AN ABSTRACT OF THE THESIS OF

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Title: Effects of pollen collected by honey bees from pollination dependent agricultural cropping systems on honey bee nutrition

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Managed honey bee (*Apis mellifera* L.) colonies are important pollinators of many cultivated crops. Honey bee colony declines averaging 30% annually in the United States for the past 7 years have caused significant concern and hence have been a topic of intensive investigation. These declines are reportedly due to multiple factors. Poor nutrition, which may be a result of current migratory pollination practices, is one such factor. Migratory pollination is a common practice of beekeepers from the Pacific Northwest and involves the placement of managed honey bee colonies within a series of cropping systems. There is a gap in knowledge on how migratory pollination practices impact honey bee nutrition. To understand the potential impacts of migratory pollination on honey bee nutrition, it is critical to assess the diversity of pollen collected by bees when colonies are placed adjacent to these cropping systems.

In this study, we describe the diversity of pollen collected by honey bee colonies managed by beekeepers in the Pacific Northwest region of the United States when placed in seven major cropping systems. We quantified the percent of target crop pollen and overall diversity of pollen collected by honey bees when colonies were placed in these cropping systems. We collected and identified pollen in almond (*Prunus dulcis* (Mill.)), cherry (*Prunus avium* L.), highbush blueberry (*Vaccinium corymbosum* L.), meadowfoam (*Limnanthes alba* Benth.), white clover (*Trifolium repens* L.), radish (*Raphanus sativus* L.), and carrot (*Daucus carota* (Hoffm.)) cropping systems. We found

that pollen collected from colonies placed in almond cropping systems was predominately *Prunus* sp., and hence, low in pollen diversity. At the other end of the spectrum, pollen collected from colonies placed adjacent to blueberry cropping systems did not yield any target pollen types (Highbush blueberry, *Vaccinium corymbosum* L.), but was high in overall pollen diversity. The pollen collected from colonies placed in other cropping systems was largely intermediate in diversity between these two extremes. There were not many plant species in bloom when pollen was collected from colonies placed in almond cropping system, whereas more plants were in bloom when we collected pollen from colonies placed in blueberry cropping system. The results of this study demonstrate that honey bees collected pollen from plants in the surrounding environment and collected different degrees of pollen diversity across different cropping systems in which the colonies were placed.

We further determined if pollen diversity influences colony-level protein utilization and biosynthesis of protein in nurse bees by conducting a pollen feeding experiment. Pollen collected from four different cropping systems in the first study was used to formulate four different diets, each varying in pollen diversity. We measured protein consumption in experimental colonies and by sampling nurse bees from each colony to estimate hypopharyngeal gland protein content and proteolytic enzyme activity after five weeks of feeding on the experimental pollen diets. Experimental colonies fed on pollen collected from almond cropping system exhibited a high protein consumption rate. However, low protein content as found in hypopharyngeal glands of nurse bees in these colonies. The nurse bees in these colonies also had low proteolytic enzyme activity, which indicates a lower rate of protein digestion. Overall, these results suggest that the diet representing pollen collected from almond cropping systems had low digestion rate and may have resulted in lower nurse bee hypopharyngeal gland protein. However, we cannot say this with certainty, as there were other confounding factors involved, such as presence of pesticides in the pollen collected from the cropping systems.

Effects of pollen collected by honey bees from pollination dependent agricultural cropping systems on honey bee nutrition

by
Ellen Topitzhofer

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Ellen Topitzhofer, Author

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CONTRIBUTION OF AUTHORS

Conceived and designed the experiments: Ellen Topitzhofer, Ramesh Sagili. Performed the experiments: Ellen Topitzhofer, Gretchen Jones. Analyzed the data: Ellen Topitzhofer, Lan Xue. Wrote the paper: Ellen Topitzhofer.

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Chapter 1:
General Introduction

Many crops rely on honey bee (*Apis mellifera* L.) foraging activity in order to set fruit (Klein et al., 2007). Approximately 2.5 million commercially managed honey bee colonies pollinate several cropping systems in the United States (U.S.) every year (Morse and Calderone, 2000). Many of these colonies are transported a considerable distance from overwintering sites in order to be placed into a succession of cropping systems. This type of 'migratory' beekeeping has become prevalent in the U.S. as crop producers rent the services of honey bee colonies and beekeepers accrue income primarily by satisfying the pollination needs of different cropping systems.

Compared to other countries that utilize migratory beekeeping, the scale of operations in the U.S. is unique in terms of the sheer number of colonies moved and the distances covered for pollination services. Starting in the spring, beekeepers transport their colonies to different cropping systems in bloom, such as almonds, apples, and blueberries, across the U.S. in order to meet the tremendous need for crop pollination. Each cropping system requires a different number of colonies per acre for adequate pollination (Caron and Sagili 2012).

In the Pacific Northwest, a common migratory pollination route for beekeepers includes almond (*Prunus dulcis* (Mill.)), an early blooming cropping system that exists primarily in California's Central Valley. Almond cropping systems are typically followed by stone fruit cropping systems, such as cherry (*Prunus* spp.), pear (*Pyrus* spp.), plums (*Prunus* spp.), and peaches (*Prunus persica* (L.) Stokes). In May and June, beekeepers will commonly transport colonies to highbush blueberry (*Vaccinium corymbosum* L.), meadowfoam (*Limnanthes alba* Benth.) and clover (*Trifolium* spp.) cropping systems. These colonies will then pollinate vegetable seed crops, such as radish (*Raphanus sativus* L.), watermelon (*Citrullus*

lanatus (Thunb) Matsum & Nakai), cantaloupe (*Cucumis melo* Naudin) and carrot (*Daucus carota* (Hoffm.), in the following summer months. Pumpkin (*Cucurbita pepo* L.) and buckwheat (*Fagopyrum esculentum* Moench) cropping systems are some of the last cropping systems in bloom. After pollination rental is over, the beekeeper's will typically place colonies in overwintering yards and feed them sugar syrup and protein supplement as needed to avoid starvation during the winter and enhance survival over the winter.

Although current migratory beekeeping practices contribute to the pollination needs of many cropping systems, such practices may affect the health of honey bees. The transportation of colonies on a large scale can expose honey bees to changes in forage and promote disease transmission. In addition, current cropping systems may negatively influence honey bee health. Starting in the 1950s, most U.S. small-scale farms began to consolidate into larger farming operations due to a host of reasons, including insufficient funds, extreme climatic conditions, and inefficient production (Morse and Calderone, 2000). With the consolidation, agricultural operations began producing major food crops as extensive monocultures or within enclosed greenhouse spaces, transforming common farming practices. Widespread chemical pesticide use became prevalent, and intensive weed removal and elimination of cover crops became a common practice (Nicholls and Altieri, 2013).

These changes have negatively affected honey bee and other native bee populations. Since 1982, cropland acreage, including Conservation Reserve Program land, has steadily declined (Heller and Keoleian, 2003; Naug, 2009). Loss of forage habitat for honey bees and other pollinators in the U.S. has been a concern for many years. These factors associated with migratory beekeeping and modern farming practices have the potential to negatively impact bee health by limiting the abundance and diversity of forage available to honey bees. With limited forage diversity, honey bees are at a higher risk of incomplete nutrition.

Without proper nutrition, colonies may experience stress that negatively influences other important health factors (Stanley and Linskens, 1974; Schmidt et al., 1995). Poor nutrition results in a weakened immune system in honey bees (Alaux et al., 2010). Nutritional deficiency predisposes honey bees to higher susceptibility to disease and sensitivity to pesticide exposure (Wahl and Ulm, 1983; Eischen and Graham, 2008; Foley et al., 2012; Di Pasquale et al., 2013).

Pollen diversity, in particular, may have an impact on honey bee nutritional health. Honey bees that have access to fewer pollen types in their diet may experience malnourishment (Schmidt, 1984; Schowalter, 2006). Cropping systems may thus compromise bee health as different plant species produce pollen of varying quality and quantity (Stanley and Linskens, 1974). Without the presence of cover crops and other non-target plant species, honey bees may suffer from a reduction of supplemental food sources in contemporary U.S. agricultural landscapes. Researchers speculate that the nutritional stress of low pollen diversity resulting from monocropping and habitat loss is one of the contributing factors in current honey bee colony declines (Oldroyd 2007; Naug 2009).

Honey bees rely exclusively on pollen for their protein needs (Standifer, 1967; Loper and Berdel, 1980; Winston, 1987). A colony's ability to regulate the relative proportion of foragers specializing on particular resource is crucial when a colony is faced with starvation and survival. According to Camazine (1993), environmental and inner-colony conditions influence the strength and intensity of pollen collection. If the colony does not store enough pollen in the hive, the colony will respond by increasing the proportion of pollen foragers, the frequency of foraging trips, and/or the amount of pollen collected on the foragers' hind legs (Pankiw, 2004). The amount of reserve pollen stored in a hive averages between one and two full frames in a typical hive of approximately 50,000 individual honey bees (Camazine, 1993). Because periodic pollen dearth may occur throughout the regular season, colonies need to be highly responsive to the

fluctuation of pollen availability within the landscape. They accomplish this through efficient regulation of pollen foraging to maintain pollen reserves (Seeley, 1995).

Every plant species produces pollen that is unique in its composition of protein, amino acids, sterols, lipids, vitamins, and minerals (Stanley and Linskens, 1974). These nutrients are essential for honey bee larval development. Nurse bees consume stored pollen and convert it to proteinaceous secretions that are fed to the larvae (Seeley, 1995). Nurse bees will also feed adult worker bees, primarily foragers, but the majority of feedings are larval feedings (Crailsheim, 1992). For normal growth and development, honey bees need all the essential amino acids in specific proportions (DeGroot, 1953). The probability of satisfying these specific amino acid proportions is high in the case of a polyfloral, or diverse, pollen diet, whereas low pollen diversity might be a major limiting factor (Tasei and Aupinel, 2008; Alaux et al., 2010).

In this study, we examined the diversity of pollen available to honey bees during migratory pollination events and how diversity of pollen influences honey bee nutrition. Specifically, our objectives were: (1) to describe the diversity composition of pollen collected by honey bee colonies placed in seven major cropping systems pollinated by managed colonies residing in the Pacific Northwest region of the United States and (2) to examine if pollen diversity has an effect on colony-level protein utilization and biosynthesis of protein in nurse bees.

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CHAPTER 2:

Assessment of pollen diversity collected from honey bees placed in seven cropping systems

1. ABSTRACT

Honey bee populations are currently in decline, posing a threat to United States food security. It has been suggested that poor nutrition is one of the causes of honey bee colony declines and modern cropping systems could be negatively affecting honey bee health. Cropping systems that rent honey bee colonies for pollination may provide inadequate pollen diversity for honey bees. A first step in determining whether differential collection in pollen varieties within and around cropping systems might negatively affect colony health is to determine whether honey bees situated near these cropping systems return to their colonies with different levels of pollen diversity. We identified the number of pollen types found in pollen collections from colonies placed adjacent to seven different cropping systems and quantified the percent of target crop pollen that bees collected when placed adjacent to each of these cropping systems. We also quantified pollen color pellet weights as a means of characterizing the overall diversity of pollen that honey bees brought back to colonies placed in each cropping system. We found that the pollen collected from colonies placed in almond cropping systems (*Prunus* sp.) was predominately from the target pollen type and hence low in pollen diversity. At the other end of the spectrum, pollen collected from colonies placed in blueberry cropping systems (Highbush blueberry, *Vaccinium corymbosum* L.) was high in overall pollen diversity, but did not yield any target pollen types. We sampled colonies placed in five other cropping systems, each yielding pollen diversity between these two extremes, and found both target and non-target pollen types in each cropping system. These results indicate that honey bees collect pollen from plants in the surrounding environment and collect pollen of varying degrees of diversity within the sites from which we collected.

2. INTRODUCTION

Honey bees (*Apis mellifera* L.) are major pollinators for United States (U.S.) fruit and seed crops that depend upon bee pollination for abundant and high quality crop production (Klein et al., 2007). Many crop producers rent honey bee colonies from beekeepers for the pollination services during critical crop bloom periods. These pollination contracts often constitute a significant source of beekeepers' income (Burgett 2011), and many commercial beekeepers transport their colonies throughout the year to key cropping systems in bloom that require bee pollination for crop yield (Morse and Calderone, 2000).

For colony growth and development, individual honey bee foragers gather nectar and pollen from the landscape in which they are placed. Honey bees thus serve as pollination vectors by carrying pollen while foraging on multiple flowers. Nectar and pollen provide honey bees with essential nutrients. Nectar serves as a carbohydrate source and primarily consists of monosaccharides and oligosaccharides (Ball, 2007). Vitamins, minerals, and amino acids are also found in nectar but in trace amounts (Ball, 2007). Honey bees raise their young with the pollen they collect from their environment. Pollen is comprised of varying amounts of protein, amino acids, lipids, vitamins, and minerals, which are essential for larval development (Stanley and Linskens, 1974). Pollen largely contributes to growth of the fat body in larvae and egg development in the queen (Pernal and Currie, 2000; 2001). Nutritional value, specifically protein content, varies greatly among different species of pollen (Standifer, 1967; Roulston et al., 2000). Every plant species produces pollen with a unique nutritional composition, and any one pollen species may not provide full nutritional requirements for honey bees (Stanley and Linskens, 1974). Little is known about how differences in nectar and pollen diversity might influence honey bee colony health.

Since 2007, there have been colony losses averaging over 30% by commercial beekeepers in the U.S. (vanEngelsdorp and Meixner, 2010). Scientists have

attributed this phenomenon to an interactive combination of chronic health problems, which include lack of proper nutrition, pesticide exposure, and prevalence of common pests and disease (vanEngelsdorp et al., 2010). Pollen is an important factor in maintaining nutritional colony health. A diet low in pollen diversity negatively affects a colony's defense system, which consequently increases disease susceptibility and pesticide sensitivity (Wahl and Ulm, 1983; Alaux et al., 2010; DeGrandi-Hoffman et al., 2010; Foley et al., 2012; Di Pasquale et al., 2013). Thus, cropping systems that require bee pollination may put bees at risk for nutritional deficiency if that pollen source is deficient in certain nutrients and bees are unable to find alternatives means of acquiring them. Moving colonies to different cropping systems for pollination may impact what diversity of pollen honey bees bring back to the colony.

Oregon is an ideal place to answer this research question because beekeepers in Oregon pollinate several cropping systems throughout the season. In Oregon, a commercially owned colony will pollinate an average of 5.8 crops each year (Caron and Sagili, 2012). This study provided a representation of pollen sources in seven common cropping systems pollinated by honey bees from the PNW region. This study aimed to document the ability of honey bees to obtain pollen and the extent of diversity of that pollen collection in seven major crops pollinated by managed colonies in the PNW region of the U.S. The objectives of this study were to (1) describe the potential of pollen availability by measuring quantity collected in colonies placed by seven different cropping systems and (2) describe pollen diversity by quantifying richness of plant taxa and abundance of pollen sources.

