideal world, more replicate apiaries across these two islands would have allowed a replicated spatial analysis at honey bee health in Maui and O'ahu. This would permit a

robust analysis of the interaction between Varroa mite infestation (or lack thereof), nutrition, and bee colony health. The current study thus provides a first approximation of the possible effects of these factors on bee health, highlighting potential future aspects that should be addressed in larger replicated studies.

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