3. METHODS

To get a detailed description of what pollen loads bees collect while engaged in migratory pollination services, we collaborated with 18 PNW beekeepers to

sample pollen loads collected by honey bees within each selected cropping system.

3.1 Pollen collection

Almond (*Prunus dulcis* (Mill.)), cherry (*Prunus avium* L.), highbush blueberry (*Vaccinium corymbosum* L.), meadowfoam (*Limnanthes alba* Benth.), white clover (*Trifolium repens* L.), radish (*Raphanus sativus* L.), and carrot (*Daucus carota* (Hoffm.)) constituted the seven cropping systems that we targeted for pollen collection from colonies. These cropping systems are some of the most frequently pollinated cropping systems by commercial beekeepers residing in the Pacific Northwest region (Caron and Sagili, 2012). Each sampling site (n = 19) contained colonies belonging to a different beekeeper. We chose sampling sites based on where each beekeeper held pollination contracts and was at least three miles away from any other sampling site (Fig. 2.2 and 2.3 and Appendix A.1). The number of managed colonies within each sampling site was dependent on specifications in each individual pollination contract, reflecting specifications of the beekeeper and crop producer (refer to Appendix A.1). We collected pollen from colonies when the intended cropping system was visually estimated to be between 80 and 100 percent in bloom.

We collected pollen loads from returning foragers by fitting standard pollen traps onto commercial colonies placed in each cropping system, leaving pollen traps on colonies for a maximum of seven days. In all sampling sites except white clover cropping systems, the collection period exceeded seven days. In these cases, we removed pollen traps on day 7 and reinstalled them onto colonies that received the same selection protocol (Fig. 2.1 and Appendix A.1).

We selected colonies that had entrances facing south for maximum foraging activity for pollen trap collection. We also selected colonies for highest frequency of pollen foragers within colonies for each sampling site. We observed foragers

within a two-minute period at each hive entrance of interest (Pettis et al., 2013). We thoroughly pooled and mixed pollen in plastic bags after collecting from traps in the same site and selected a 30-gram composite sample for each week of each collection in order to analyze pollen diversity.

3.2 Pollen identification

We gathered anthers from several individual plants of each species that were in bloom around each cropping system where pollen traps were placed. Anthers served as reference samples for plant species for the pollen identification process. Pollen and anther samples were stored at -20°C within 6 hours from the time it was collected. We were not able to sample every flowering species within the foraging radius. The identity of pollen from plants not included in our own collecting efforts, were confirmed using pollen voucher specimens (Jones et al., 1995).

We took a 30-gram sample from individual locations of each cropping system, and sent to Dr. Gretchen Jones at USDA-ARS (College Station, TX) to identify pollen grains down to the lowest possible taxonomic rank. Dr. Gretchen Jones conducted pollen identification analysis through acetolysis and scanning electron microscopy. Pollen analysis involved comparing scanning electron micrographs of pollen samples to collected anther and voucher specimens (Jones et al., 1995). Scanning electron micrographs allowed detection of some diagnostic characteristics that were otherwise undetectable by light microscopy identification techniques.

Pollen identification techniques followed laboratory protocols of the USDA-ARS (College Station, TX). Dr. Gretchen Jones acetolyzed each sample with a 9:1 ratio of acetic anhydride to sulfuric acid (Erdtman, 1960; 1963) and added glacial acetic acid to stop the acetolysis reaction, rinsing, centrifuging, and decanting the samples each time. She then stained pollen samples with three

drops of Saffrinin O and mixed each sample with seven drops of glycerin (Jones, 2012).

Dr. Gretchen Jones prepared samples for SEM by adding a drop of each sample's pollen residue onto a marked stub. She then allowed this pollen drop to dry before coating it with 400 Å of gold palladium before finally examining the result using a JEOL T330-A scanning electron microscope (JEOL Ltd.).

Dr. Gretchen Jones (USDA-ARS, College Station, TX) took micrographs of pollen grains in several diagnostic positions with an Aus Jena Jenaval compound light microscope at 400x using bright field, phase contrast, and Nomarski phase techniques (Fig. 2.4).

3.3 Target crop proportion

Pollen collected from honey bees are in the form of a pellet when trapped from foragers. Pollen color pellet weights were measured at Oregon State University. We analyzed composite samples of approximately 30 grams from each site. If we collected pollen from colonies placed in crops for multiple weeks, we separated pollen collection samples for each seven-day period. We analyzed one 30-gram sample per week for each site. Measuring abundance of each color pellet followed modified methods from Pettis et al. (2013). We sorted pollen pellets by color, following the designated color labels listed in Appendix A.1. Dr. Gretchen Jones qualitatively assigned the pellet color labels (USDA-ARS, College Station, TX). Wet weight was recorded for each subset of color groups per sample.

For each color label, we dissolved two or three color pellets in DI water in separate microcentrifuge tubes and mounted them onto slides. We then viewed pollen grains with a Leica DM750 light microscope under 400x magnification (Leica Microsystems) and identified the grains to the lowest taxonomic level possible. To identify pollen grains, we compared our samples to vouchers of

collected flowers, published reference collections, and confirmed by results of previous pollen identification (Jones, unpublished data; Jones et al., 1995; McKenney, 2011).

We quantified pellet color weight as a means to estimate pollen diversity. We did this by quantifying pellet color by weight in pollen collections. We calculated the proportion of the target crop pollen collected by percent wet weight of color pellet subsets that contained the target crop's plant species (Pettis et al. 2013). Because of difficulties identifying *Prunus* sp. pollen to species, target crop proportions were determined at the genus level for almond and cherry cropping system collections.

3.4 Shannon's diversity index values

We calculated pollen diversity estimation values using the Shannon Diversity Index for color pellet weights from section 3.3. The Shannon Diversity Index accounts for taxonomic richness and evenness (Shannon, 1948; Shannon and Weaver, 1949). A Shannon Diversity Index value lower than another would indicate a lower level of diversity present in the sample. Shannon Diversity Index values were calculated for each site of pollen collection from colonies placed in the different cropping systems using R statistical software (v. 2.14.1, R Development Core Team).

4. RESULTS

4.1 Pollen collection

Different quantities of pollen were collected from colonies placed in the different cropping systems (Appendix A.1). However, our study design precluded quantitative analysis of differences between cropping systems. Variability in pollen quantity existed between collection sites within each cropping systems. Honey bees placed in almond, cherry, and meadowfoam cropping systems

collected considerable amounts of pollen in each collection site. Colonies placed in blueberry and carrot cropping systems collected less substantial amounts of pollen from sites.

4.2 Pollen identification

Pollen types from all samples are listed in Table 2.1. Color pellets contained a single plant taxa or a mixture of several plant taxa (refer to Appendix A.2). An average of 1.4 plant taxa were found in color pellets across all cropping system collections. Samples from colonies placed in almond and carrot cropping systems contained relatively low amounts of color pellets and taxa compared to the other sampled cropping systems. Pollen collected from colonies placed in almond cropping systems contained 3.2 ± 0.5 color pellets and 3.8 ± 1.2 plant taxa (Table 2.1). Cream, tan, and yellow gold-labeled color pellets all contained only *Prunus* sp. from almond cropping system collection samples. Pollen collected from colonies placed in carrot cropping system contained 4.8 ± 0.3 color pellets and 6.8 ± 0.6 plant taxa (Table 2.1). Pollen samples from colonies placed in blueberry cropping systems contained a high amount of color pellets and plant taxa (11.0 ± 1.7 color pellets and 16.3 ± 3.2 plant taxa). The other cropping system sites had pollen collections that fell in between these extremes. Pollen collected from colonies placed in cherry cropping systems contained 7.6 ± 1.8 color pellets and 7.3 ± 1.8 plant taxa, meadowfoam cropping systems contained 8.6 ± 1.4 color pellets and 8.2 ± 1.8 plant taxa, and radish cropping systems contained 7.0 ± 1.0 color pellets and 6.5 ± 0.5 plant taxa (Table 2.1).

Regardless of the cropping system in which honey bees were foraging, the collected pollen pellets contained pollen from *Trifolium* spp. (clovers), which were *Trifolium repens* L. (white clover), *Trifolium incarnatum* L. (crimson clover), or *Trifolium arvense* L. (rabbitfoot clover). Additionally, honey bees foraging in all cropping systems, except almond and carrot, returned to the colony with pollen containing material from *Taraxacum officinale* F.H. Wigg (common

dandelion). Other non-target pollen types commonly seen in the course of this study were as follows: (a) *Cornus stolonifera* L. (red stem dogwood), (b) *Viburnum* sp. (viburnums), (c) *Crataegus* sp. (hawthorns), and (d) *Medicago sativa* L. (alfalfa). We found no highbush blueberry (*Vaccinium corymbosum* L.) in any highbush blueberry cropping system samples (refer to Appendix A.2).

4.3 Target crop proportion

Pollen samples collected from colonies placed in almond cropping systems contained a relatively high proportion of target crop genus. Almond cropping system samples yielded 99.5 ± 0.3 percent of color pellets with *Prunus* sp. genera found (Table 2.2). Colonies placed in blueberry and carrot cropping systems had relatively low target crop proportions in collected samples. Colonies placed in blueberry cropping system samples yielded 0.0 ± 0.0 percent of color pellets with highbush blueberry (*Vaccinium corymbosum* L.) species found (Table 2.2), whereas samples from colonies placed in carrot cropping systems contained an average of 1.1 ± 1.1 percent of color pellets with carrot (*Daucus carota* (Hoffm.)) species found (Table 2.2). Cherry cropping systems samples had 53.1 ± 17.8 percent of color pellets with *Prunus* sp. genera. Meadowfoam cropping system samples contained 47.3 ± 13.9 percent of color pellets with meadowfoam (*Limnanthes alba* Benth.). White clover cropping system samples had 24.5 ± 21.5 percent of color pellets with white clover (*Trifolium repens* L.). Lastly, pollen from colonies placed in radish cropping systems collected 40.3 ± 15.7 percent color pellets from Brassicaceae spp. (Table 2.2).

4.4 Shannon's diversity index values

We estimated diversity by calculating values of the Shannon Diversity Index for each crop. Samples from colonies placed in almond cropping system sites had relatively lower Shannon Diversity Index values (*H*) than samples from colonies placed in the other cropping systems. Colonies placed in almond cropping system samples had an average index value of 0.39 ± 0.12 *H* (Table 2.3). The average

index value of pollen collected from colonies placed in cherry cropping systems was 1.04 ± 0.23, 1.2 ± 0.10 for blueberry cropping systems, 1.13 ± 0.11 for meadowfoam cropping systems, 1.31 ± 0.0 for white clover, 1.14 ± 0.11 for radish cropping systems, and 1.1 ± 0.04 for carrot cropping systems (Table 2.3). A visual representation of pollen pellet weights appears as a series of histograms (Fig. 2.5).

5. DISCUSSION

We intended this study to be an empirical description of the pollen diversity found in colonies placed near cropping systems pollinated by PNW beekeepers. However, caution should be used when comparing the results associated with the various cropping systems due to the number of confounding variables inherent in the study. First, differences in climatic conditions and seasonal periods between field sites influenced pollen diversity. Second, each cropping system differed in field size and spatial placement. Third, because we collaborated with several beekeepers, not all colonies received the same hive management practices and were likely very different among all sampled colonies. The seasonality of cropping systems influenced pollen collection. Weather conditions, such as temperature, wind, and humidity, greatly influence foraging activity levels in colonies. Each location and cropping system contained colonies from different beekeepers. Therefore, hive management practices were likely very different among all sampled colonies. This includes different queen age, size and strength of adult population, and amount of brood and food stored in the hive. However, despite the inability to analyze these data using quantitative methods, interesting patterns related to pollen foraging emerged. These patterns could be significant to beekeepers in relation to how they manage their bees for particular cropping systems and to future investigators seeking to study this important problem.

Although our study design precluded quantitative analysis of the data, intriguing patterns emerged from analysis of quantities collected. Honey bees collected varied amounts of pollen from their foraging environment when placed adjacent to different cropping systems. It appeared that honey bees had collected substantially low quantities of pollen in highbush blueberry and carrot systems. A low amount of pollen sampled from colonies placed in blueberry cropping systems could have been influenced by the fact that we did not find any of the target crop, blueberry (*Vaccinium* spp.), pollen in these sample collections. This finding is consistent with results of Pettis et al. (2013). In the field, we observed foragers having difficulty maneuvering around blueberry's bell-shaped flowers. Honey bees' inability to provide buzz pollination may have contributed to honey bees being unable to collect pollen onto their hind legs. Colonies may have been unable to collect large quantities of pollen in the landscape because a large portion of the flowering plants in the area, the target crop, were not utilized by honey bees for pollen collection. Colonies placed in carrot cropping systems also produced very little pollen quantity. This could have been due to the high desert climatic conditions where we sampled (Madras, OR). When we were working in the high desert sampling sites, we observed very little flowering plants in the area. Low amounts of pollen collection in colonies can dramatically affect brood rearing and colony strength. Beekeepers that bring their colonies to pollinate cropping systems like highbush blueberry and carrot should be aware of the amount of pollen stores in their colonies, and may have to artificially feed colonies with protein supplement if they observe low pollen collection. Although protein supplements are not identical to pollen in biochemical composition, researchers have observed increased brood rearing and decreased disease susceptibility associated with the use of supplements (Waller et al., 1981; Nabors, 2000; van der Steen, 2007). In the colonies placed in almond, cherry and meadowfoam cropping systems, we observed relatively high quantities of pollen collected. This indicates a potentially high amount of flowering plants for honey bees in the surrounding area where the colonies were placed for pollination. This

is beneficial to both the beekeepers and crop producers because high amounts of pollen stores in colonies will increase adult population and brood production, which ultimately encourages a large foraging population.

Because pollen collections took place during different locations and times of the year, weather conditions likely varied within each cropping system, potentially altering the preference of foraging and the distance foragers travelled for pollen collection. Each cropping system also differed in field size. This most likely influenced to a large degree the diversity of other non-target pollen types within sampled pollen collections. We did not collect all flowering plants (which we used to facilitate pollen identification) within the foraging radius of each sampled colony. Nonetheless, our results indicate that honey bees foraging in different cropping systems can return to the colony with different levels of pollen diversity. As previously discussed, honey bees foraging in almond cropping systems collected few pollen varieties, which raises the question of whether low pollen diversity negatively affects the colonies used to pollinate this crop. And even though pollen collected from colonies placed in highbush blueberry fields contained no target crop pollen, honey bees collected the highest amount of plant taxa in this cropping system, compared to the other cropping systems we examined.

The pollen collected by foragers from colonies in almond cropping systems had a higher proportion of target crop pollen compared to all other cropping systems evaluated. Almond cropping systems in California present a consistent monofloral landscape for bees due to early bloom and large acreage planted (Blue Diamond Growers, 2012; National Agricultural Statistics Service, 2012). These results support the fact that there is high flower visitation in almond trees when colonies are pollinating. Honey bees can thus be considered effective in providing pollination services to almonds. The pollen collection for highbush blueberry fields also presented a unique agricultural landscape in that no target pollen was

found in our pollen collection samples. Such observations support previous studies that suggested honey bees may not be the most efficient pollinator for blueberry (Stubbs and Drummond, 2001; Javorek et al., 2002). Instead, wild bee species that provide buzz pollination may be more efficient pollinators when compared to honey bees in highbush blueberry fields (Cane et al., 1985; Garibaldi et al., 2013). Wild bee populations can be encouraged with the installment of hedgerows or intercropping with bee forage. However, researchers have shown that lowbush blueberry fields produce higher yield when honey bee colonies are placed for pollination (Aras, 1996; Sampson and Cane, 2000). This suggests the possibility that honey bees may be utilizing highbush blueberry flowers for nectar but do not collect pollen. By collecting nectar from these flowers, foragers may still be able to provide cross-pollination by transferring pollen along the body hairs. Thus, pollen returning to the colony is not a true estimate of their efficacy at pollination.

It is important that crop producers and beekeepers are aware of the diversity of pollen that honey bees collect within cropping systems that require pollination services. Honey bee colonies placed in cropping systems where they are unable to collect pollen from the target crop may need additional pollen sources in order to maintain optimal colony growth. Strong colonies resulting from availability of supplemental pollen resources will benefit both beekeepers and the crop producers that rely on these colonies for optimal pollination. This information pertaining to availability of foraging resources during specific time periods can also be used for native bee conservation efforts as several native bee species also forage on many of the same resources that honey bees use.

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Table 2.1 Mean number of plant taxa and color types in pollen collection of each cropping system with the total amount of taxa identified to the level of family, genus, or species.

			Number of Taxa Identified		
Cropping system	Mean number of pollen color types (SE)	Mean number of plant taxa (SE)	Family	Genus	Species
Almond	3.2 (0.5)	3.8 (1.2)	3	4	5
Cherry	6.7 (1.8)	7.3 (1.8)	4	7	5
Blueberry	11.0 (1.7)	16.3 (3.2)	7	11	14
Meadowfoam	8.6 (1.4)	8.2 (1.8)	5	3	14
White clover	10 (0.0)	13 (0.0)	3	4	6
Radish	7.0 (1.0)	6.5 (0.5)	2	2	5
Carrot	4.8 (0.3)	6.8 (0.6)	2	5	6

Table 2.2 Mean percent (+/- SE) of target crop pollen collected from hives placed into each of the seven cropping systems included in this study. Target pollen was identified to the lowest taxonomic level possible.

Cropping system	Level of taxa identification	Target pollen taxa	Mean percent of target crop (SE)
Almond	Genus	*Prunus* sp.	99.5 (0.3)
Cherry	Genus	*Prunus* sp.	53.1 (17.8)
Blueberry	Species	*Vaccinium corymbosum*	0.0 (0.0)
Meadowfoam	Species	*Limnanthes alba*	47.3 (13.9)
Clover	Species	*Trifolium repens*	24.5 (21.5)
Radish	Family	Brassicaceae spp.	40.3 (15.7)
Carrot	Species	*Daucus carota*	1.1 (1.1)

Table 2.3 Mean Shannon's diversity index values of sample site locations for pollen collection from colonies placed in each cropping system.

Sample Site Location	Shannon's diversity index (*H*)	Mean Shannon's diversity index per cropping system (SE)
Almond		
Turlock, CA	0.71	0.39 (0.12)
Chowchilla, CA	0.34	
Gustine, CA	0.21	
Ripon, CA	0.08	
Madera, CA	0.61	
Cherry		
Gervais, OR	1.5	1.04 (0.23)
St. Paul, OR	0.82	
Salem, OR	0.79	
Blueberry		
Jefferson, OR	0.93	1.2 (0.10)
Sheridan, OR	1.15	
Newberg, OR	1.32	
Forest Grove, OR	1.4	
Meadowfoam		
Eugene, OR	1.09	1.13 (0.11)
Eugene, OR	1.43	
Monmouth, OR	0.94	
Monroe, OR	1.04	
White clover		
Corvallis, OR	1.31	1.31 (0.0)
Radish		
Shedd, OR	1.24	1.14 (0.11)
Shedd, OR	1.03	
Carrot		
Madras, OR	1.13	1.1 (0.04)
Madras, OR	1.05	

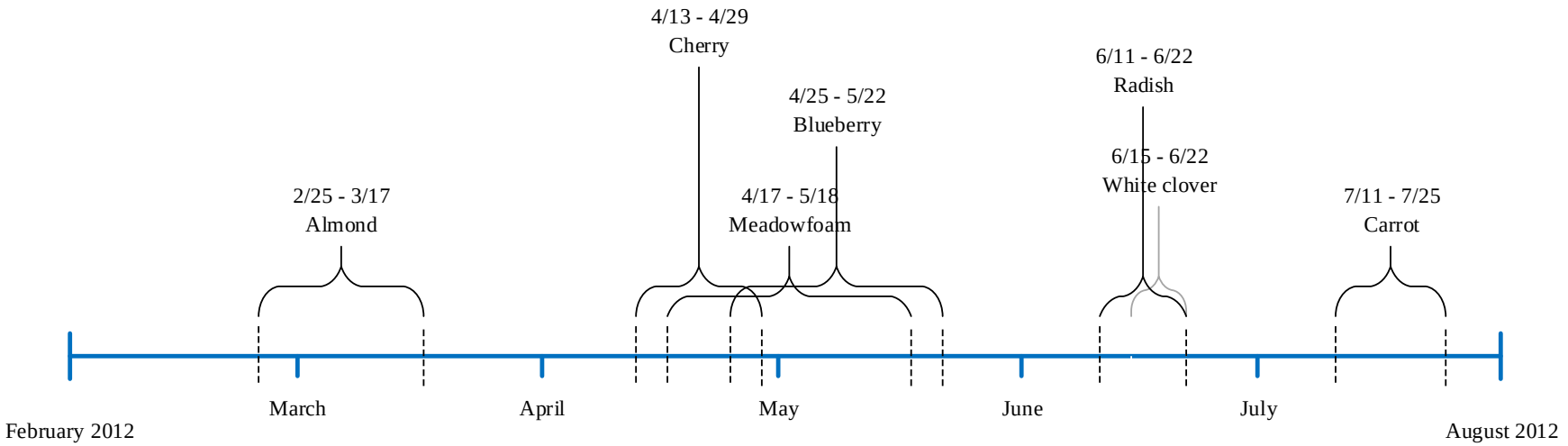


Figure 2.1 Timeline of pollen sampling dates when colonies were placed in seven different cropping systems. All dates are 2012. Graphic was made with Microsoft Visio (Microsoft Office).

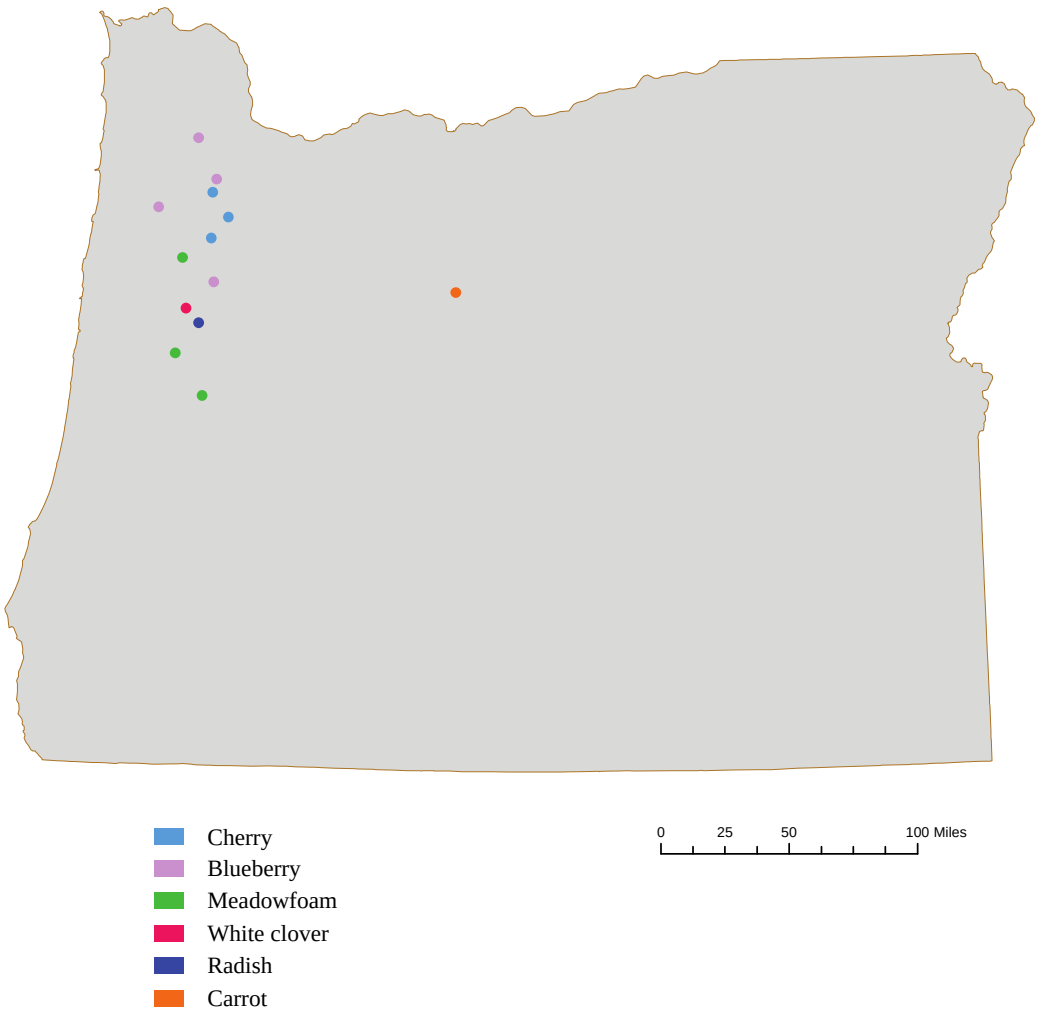


Figure 2.2 Map of Oregon pollen collection sites. Each color represents a different cropping system. Two separate sites were in Madras, OR for carrot cropping system sites. Figure was made in Adobe Illustrator with a digital vector map of Oregon state.

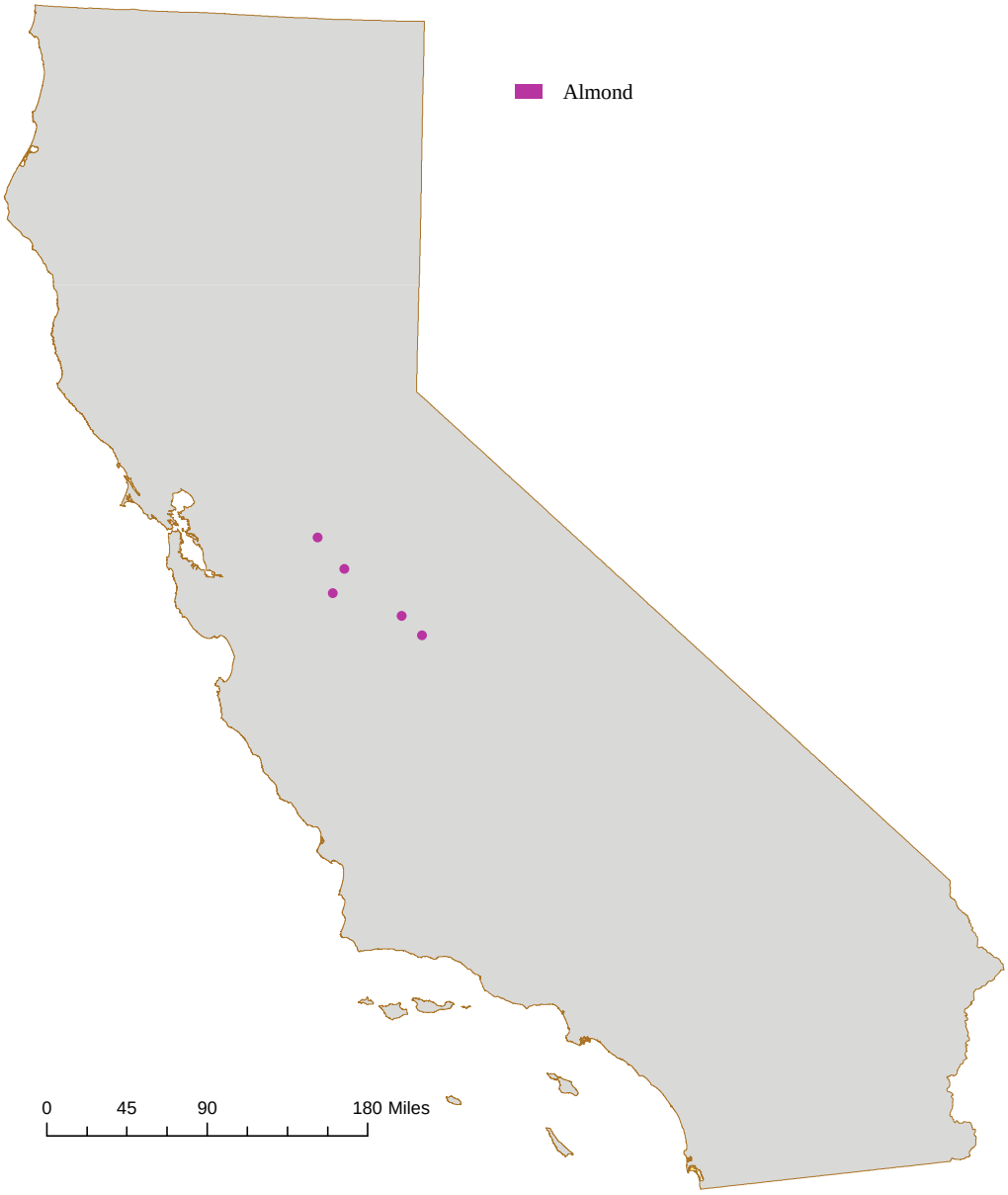
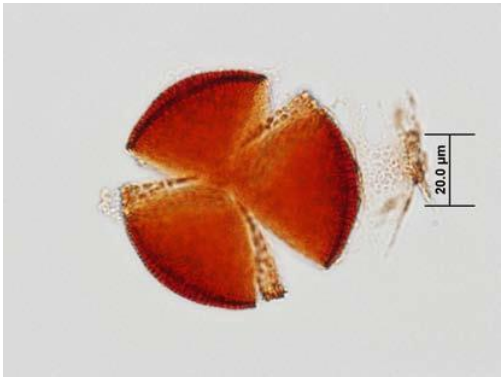
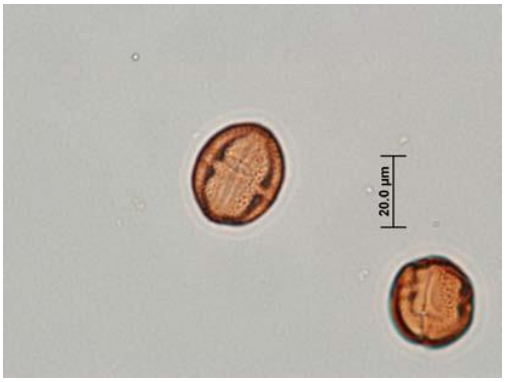


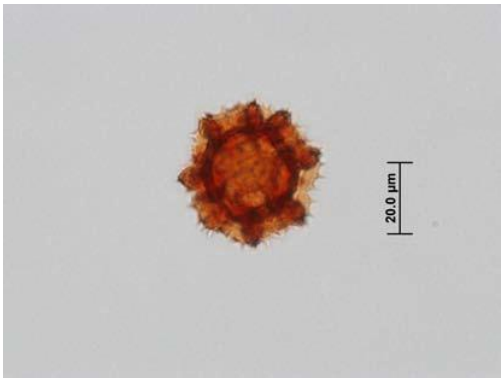
Figure 2.3 Map of California pollen collection sites. Color of sites represent almond cropping systems. Figure was made in Adobe Illustrator with a digital vector map of California state.



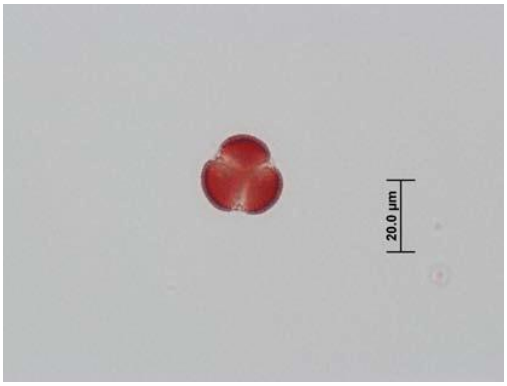
Acer microphyllum



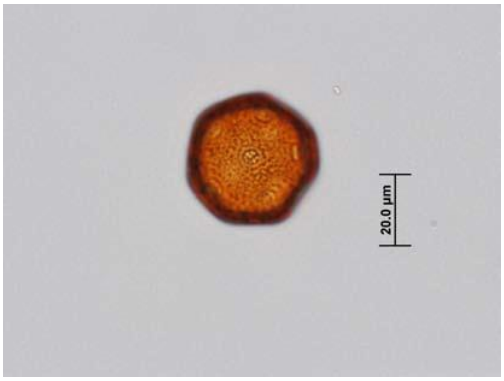
Anacardiaceae sp.



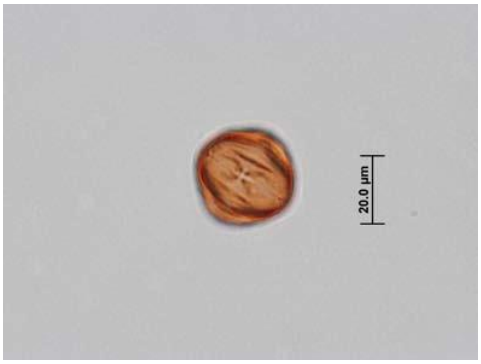
Cichorea sp.



Brassicaceae sp.

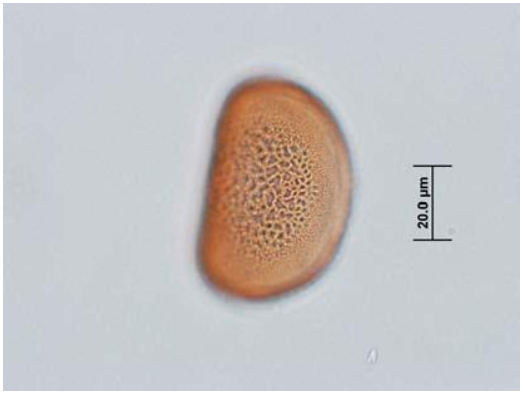


Caryophyllaceae sp.

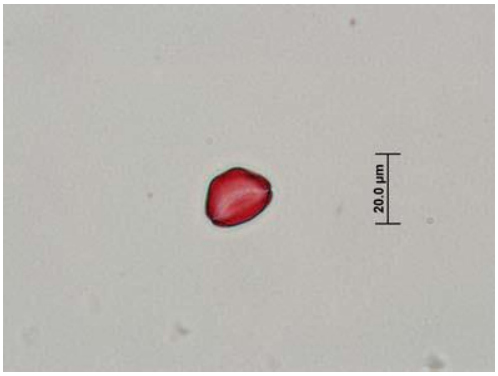


Cornus stolonifera

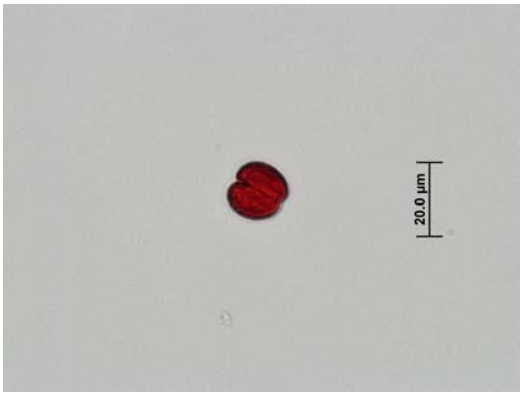
Figure 2.4 Examples of acetolysized pollen light micrographs from this study. Micrographs were produced by Dr. Gretchen Jones (USDA-ARS).



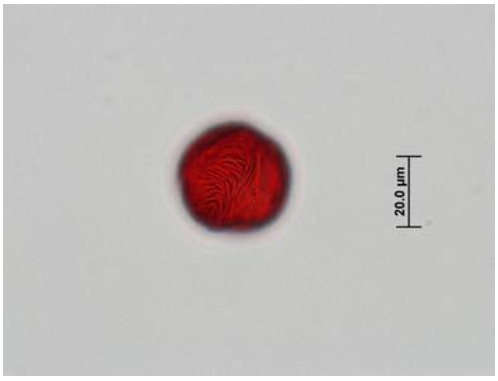
Liliaceae sp.



Limnanthes alba



Pedicularis sp.



Fragaria sp.

(Figure 2.4 Continued)

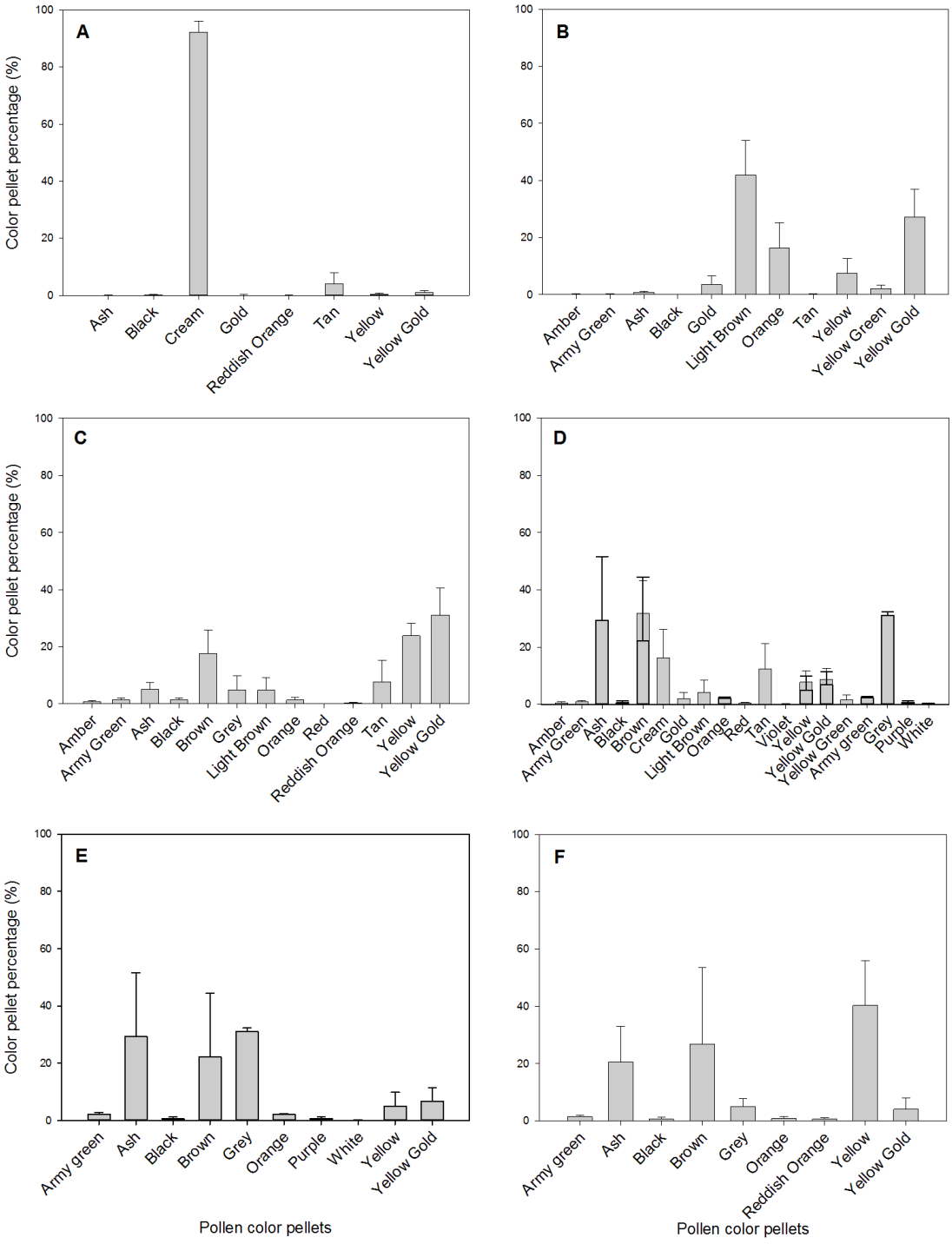
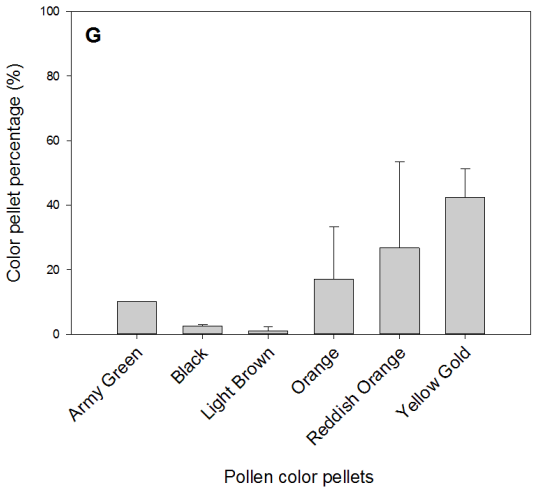


Figure 2.5 Graph series of mean percent pollen color pellet weight in **(A)** almond, **(B)** cherry, **(C)** blueberry, **(D)** meadowfoam, **(E)** white clover, **(F)** radish, and **(G)** carrot. Bars represent standard error.



(Figure 2.5 Continued)

CHAPTER 3:

Effects of pollen collected from agricultural cropping systems on protein digestibility and utilization in honey bees

1. ABSTRACT

Current practices of migratory honey bee (*Apis mellifera* L.) pollination services expose honey bees to a highly polarized pollen landscape that may impact foragers' ability to obtain a diversity of pollen types. Lack of pollen diversity could impact honey bee nutrition. Poor nutrition is one of the factors suggested as contributing to honey bee decline. To examine the potential effects of low pollen diversity on honey bees, we conducted a feeding experiment to assess protein digestibility in honey bees fed on pollen collected from colonies placed in various cropping systems. We supplied honey bees with one of four diet treatments that were established from field-collected pollen. First, we examined the effects of pollen diversity on colony-level protein utilization and biosynthesis of protein in hypopharyngeal glands and digestive tracts of nurse bees. Second, we investigated the effect of pollen diversity on colony fitness by measuring colony growth parameters.

We found that honey bees in experimental colonies fed on pollen collected from colonies placed in almond cropping systems exhibited a high protein consumption rate compared to the other diet treatments. However, we discovered relatively low levels of protein in the hypopharyngeal glands of nurse bees in this treatment. A lower proteolytic enzyme activity level within nurse bee digestive tracts indicated a lower digestion rate of protein when fed on the diet consisting of pollen collected from colonies placed in almond cropping systems compared to the other diet treatments. Further, we found no differences in the amount of brood or adult bees between the treatments for a 5-week period. We concluded that the pollen collected by honey bees in California (CA) almond cropping systems for

this study influenced honey bees' ability to utilize the protein provided by the pollen.

2. INTRODUCTION

Commercial agriculture that requires bee pollination is highly dependent on honey bee (*Apis mellifera* L.) colonies as manageable pollinators in the United States (U.S.) (Aizen et al., 2008). Beekeepers transport colonies to different crops to satisfy the pollination needs for U.S. food production. Since 2007, there have been colony losses averaging over 30% by commercial beekeepers in the U.S. (vanEngelsdorp and Meixner, 2010). Scientists have attributed the decline in honey bee colonies to an interactive combination of factors, which include lack of proper nutrition, pesticide exposure, and prevalence of common pests and disease (vanEngelsdorp et al., 2010). Of these factors, honey bee nutrition has been an understudied aspect of honey bee health. Nutritional quality of pollen diets can be observed in numerous ways: (1) the properties of the pollen grains, (2) nutritional composition of the pollen sources, and (3) diversity of pollen sources. Pollen properties consist of wall porosity, thickness, and composition that may affect the digestibility of different pollen types (Roulston and Cane, 2000). Nutritional composition refers to diet quality in terms of the completeness in levels of protein, amino acids, sterols, lipids, vitamins, and minerals within the diet. And lastly, the amount of diversity in pollen sources consumed by honey bees could also impact diet quality. The diet of commercial honey bees is impacted by the food resources they collect when employed in cropping systems for pollination.

Although we know relatively little about how honey bee diet impacts pollinator health, we can infer hypotheses from patterns documented for other insect guilds. Generalist herbivores, for example, are healthier when presented with a diet of mixed plant species rather than a single species (DeGroot, 1953; Bernays et al., 1994). Large monocropped, agricultural landscapes can reduce forage availability

for honey bees by intensive weed removal or loss of wild habitat (Naug, 2009; Nicholls and Altieri, 2013). Pollen is the exclusive source of protein for honey bees. There is evidence that some cropping systems pollinated by honey bees are lacking in pollen diversity for honey bee colonies, and hence there is a need to understand the impact of low pollen diversity on honey bee health. (Pettis et al., 2013).

Potential diet limitations pertain to the gathering and metabolism of pollen. Honey bees feed larvae with pollen they collect, which greatly contributes to larval development. Pollen contains nutrients that are essential for brood development. Every plant species produces pollen that is unique in its composition of protein, amino acids, sterols, lipids, vitamins, and minerals, but not every species produces pollen that meets nutritional requirements for honey bees (Stanley and Linskens, 1974). Nutritional deficiencies often affect colonies in foraging environments that present meager or poor quality pollen sources (Schmidt et al., 1995). Research has shown that nutritional health can influence a colony's defense system (Alaux et al., 2010; DeGrandi-Hoffman et al., 2010). When honey bees experience malnourishment, they can become more susceptible to infection and sensitive to pesticide exposure (Wahl and Ulm, 1983; Foley et al., 2012; Di Pasquale et al., 2013). Pesticide exposure, including insecticides, fungicides, and miticides, has been recorded in pollen collected from honey bees in agricultural settings, and we considered this when evaluating diet quality in our study (Pettis et al. 2013).

Honey bees must process pollen before it is consumed and fed to larvae. Worker bees perform several different tasks as they age and can be grouped into the following four categories (from oldest to youngest): (a) forager, (b) middle-aged, (c) nurse, and (d) newly emerged bees (Seeley, 1995). Once foragers bring pollen back to the colony, middle-aged bees compress it in a comb cell. When the pollen cell is full, middle-aged bees will initiate lactic acid fermentation by secreting a thin sugary layer over the pollen (Gilliam et al., 1989). Nurse bees will then

consume and digest pollen after fermentation is complete. Pollen will breakdown in the midgut via enzyme activity and osmotic shock before being absorbed through the peritrophic membrane (Jimenez and Gilliam, 1990). Proteolytic enzymes within the gut specifically break down protein from the pollen into peptides and amino acids. Nurse bees have highly developed hypopharyngeal glands and high proteolytic enzyme activity in their midguts compared to foragers and bees performing other tasks in the colony (Crailsheim et al., 1992). Nurse bees then biosynthesize the absorbed protein into a digestible food for larvae through their hypopharyngeal glands (Crailsheim, 1990). The majority of nurse bee's feedings will be to larvae, but adult workers, mainly foragers, are also fed via trophallaxis (Crailsheim, 1992).

To date, most nutritional studies have been performed in small laboratory experiments for a short duration of time (Standifier, 1967; Wahl and Ulm, 1983; Pernal and Currie, 2000; Alaux et al., 2010; Di Pasquale, 2013). The time a colony spends in each cropping system usually exceeds the amount of time it takes to raise several generations of brood. For this study, we examined the effects of pollen diet on honey bee colonies over a period of five weeks, maintaining colonies in semi-field conditions to ensure a realistic environment and social context.

This study aims to evaluate diet effects of pollen diversity using pollen collected from colonies placed in cropping systems for pollination services. Specifically, we observed the individual and collective nutritional effects of protein utilization on honey bees with diets differing in pollen diversity. Our objectives were to (1) determine if correlations exist between pollen diversity and both colony-level protein utilization and biosynthesis of protein in hypopharyngeal glands and digestive tract of nurse bees and to (2) investigate the effect of pollen diversity on colony fitness by measuring colony growth.

3. METHODS

3.1 Field collected pollen

To create our diet formulations, we used pollen brought back to the colony from honey bees placed near almond (*Prunus dulcis* (Mill.)), cherry (*Prunus avium* L.), blueberry (*Vaccinium corymbosum* L.), and meadowfoam (*Limnanthes alba* Benth.) cropping systems. We chose to formulate our diet treatments using pollen collected from colonies placed in these four cropping systems because they were the most frequently pollinated crops by commercial beekeepers in the PNW region (Caron and Sagili, 2012). In order to collect pollen loads from foragers, we fitted standard pollen traps onto commercial colonies placed in each cropping system (see Chapter 2 for details). We installed pollen traps when the target crop was visually estimated to be 80 to 100 percent in bloom and grouped colonies on pallets placed along the edges of the crop field. Pollen traps were placed on colonies with south facing entrances, and had highest frequency of pollen foragers when observed for a 2-minute time period. Pollen traps remained on colonies for a maximum of five days. All pollen that we collected from traps in the same orchard was thoroughly mixed and pooled in plastic bags. Pollen was immediately stored at -20°C after collection. Dr. Gretchen Jones at USDA-ARS, College Station, TX (see Chapter 2 for details of the protocols that were followed) identified pollen samples using techniques listed in Appendix A.1.

3.2 Pollen diet formulation

For this study, we created four distinct pollen diets. These formulated diets reflected pollen diets that honey bees in managed PNW colonies might consume as commercial beekeepers sequentially migrate through agricultural regions to carry out pollination contracts. Each of the four treatments represented a different level of diversity in amount of pellet color and plant taxa present (Table 3.2). Diet 1 consisted of pooled pollen collected from colonies placed in almond cropping systems. Diet 2 contained pooled pollen collected from colonies placed from meadowfoam cropping systems. The two treatments that represented the highest

range in diversity, namely, Diets 3 and 4, were mixtures of pollen we collected from colonies placed in cropping systems. These diet treatments were mixed with the ratio calculated by wet weight. Diet 3 was an equal mixture of pollen from colonies placed in almond and meadowfoam cropping systems. Diet 4 was an equal mixture of pollen collected from colonies placed in almond, meadowfoam, cherry, and blueberry cropping systems.

To assess the composition of the diets, we sent the samples of the diets to two labs. Two 3-gram composite subsamples from each diet treatment were sent to University of California-Davis' Analytical Laboratory (Davis, CA) for total nitrogen analysis. The laboratory analyzed total nitrogen content by combustion method of the dry weight of duplicate 3-gram composite samples (AOAC Official Method 990.03). The laboratory also calculated the crude protein percentage of each treatment diet by multiplying total nitrogen content of each sample by 6.25, the most frequently used conversion factor for pollen (Roulston and Cane, 2000). We also sent a 30-gram sample of each diet treatment to the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO) to quantify ratios of 10 essential amino acids. The amino acid profile was analyzed by calculating the proportion (weight/weight) of each individual amino acid (AOAC Official Method 982.30 E(a,b,c), chp. 45.3.05, 2006).

3.3 Experimental design

Flight cage

We established colonies in five-frame nucleus boxes with naturally-mated sister queens and equal numbers of adult bees and honey stores, randomly assigning colonies to individual flight cages (30 x 20 x 6 m) placed within the same apiary (Figure 3.1). We applied top feeders and external feeders, both types containing a 50% sucrose solution (weight by volume), to all colonies. We also provided water to these colonies in external feeders.

Diet treatment

We replicated each treatment (diet) five times with a single honey bee colony serving as the experimental unit. To administer the pollen, we provided one frame of empty comb in each colony. We then added pollen treatments to empty-comb frames by distributing the raw pollen into the cells followed by a spray of 50% (w/v) sucrose solution. The sucrose solution initiates the pollen processing procedure by simulating the packing of pollen into the cells by workers (Dreller and Tarpy, 2000).

Mite loads

Varroa mite (*Varroa destructor* Anderson and Trueman, 2000) infestation levels were tested at the beginning of the experiment to determine if any experimental colonies had high levels of infestation. We obtained *Varroa* mite counts through the standard ethanol wash method (DeJong, 1982).

3.4 Colony protein use

We calculated colony protein consumption of each diet treatment by modifying methods from Pernal and Currie (2000). We estimated protein consumption by multiplying the mean percent crude protein content of pollen treatments by the total amount of pollen consumed by the colony. We recorded pollen consumption by determining the grams of pollen fed to each colony per week for the 5-week duration of the experiment. Each week, we fed 500 grams of pollen of the specified diet treatment to each colony.

3.5 Hypopharyngeal gland protein content

The selection process for identifying nurse bees was derived from Schmickl et al. (2003). We collected nurse bees after observing a worker bee placing her head into a larval cell for more than three seconds, but less than three minutes. Selecting bees within this period avoided selecting for other behavioral characteristics, such as cell inspection, napping, and cell cleaning (Huang and

Otis, 1991). We sampled 20 nurse bees from each colony after five weeks (35 days) of feeding on diet treatments. Nurse bees were stored in 300mL jars, transferred into an ice cooler, and stored at -20°C for later analysis. We used the heads from the sampled nurse bees to quantify the protein content in their hypopharyngeal glands. We dissected hypopharyngeal glands from each head and stored them in PBS buffer at -20°C for further analysis (Figure 3.2). The supernatant of the glands was extracted after the glands were homogenized and centrifuged at 13,000 rpm for two minutes. Samples were prepared in a 96-well plate along with a sequence of dilutions of known bovine serum albumin concentrations to formulate a reference standard curve. We used the bicinchoninic acid (BCA) method to quantify protein content, using the BCA Protein Assay Kit (Pierce Biotechnology, Inc.). We then added a working reagent to each sample and determined the standard curve. The working reagent contained BCA to initiate the biuret reaction, which involved the Cu^{+2} ions of the BCA reagent binding to peptides in the solution (Smith et al., 1985). Colorimetric detection of the purple-colored Cu^{+1} ions, the product of the biuret reaction, is directly correlated with protein concentration (Calloway, 1997). The absorbance of the samples and standard curve were quantified at 562 nm in a microplate reader (BioTek, Synergy^{TM} 2). Duplicate absorbance values of standard curve and samples were corrected by path length and duplicate blanks using microplate reader software (BioTek, Gen5^{TM}). We calculated the protein concentration of each sample using a linear regression equation extracted from the standard curve. Due to high protein amounts, we diluted samples and subsequently calculated the dilution factor in order to obtain absorbance values to fit into the standard curve concentration range.

3.6 Gut proteolytic enzyme activity level

We used the abdomens from the sampled nurse bee to quantify the gut proteolytic enzyme activity level, performing assays based on protocols described in Michaud et al. (1995) and Sagili et al. (2005); however, we used higher

centrifugation speeds and shorter incubation times. We removed abdomens from each bee before homogenizing and centrifuging them at 13,000 rpm for 5 minutes to remove the supernatant. To test proteolytic enzyme activity levels, we exposed the supernatant to azocasein, a dyed protein substrate, to initiate hydrolysis of the protein substrate caused by proteolytic enzymes present in the supernatant. The supernatant was mixed with a solution of 2% azocasein (w:v) and Tris-HCl buffer (0.1 M, pH 7.9) and incubated for 4 hours at 37°C. The proteolytic enzymes reacted in the solution and caused the dye from the azocasein to be released into the solution as it broke down. This allowed the sample to be quantified through spectroscopy. We subsequently added trichloroacetic acid to each sample in order to stop proteolysis and then analyzed the activity level by recording absorbance values with a spectrophotometer at 440 nm (Thermo Scientific, GENESYS^{TM} 20). Finally, we corrected duplicate absorbance values of every sample by a blank reading that consisted of the sample without undergoing incubation at 37°C.

3.7 Colony Growth

We constructed a standardized grid by fitting 2.54-cm mesh onto a Langstroth wooden frame (23.18X50.48 cm; Pankiw et al., 2004) and used the grid to estimate brood area, which included eggs, open larvae, and capped brood cells. We estimated adult population by counting the percentage of occupied comb on both sides of each frame and performed colony assessments every 7 days for 5 weeks.

3.8 Pesticide analysis

Although not part of the original experimental design, we sent a 10-gram sample to the USDA-AMS National Science Laboratory (Gastonia, NC) to be screened for 171 common pesticides. Pesticide residues were reported in parts per billion (ppb) for each of the samples obtained from the four respective cropping systems that were used for designing the treatment formulations (Appendix B.1).

3.9 Statistical analysis

We employed five different statistical analyses for this study. First, we assessed differences between the pollen samples formulated in this study using a one-way ANOVA, which allowed for the comparison of the number of pollen pellet colors and number of pollen taxa in each of the crops from which pollen was gathered (Table 3.1). The ten essential amino acids for the four treatment diets were qualitatively compared to their corresponding threshold levels described in DeGroot (1953) [Table 3.4].

Second, we performed a non-parametric Kruskal-Wallis test due to a non-normal distribution to determine if there was a difference in *Varroa* infestation between treatment groups. Third, we further made median comparisons based on unplanned comparisons of mean ranks, the equivalent to post-hoc Tukey adjustment for parametric tests, for *Varroa* infestation levels. Fourth, we used three separate ANOVA tests to determine the difference in colony protein consumption, mean hypopharyngeal gland protein content, and mean gut proteolytic enzyme activity levels between pollen diet treatments. Nurse physiology parameters modeled treatment as the main effect. Fifth, differences in diet treatment of adult bee population and brood area were determined by two separate repeated measures ANOVA tests with an AR1 covariance structure.

Statistical analysis of the pesticide residues was not possible because funds were only available to screen a small set of samples. We sent one sample per cropping system. We conducted all statistical analyses in R (v. 2.14.1, R Development Core Team) and generated all figures in SigmaPlot (v. 12.5, Systat Software) and Adobe Illustrator.

4. RESULTS

4.1 Field collected pollen

For pollen collected within each cropping system, we found multiple plant taxa in some of the pollen pellet colors. There were significant differences between total counts of pollen color pellets (ANOVA, $F_{3, 12} = 6.78$, $p = 0.006$; Table 3.1). Differences in number of plant taxa were also significant (ANOVA, $F_{3, 12} = 6.88$, $p = 0.006$; Table 3.1). Almond cropping system samples had 3.2 ± 0.5 number of color pellets and was lower than meadowfoam and blueberry cropping systems. Almond cropping systems were lower than blueberry cropping systems in plant taxa. Almond cropping systems contained 3.8 ± 1.2 plant taxa, and blueberry cropping systems contained 16.3 ± 3.2 plant taxa in samples.

4.2 Pollen diet formulations

All 10 amino acids of each diet treatment exceeded the minimum threshold required for honey bees as reported by University of Missouri Agricultural Experiment Station Chemical Laboratories (Table 3.3). Crude protein content was highest in Diet 1 with 30.5% crude protein. Diet 2 was lowest in crude protein content with 25.3% crude protein. Diet 3 contained 27.7% and Diet 4 contained 26.5% crude protein (Table 3.3). We observed no queen supersedure or disease in the colonies for the duration of the study, hence colony dynamics pertaining to brood rearing were equal among experimental colonies.

4.3 Colony protein use

There were no significant differences in total pollen consumption between the treatments for the duration of the experiment (ANOVA, $F_{3, 12} = 0.422$, $p = 0.740$; Fig. 3.3A). We found significant differences in mean protein consumption among diet treatments ($F_{3, 12} = 7.24$, $p = 0.005$). Mean colony protein consumption rate was higher in Diet 1 compared to all other diet treatments. Diet 1 protein consumption was 121 g greater than Diet 2 (95% CI:[92.1, 149]; Fig. 3.3B), 88.8

g greater than Diet 3 (95% CI:[60.4, 117]; Fig. 3.3B), and 98.8 g greater than Diet 4 (95% CI:[70.4, 127]; Fig. 3.3B).

4.4 Hypopharyngeal gland protein content

There was a significant difference in mean hypopharyngeal gland protein in relation to colony protein consumption (ANOVA, $F_{3, 9} = 11.27$, $p < 0.001$). The mean protein utilization rate of hypopharyngeal glands was less in Diet 1 compared to all other diet treatments. Diet 1 protein consumption was 0.009 mg/g(mL) less than Diet 2 (95% CI:[0.002, 0.015]; Fig. 3.4), 0.007 mg/g(mL) less than Diet 3 (95% CI:[0.001, 0.014]), and 0.009 mg/g(mL) less than Diet 4 (95% CI:[0.002, 0.015]).

4.5 Gut proteolytic enzyme activity level

An ANOVA test showed that the mean gut proteolytic enzyme activity level was correlated with the proportion of diet consisting of pollen collected from almond cropping systems ($F_{3, 12} = 5.19$, $p = 0.035$). Proteolytic enzyme activity was negatively correlated with the proportion of diet consisting of pollen collected from almond cropping systems ($r^2 = 0.22$, $p = 0.002$; Fig. 3.5).

4.6 Colony growth

For both repeated measures of ANOVA tests performed on mean adult population and brood area, the best model of fit was with an AR1 covariance. No interaction effect between diet treatment and week was observed (adult population, $F_{16, 80} = 1.24$, $p = 0.328$; brood area, $F_{16, 80} = 0.787$, $p = 0.688$). In addition, no significant differences existed between the mean total adult population growth among diet treatments ($F_{16, 80} = 1.24$, $p = 0.328$). Nor did significant differences exist between the mean brood area among diet treatments ($F_{16, 80} = 0.093$, $p = 0.963$). Results from the Kruskal-Wallis test indicated no significant differences among mean ranks of *Varroa* mite infestation rate per colony across treatment groups ($H_3 = 0.691$, $p = 0.875$).

5. DISCUSSION

5.1 Field sampled pollen

The pollen samples collected from the four selected cropping systems were distinct from each other in several aspects. Foragers collected pollen from numerous plant species, along with the pollen from the target cropping system, but to varying levels. This finding supports previous studies, which suggest that honey bees prefer gathering pollen from multiple floral sources, even in landscapes characterized by single floral sources that are near and abundant (Phillips, 2011; Pettis et al., 2013). When foragers collect pollen, they detect olfactory cues of phagostimulants located in the pollenkitt, that is, the pollen's external surface, which serves as a feeding attractant in pollen (Doull and Standifer, 1970; Pernal and Currie, 2002; Schmidt and Hanna, 2006). Pollen's makeup of phagostimulants produces a species-specific odor that can be detected by foragers, allowing them to distinguish pollen from non-pollen substances and between different plant sources (Schmidt and Johnson, 1984; Dobson et al., 1996; Dobson and Bergström, 2000). Our findings regarding forager preferences show that honey bees gather pollen from multiple sources, which is indicative of mixed-pollen collection habits, instead of collecting the maximum amount of pollen available in the environment from a single source (Schmidt, 1984). Consequently, all of our pollen collected from colonies placed in cropping systems constitutes an accurate reflection of a colony's ability to obtain pollen from multiple sources in actual field settings.

The pollen received by colonies placed in almond cropping systems had significantly fewer numbers of color pellets and plant taxa compared to all other sites examined. Almonds in California constitute one of the largest monofloral agricultural landscapes for which honey bees are rented to pollinate in the U.S.. Because almond trees bloom early in the year when few other sources of pollen are available, the almond orchards in California represent a unique agricultural landscape. In 2012, full bloom ranged from approximately February 24th to March

7th, depending on almond cultivar and region of California (Blue Diamond Growers, 2012). Furthermore, California almonds also cover an extensive amount of acreage. In 2012, California had 780,000 acres of fruit-bearing almonds that yielded about 4.35 billion dollars in utilized production (NASS, 2012). Observations about nutritional effects described in this study may be useful to beekeepers that utilize almond cropping systems as part of their pollination contracts. This lack of pollen diversity in cropping systems, such as almonds, may be associated with nutritional deficiencies experienced by honey bees, which beekeepers should consider.

5.2 Colony protein use

The overall consumption by members of the colony did not differ with respect to pollen diets of varying crude protein percent. However, they did significantly differ with respect to the amount of protein consumed, which is a more realistic representation of the flow of protein to larvae and is consistent with previous findings (Schmidt, 1984; Schmidt and Johnson, 1984; Li et al., 2012). The results reinforce previous findings describing a colony response in which inadequate pollen availability tended to lead to colony consuming pollen at a higher rate instead of foraging for pollen with higher quality protein (Pernal and Currie, 2001). Researchers have shown that some insects can optimize nutritional uptake by overeating a limited nutrient and undereating an overabundant nutrient (Schowalter, 2006). Our study's results suggest that nurse bees consumed and possibly overate pollen in Diet 1 because they were not obtaining protein as effectively as nurse bees on other treatment diets.

5.3 Hypopharyngeal gland protein content

Results pertaining to the flow of protein from flower to larvae show that colonies consuming Diet 1 did not utilize protein as efficiently as other diet treatments within this study. Protein content in the hypopharyngeal glands is a direct indicator of the amount of protein that is contributing to the feeding cycle for

developing larvae. As seen in the experiment, the overall consumption by members of the colony were at a high level when fed Diet 1, but nurse bees had significantly lower amounts of protein content in their hypopharyngeal glands. There is a direct correlation between proteolytic enzyme activity level and hypopharyngeal gland protein content in honey bees (Sagili et al., 2005). Our results suggest that nutritional makeup of the pollen could be disrupting the pollen digestion process, making protein less available for hypopharyngeal glands to convert into food for larvae. Additionally, our results suggest that looking at the hypopharyngeal gland protein content relative to colony protein consumption may be a more accurate way to view nutritional status instead of solely relying on hypopharyngeal gland protein content.

All four of our diet treatments, including the treatment derived from mono-floral almond cropping systems, had crude protein content that exceeds the current recommended amount for honey bees. These four treatments also exceeded the minimum requirements of 10 designated essential amino acid ratios (DeGroot, 1953). This calls into the question whether current thresholds of pollen protein content are adequate for colony health. Crude protein and amino acid profile may not be an accurate portrayal of protein quality for honey bees. Instead of looking at the absolute value of protein and each amino acid ratio, nutritional requirements might be more sensitive to how the amino acids differ relative to each other (DeGroot, 1953). Since all diet treatments in this study exceeded the nutritional minimum for honey bees, in regard to crude protein level and 10 essential amino acids, this study is able to focus on the effects of pollen diversity, itself, as means of colony health.

5.4 Gut proteolytic enzyme activity level

Pollen can be difficult to digest and is not completely utilized in the gut (Schmidt and Buchmann, 1985). Our results suggest that Diet 1 was not digested well when compared to other diet treatments due to lower proteolytic enzyme activity. There

are a number of possible explanations for the lower proteolytic enzyme activity observed with Diet 1. Proteolytic enzyme activity can be sensitive to pH. For instance, maximum performance of enzymes occurs between a pH level of 8.0 and 8.5 (Moritz and Crailsheim, 1987). Different pollen diets may have influenced the pH level within the midgut, resulting in different performance levels of proteolytic enzymes. Crude protein content and amino acids have been observed to significantly degrade when stored and processed in comb (Standifier et al., 1980). Differences in degradation of crude protein and amino acid content of the different pollen diets may have occurred after storage. In addition, pollen wall porosity, thickness, and composition may also affect the digestibility of different pollen types (Roulston and Cane, 2000).

The decrease in proteolytic enzyme activity observed in diet treatments with increase in almond cropping system pollen in the diet formulations could also be attributed to pesticides detected in the pollen collected from almond cropping systems (Appendix B.1). It is difficult to say whether low proteolytic enzyme activity is a result of inherent nutritional deficits of pollen collected from colonies placed in almond cropping systems, pesticides levels detected in the pollen or a combination of both. However, we can conclude that if fungicide levels were typical for California almond cropping systems, the bees foraging in those cropping systems would experience a drop in protein metabolism. Although we could not statistically determine the effects of the pesticide levels, it is interesting to note that proteolytic enzyme activity level was the highest in the pollen diets composed of pollen collected in meadowfoam cropping systems, which had no detectable fungicide loads.

Generally, a single food source does not satisfy adequate requirements for optimal nutrition in organisms (Schowalter, 2006). The results of this study support optimal foraging theory, which posits that organisms select multiple food sources to maximize their diet intake efficiency and digestion rate (MacArthur and

Pianka, 1966; Zimmerman, 1981; Verlinden and Wiley, 1989). A branch of this theory explains the balancing act between nutrient-toxin interactions by claiming that an organism maximizes nutrient uptake by foraging within a variety of food sources that offer different amounts of nutrients and toxins in order to increase the likelihood of nutritional fulfillment and toxin dilution (Bernays et al., 1994; Hägele and Rowell-Rahier, 1999; Provenza et al., 2003). Honey bee forager preferences do not reflect intrinsic qualities, such as protein content or toxicity (Schmidt, 1984; Pernal and Currie, 2002). Honey bees naturally collect a mixed-pollen diet possibly to confront nutrient-toxin interactions (Schmidt, 1984). Wahl and Ulm (1983) found that honey bees raised on single-source pollen diets exhibited higher pesticide sensitivity than mixed pollen diets. The pollen collected in almond cropping systems for this study and others calls into question the feasibility for honey bees to achieve nutrient satisfaction and avoid toxin exposure (Pettis et al., 2013). The associational effect seen with proteolytic enzyme activity showed that almond pollen may have a toxic effect, and when diluted with more pollen sources, colonies exhibited a higher level of digestion (Fig. 3.5A). Regardless of whether the pollen diet in the almond orchards is nutritionally sufficient, the low amount of forage diversity increases the probability of toxic exposure. Colonies placed in bee pollinated cropping systems may risk toxic exposure because of routine conventional pesticide applications in these cropping systems.

Another potential toxic effect could result from plant-producing allelochemicals, phytotoxins, or secondary metabolites. For example, almond trees naturally produce a cyanogenic glycoside called amygdalin. In previous studies, honey bees that were fed on amygdalin exhibited negative effects (London-Shafir et al., 2003; Schowalter, 2006). Amygdalin concentrations present in the diet treatments of this study may have caused a reduction in pollen digestion.

5.5 Colony growth

Hypopharyngeal gland protein content was not directly correlated with larval diet. It is uncertain from this study whether the larvae were consuming more or less protein in the different experimental diets. The protein levels being received by larvae may have stayed the same because colony growth measurements did not differ during the five week experimental period. Nurse bees could have been compensating somehow to maintain larval populations. When nurse bees cannot obtain enough protein from what they consume, they use protein from their own body tissue to feed larvae (Haydak, 1934). This could be a possible method of compensation by having nurse bees overeat the almond diet. Honey bee colonies exhibit behavioral flexibility in the distribution of worker tasks in order to provide a buffer to negative environmental influences (Seeley 1982). Therefore, another possible method of compensation could be an extension of the time nurse bees remain as nurse bees. This would increase the population of nurse bees among the worker population in the colony. This would also delay transition of nurse bees becoming foragers, and might affect colony foraging activity as a result.

However, hypopharyngeal gland development and protein biosynthesis is unaffected by the amount of brood present (Hrassnigg and Crailsheim, 1998; Pernal and Currie, 2000). Even though nurse bees consuming the almond diet had lower hypopharyngeal gland protein content, they might have used protein from their own bodies to maintain larval populations. When colonies experience wavering homeostasis, they respond by fluctuating population sizes of each worker task force (Huang and Robinson, 1996). The colonies fed on the almond diet may have responded by generating more nurse bees than usual.

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Table 3.1 Floral diversity in pollen collected from colonies placed adjacent to select cropping systems. Floral diversity was measured by the mean number of pellet colors and mean number of plant taxa found in pollen collection samples. Standard errors are represented to display variation between locations. Significant differences among crops are indicated by letters ($p = 0.006^{1}$, $p = 0.006^{2}$).

Pollen collection from colonies placed in listed cropping system	Number of sites (n)	Mean number of pellet colors (SE)[1]	Mean number of taxa (SE)[2]
Almond	5	3.2 (0.5)[a]	3.8 (1.2)[a]
Cherry	3	6.7 (1.8)[ab]	7.3 (1.8)[ab]
Meadowfoam	5	8.6 (1.4)[b]	8.2 (1.8)[ab]
Blueberry	3	11.0 (1.7)[b]	16.3 (3.2)[b]

Table 3.2 Floral diversity factors of pollen diet formulations. Diet treatments were from pooled pollen collections from colonies placed adjacent to select cropping systems.

Diet label	Treatment diet source formulation[1]	Number of pellet colors (n)	Number of plant taxa (n)
Diet 1	100% Almond cropping system collection	3.2	3.8
Diet 2	100% Meadowfoam cropping system collection	8.6	8.2
Diet 3	50% Almond cropping system and 50% Meadowfoam cropping system collection	11.8	12.0
Diet 4	25% Almond, 25% Meadowfoam, 25% Cherry, and 25% Blueberry cropping system collection	29.5	35.7

[1]Percentages indicate pollen blend by mixing listed pollen collections (w/w)

Table 3.3 Crude protein content of pollen diet formulations. Standard errors associated with crude protein content are based on four composite samples for each diet.

Diet label	Percent crude protein content (SE)[1]
Diet 1	30.5 (0.05)
Diet 2	25.3 (0.06)
Diet 3	27.7 (0.03)
Diet 4	26.5 (0.04)

[1]Crude protein analyzed by combustion method of sample dry weight

Table 3.4 Profile of required amino acids of each pollen diet formulation based on mean of duplicate samples. Units are measured in proportion (w/w% in grams per 100 grams).

	Essential amino acids[1]									
	Arg	His	Iso	Leu	Lys	Met	Phe	Thr	Try	Val
Minimum requirements[2]	*3.0*	*1.5*	*4.0*	*4.5*	*3.0*	*1.5*	*1.5*	*3.0*	*1.0*	*4.0*
Almond	4.8	2.1	4.3	7.1	7.4	2.0	4.2	3.9	1.2	5.5
Meadowfoam	4.7	2.2	4.7	7.5	7.3	2.1	4.4	3.9	1.1	5.6
50% Almond 50% Meadowfoam	4.8	2.2	4.6	7.3	7.5	2.0	4.3	3.8	1.2	5.7
25% Almond 25% Cherry 25% Meadowfoam 25% Blueberry	4.9	2.2	4.5	7.2	7.4	2.1	4.3	3.8	1.2	5.6

[1]Amino acid abbreviations: Arg: arginine, His: histidine, Iso: isoleucine, Leu: leucine, Lys: lysine, Met: methionine, Phe: phenylalanine, Thr: threonine, Try: tryptophan, Val: valine

[2]Minimum requirements of 10 essential amino acids for honey bee diet are based on minimum threshold levels from DeGroot (1953)

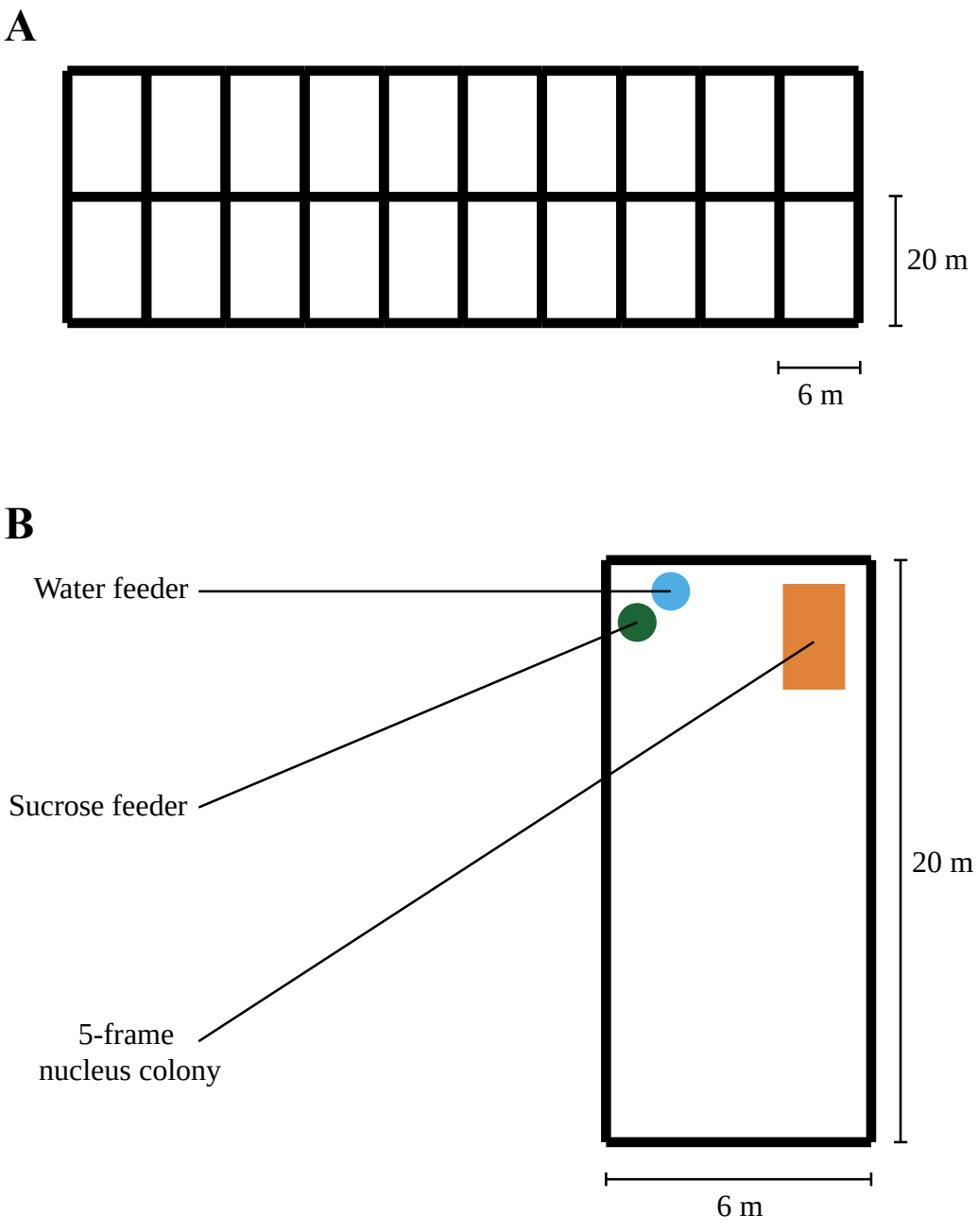

Figure 3.1A Layout of entire flight cage design. **3.1B** Layout of single flight cage with labeled colony and feeders from an aerial viewpoint.

Figure 3.2 Dissection of hypopharyngeal glands from a nurse bee (Photo credit: Ellen Topitzhofer and Phil Schapker).

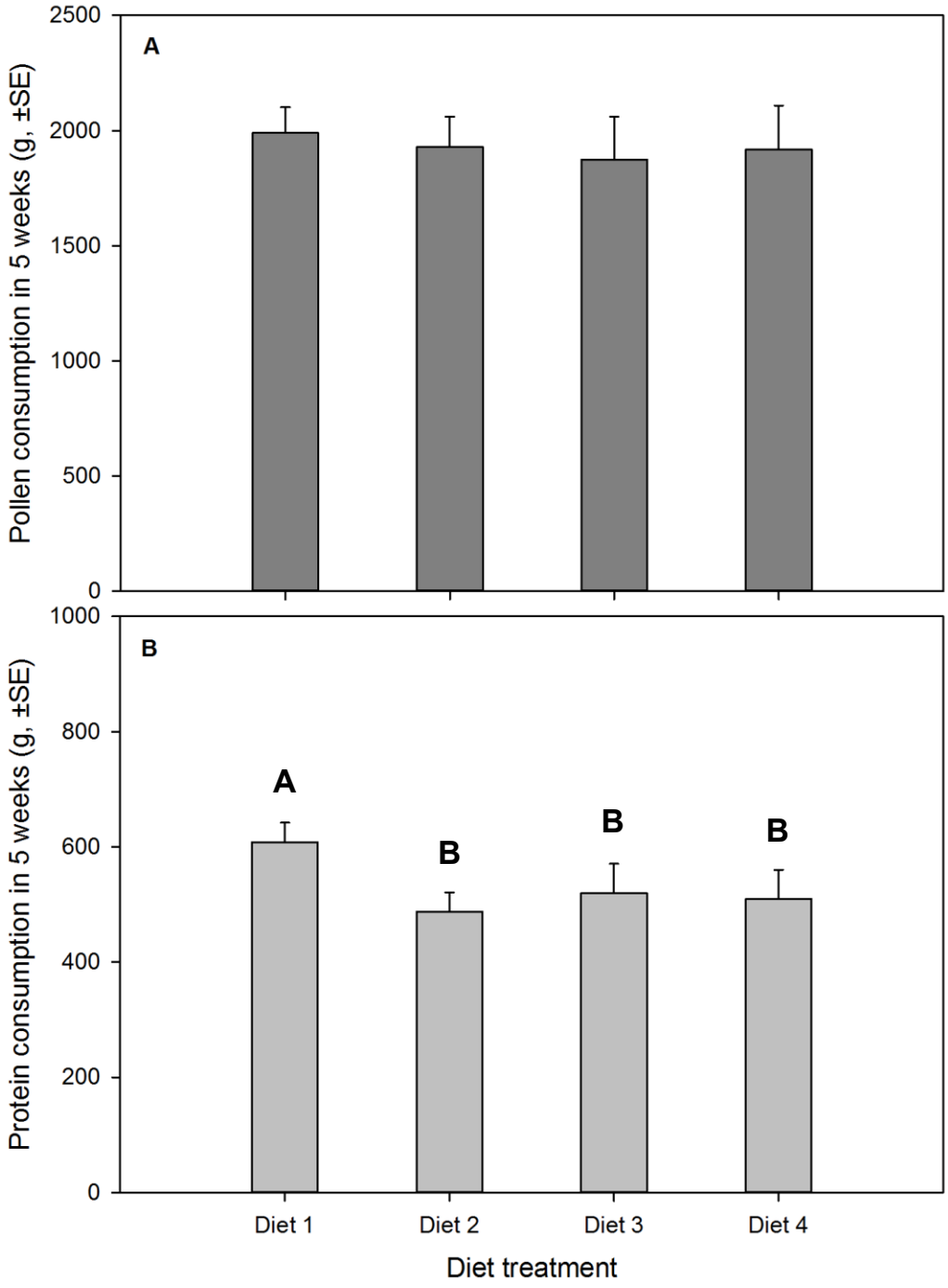

Figure 3.3A Colony pollen consumption in 5 weeks. No significant differences were found between treatment groups (p = 0.740). **3.3B** Protein consumption in 5 weeks. Protein consumption was calculated by total pollen consumption (g) and crude protein content of pollen (%). Bars represent one standard error. Letters indicate significance (p = 0.013).

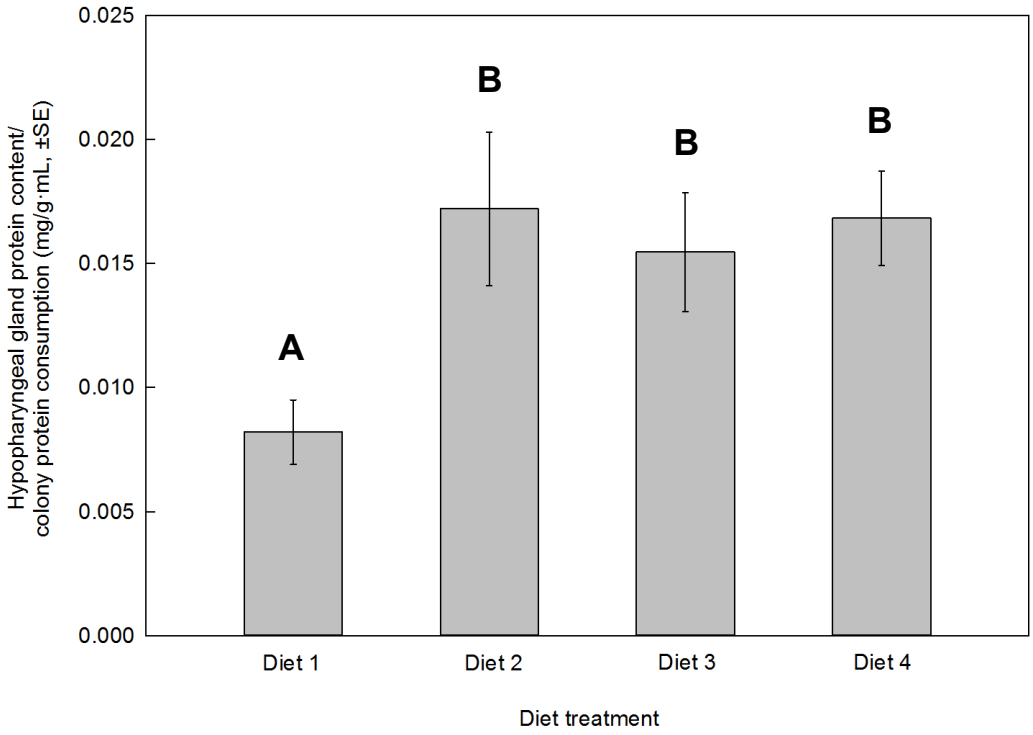

Figure 3.4 Mean hypoharyngeal gland protein relative to colony protein consumption. Letters indicate significance ($p = 0.018$). Bars represent one standard error.

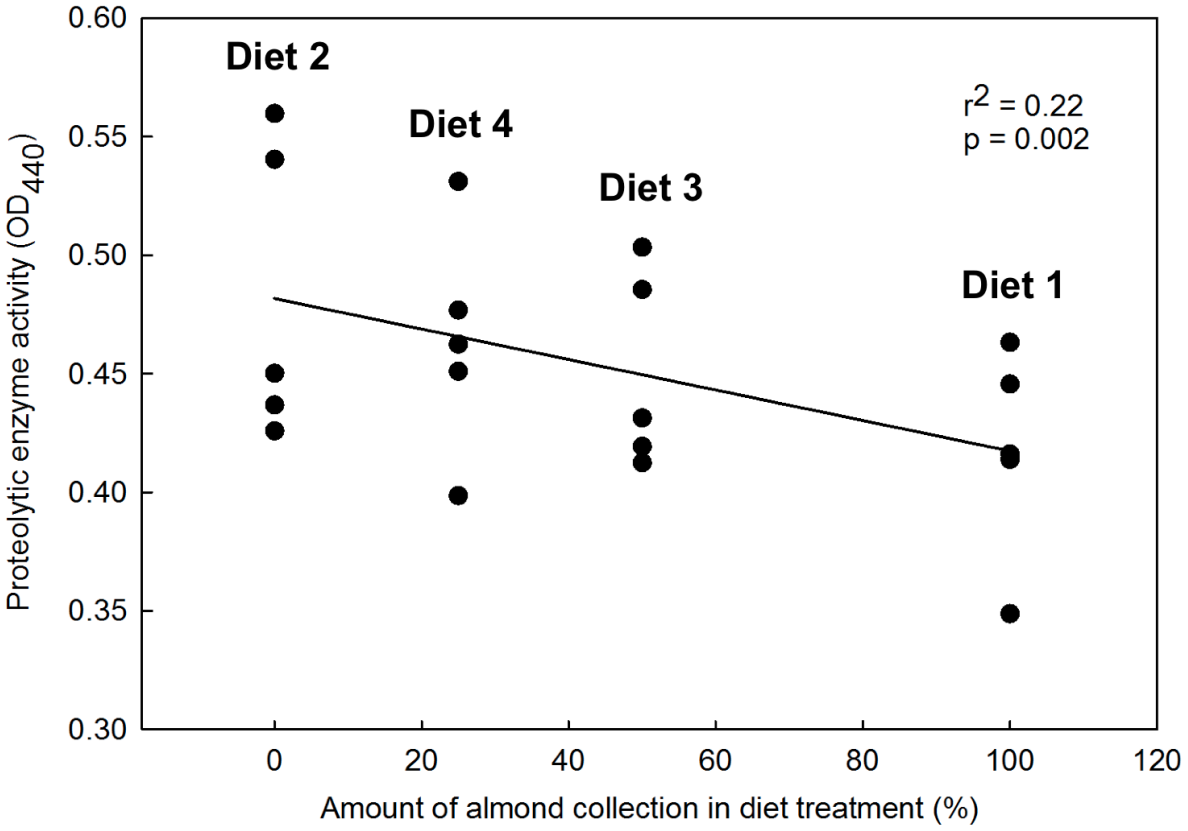

Figure 3.5 Proteolytic enzyme activity level (mean of 20 nurse bees per colony) versus percentage of pollen in diet treatment from pollen collected from colonies in almond cropping systems. Line indicates regression line ($r^2 = 0.22$, $p = 0.002$).

Chapter 4:
Summary

Managed honey bee colonies provide important pollination services for various cultivated cropping systems. Poor nutrition could affect honey bee health and is considered to be involved in recent honey bee colony declines along with few other stress factors. The current practice of migratory beekeeping within a sequence of cropping systems may contribute to poor nutrition in managed honey bee colonies. In this study, we first described the diversity of pollen collected by honey bee colonies when placed in seven cropping systems pollinated by honey bees (Chapter 2). We further examined if pollen diversity influences colony-level protein utilization and biosynthesis of protein in nurse bees by conducting a pollen feeding experiment (Chapter 3).

Because many commercial beekeepers from the Pacific Northwest region rent their colonies for pollination services, it is important to address nutritional health in cropping systems that colonies frequently pollinate. Findings from Chapter 2 indicated that the distinct cropping systems in this study contained different degrees of pollen diversity. Specifically, we found that pollen collected from colonies placed in almond cropping systems presented a low degree of pollen diversity and a high percentage of the target pollen type (*Prunus* sp.). At the other end of the spectrum, pollen collected from colonies placed adjacent to blueberry cropping systems did not yield any target pollen types (Highbush blueberry, *Vaccinium corymbosum* L.), but was high in overall pollen diversity. The pollen collected from colonies placed in other cropping systems was largely intermediate in diversity between these two extremes.

The objective in Chapter 3 was to determine the effect of pollen diversity on colony-level protein use, biosynthesis of protein in nurse bees, and colony growth. To

accomplish this objective, we designed a feeding experiment. Pollen collected from four different cropping systems in the first study was used to formulate four different diets, each varying in pollen diversity. Among the four diets, colonies were fed on pollen collected from colonies placed in almond cropping systems exhibited high protein consumption use by the colony. However, we found low amounts of protein content in hypopharyngeal glands of nurse bees fed on pollen collected from colonies placed in almond cropping systems. A lower proteolytic enzyme activity level within nurse bee digestive tracts indicated a lower digestion rate of protein. The pollen diet from almond cropping systems caused a decreased digestion rate of protein, which may have contributed to the lower amount of protein content in the nurse bee hypopharyngeal glands. These findings highlight the importance of assessing protein availability to larvae after nurse bees have consumed pollen as opposed to assessing protein from raw pollen.

The data from this study provided insights on some cropping systems that lack in pollen diversity and how that might affect the degree of protein digestibility for honey bees. Pettis et al. (2013) found that pollen collected from almond cropping systems presented a high amount of target crop pollen. Determining target crop proportions in pollen collections can be an indicator of pollen diversity between target and non-target plant species (Pettis et al., 2013). However, we found no target crop pollen in collections from honey bees in blueberry cropping systems. This suggests that quantifying the target crop proportion of pollen collected from honey bees may not provide the degree of pollen diversity in some cropping systems. The data in our study indicates that pollen from almond cropping systems presents a low level of diversity. Although almond pollen has been seen as a highly nutritious pollen diet for brood rearing in previous studies, information is lacking on the quality of the diet in relation to its degree of pollen diversity (Loper et al., 1980). As seen in this study, protein found in the collected pollen does not necessarily provide a direct translation to the protein content found in hypopharyngeal glands of nurse bees. In contrast to our study, previous studies typically observed diets solely based on protein in the

form of raw pollen (Herbert et al., 1977; Alaux et al., 2010; Degrandi-Hoffman et al., 2010; Li et al., 2012; Di Pasquale et al., 2013; Pettis et al., 2013). The results of this study suggest that a more comprehensive approach is needed to better understand the influence of the nutritional quality of collected pollen on honey bee health.

Researchers and commercial beekeepers should pay close attention to pollen diversity available to honey bee colonies to maintain optimal health in California (CA) almond cropping systems. As a large portion of U.S. commercial colonies travel to CA almond regions every year, a significant population of U.S. honey bees may interact with landscapes that lack sufficient pollen diversity. Colonies that face nutritional limitation due to monofloral environments may become more susceptible to pests and diseases, a situation exacerbated when such environments are marked by high-density of honey bee populations. Factors of density and potential nutritional deficiencies may influence cross-contamination dynamics and the rate of disease spread when honey bee colonies pollinate almonds. Studying the interaction of these variables may provide useful insights that may help in designing management strategies to improve honey bee health.

There is also a need for additional research on the affects of fungicides and other commonly applied pesticides on almond trees in relation to honey bee pollen consumption rates and protein absorption. As honey bee gut microflora play an important role in digestion, certain fungicides could possibly have a negative impact on beneficial fungi in their guts. In this study we did not investigate the effects of pollen diversity on honey bee immune system. This is an important parameter that future studies should explore to better understand the role of pollen diversity on honey bee immunity. This research also suggests that, in order to understand the nutritional status of honey bee populations, there should be an effort to determine an acceptable range for hypopharyngeal gland protein content in nurse bees for commercial honey bee colonies that are placed in a given cropping system.

In cropping systems where bees have limited access to diverse pollen or amounts of pollen, alternate forage for bees should be provided by either planting hedgerows near these fields or cultivating few acres of alternate crops to enhance pollen diversity and availability. Such management practices will not only benefit honey bees, but also other native bees in those areas.

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APPENDIX

Appendix A: Chapter 2 Supporting Information

Table A.1 Details of the locations of pollen collection within each cropping system. Collection time period, total number of colonies selected, total quantity of pollen collected, and average quantity per collection site are included. Standard error is indicated with mean quantity collected when multiple collection samples were taken.

Location of pollen collection	Collection dates (month/day, year is 2012)	Number of Colonies	Total quantity collected (g)	Mean quantity collected per site (SE)
Almond				
Turlock, CA	2/27 – 3/5	15	4762.72	3798.84 (963.89)
Turlock, CA	3/5 – 3/12	15	2834.95	
Chowchilla, CA	3/27 – 3/5	5	1360.78	
Gustine, CA	2/25 – 3/3	10	566.99	
Ripon, CA	3/26 – 3/6	5	3628.74	3458.64 (170.10)
Ripon, CA	3/6 – 3/13	5	3288.54	
Madera, CA	3/1 – 3/8	15	7030.68	3628.74 (3401.94)
Madera, CA	3/8 – 3/17	15	226.80	
Cherry				
Gervais, OR	4/13 – 4/20	10	226.80	2494.76 (2267.96)
Gervais, OR	4/20 – 4/27	10	4762.72	
St. Paul, OR	4/14 – 4/21	10	17123.10	10999.61 (6123.50)
St. Paul, OR	4/21 – 4/28	10	4876.11	
Salem, OR	4/22 – 4/29	20	4989.51	
Blueberry				
Sheridan, OR	4/25 – 5/2	15	2267.96	1133.98 (1133.98)
Sheridan, OR	5/2 – 5/9	15	0	
Jefferson, OR	5/1 – 5/8	15	1587.57	1360.78 (130.94)
Jefferson, OR	5/8 – 5/15	15	1133.98	
Jefferson, OR	5/15 – 5/22	15	1360.78	

Table A.1 (Continued)				
Forest Grove, OR	4/26 – 5/3	10	680.39	
Newberg, OR	5/8 – 5/15	15	1814.37	1360.78 (453.59)
Newberg, OR	5/15 – 5/22	15	907.18	
Meadowfoam				
Eugene, OR	5/11 – 5/18	15	10999.606	
Eugene, OR	5/11 – 5/18	10	5329.706	
Monmouth, OR	4/23 – 4/30	10	11793.392	
Monroe, OR	4/17 – 4/24	10	2041.164	2664.85 (623.69)
Monroe, OR	4/24 – 4/31	10	3288.542	
White Clover				
Corvallis, OR	6/15 – 6/22	5	453.6	
Radish				
Shedd, OR	6/11 – 6/18	5	566.99	
Shedd, OR	6/15 – 6/22	5	907.18	
Carrot				
Madras, OR	7/11 – 7/18	5	0	22.68 (22.68)
Madras, OR	7/18 – 7/25	5	45.36	
Madras, OR	7/11 – 7/18	5	0	113.40 (113.40)
Madras, OR	7/18 – 7/25	5	226.80	

Table A.2 List of color pellets and plant taxa identified in samples from each cropping system. Each cropping system section represents pooled results from sampled cropping system. Number of sites and taxa detected within each color pellet is indicated.

Almond				
Color pellet label	*n* sites detected	*n* Taxa	Listed taxa	Plant family
Gold	1	1	Brassicaceae sp.	Brassicaceae
Yellow	3	3	Brassicaceae sp., *Cornus stolonifera*, *Trifolium repens*	Brassicaceae, Cornaceae, Fabaceae
Reddish Orange	1	3	*Viburnum* sp., *Trifolium incarnatum*, *Crataegus* sp.,	Caprifoliaceae, Fabaceae, Rosaceae
Ash	1	1	Rosaceae sp.	Rosaceae
Cream	5	1	*Prunus* sp.	Rosaceae
Tan	1	1	*Prunus* sp.	Rosaceae
Yellow Gold	2	1	*Prunus* sp.	Rosaceae

Cherry (Table A.2 continued)				
Color pellet label	*n* sites detected	*n* Taxa	Listed taxa	Plant family
Gold	2	6	*Acer microphyllum, Acer* spp., Brassicaceae sp., *Trifolium arvense, Allium* sp., *Prunus* sp.	Aceraceae, Brassicaceae, Fabaceae, Liliaceae, Rosaceae
Light Brown	4	5	*Acer microphyllum, Viburnum* sp., *Quercus* spp., *Prunus* sp., Rosaceae sp.	Aceraceae, Caprifoliaceae, Fagaceae, Rosaceae
Ash	2	1	Anacardiaceae sp.	Anacardiaceae
Tan	1	1	*Anethum graveolens*	Apiaceae
Orange	5	2	*Taraxacum officinale, Prunus* sp.	Asteraceae, Rosaceae
Yellow	3	2	Brassicaceae sp., *Prunus* sp.	Brassicaceae, Rosaceae
Amber	1	1	*Viburnum* sp.	Caprifoliaceae
Yellow Gold	4	3	Brassicaceae sp., *Viburnum* sp., *Fraxinus* sp., *Prunus* sp.	Brassicaceae, Caprifoliaceae, Oleaceae, Rosaceae
Yellow Green	2	2	*Viburnum* sp., *Crataegus* sp.	Caprifoliaceae, Rosaceae
Army Green	1	2	*Viburnum* sp., *Prunus* sp.	Caprifoliaceae, Rosaceae
Black	1	1	Caryophyllaceae sp.	Caryophyllaceae

Blueberry (Table A.2 continued)				
Color pellet label	*n* sites detected	*n* Taxa	Listed taxa	Plant family
Amber	3	5	*Acer microphyllum, Trifolium incarnatum, Fraxinus* sp., *Dryas drummondii*, Scrophulariaceae sp.	Aceraceae, Fabaceae, Oleaceae, Rosaceae, Scrophulariaceae
Red	1	2	*Toxicodendron rydbergii, Limnanthes alba*	Anacardiaceae, Limnanthaceae
Orange	3	2	*Taraxacum officinale, Cichorea* sp.	Asteraceae
Ash	5	5	Brassicaceae sp., *Calycanthus floridus, Cornus stolonifera, Trifolium repens, Limnanthes alba*	Brassicaceae, Calycanthaceae, Cornaceae, Fabaceae, Limnanthaceae
Light Brown	2	5	Brassicaceae sp., *Trifolium pratense, Quercus* spp., *Aesculus hippocastanum,* Rosaceae sp.	Brassicaceae, Fabaceae, Fagaceae, Hippocastanaceae, Rosaceae
Tan	2	2	Brassicaceae sp., *Prunus* sp.	Brassicaceae, Rosaceae
Yellow	7	3	Brassicaceae spp., Liliaceae sp., *Dryas drummondii*	Brassicaceae, Liliaceae, Rosaceae
Yellow Gold	7	4	Brassicaceae spp., *Cornus stolonifera, Prunus* sp., *Halesia* sp.	Brassicaceae, Cornaceae, Rosaceae, Stryacaceae

Blueberry (continued from p. 54, Table A.2)				
Color pellet label	*n* sites detected	*n* Taxa	Listed taxa	Plant family
Army Green	4	5	*Viburnum* sp., Cheno-AM sp.[1], *Fragaria* sp., *Prunus* sp., *Pedicularis* sp.	Caprifoliaceae, Amaranthaceae, Rosaceae, Scrophulariaceae
Brown	4	5	*Viburnum* sp., *Medicago minima, Limnanthes alba, Pinus* sp., *Dryas drummondii*	Caprifoliaceae, Fabaceae, Limnanthaceae, Pinaceae, Rosaceae
Grey	1	1	*Trifolium incarnatum*	Fabaceae
Reddish Orange	2	1	*Aesculus hippocastanum*	Hippocastanaceae

[1]Cheno-AM refers to pollen samples within Chenopodiaceae or *Amaranthus* of Amaranthaceae. Due to the nature of these pollen grains, they are nearly impossible to differentiate (Martin, 1963).

Meadowfoam (Table A.2 continued)				
Color pellet label	*n* sites detected	*n* Taxa	Listed taxa	Plant family
Yellow Gold	6	6	*Acer microphyllum, Rhus glabra, Toxicodendron rydbergii, Taraxacum officinale, Liliaceae, Prunus* sp.	Aceraceae, Anacardiaceae, Asteraceae, Liliaceae, Rosaceae
Army Green	4	5	*Medicago sativa, Trifolium arvense, Trifolium incarnatum, Crataegus* sp., Scrophulariaceae sp.	Fabaceae, Rosaceae, Scrophulariaceae
Amber	2	2	*Rhus glabra, Trifolium incarnatum*	Anacardiaceae, Fabaceae
Black	3	5	*Calycanthus floridus, Limnanthes alba, Prunus* sp., Rosaceae sp., *Rhus glabra*	Anacardiaceae, Calycanthaceae, Limnanthaceae, Rosaceae
Brown	5	5	*Toxicodendron diversilobum, Toxicodendron rydbergii, Limnanthes alba, Crataegus* sp., *Holodiscus* sp.	Anacardiaceae, Limnnanthaceae, Rosaceae
Cream	3	3	*Toxicodendron rydbergii, Limnanthes alba, Prunus* sp.	Anacardiaceae, Limnanthaceae, Rosaceae

Meadowfoam (continued from pg. 56, Table A.2)				
Color pellet label	*n* sites detected	*n* Taxa	Listed taxa	Plant family
Light Brown	1	4	*Toxicodendron rydbergii*, Brassicaceae sp., *Trifolium pratense*, *Limnanthes alba*	Anacardiaceae, Brassicaceae Fabaceae, Limnanthaceae
Red	3	3	*Toxicodendron rydbergii*, *Limnanthes alba*, *Prunus* sp.	Anacardiaceae, Limnanthaceae, Rosaceae
Tan	3	2	Anacardiaceae sp., *Prunus* sp.	Anacardiaceae, Rosaceae
Orange	2	6	*Taraxacum officinale*, *Cichorea* sp., *Robinia pseudoacacia*, *Trifolium pratense*, *Limnanthes alba*, *Verbascum thapsus*	Asteraceae, Fabaceae, Limnanthaceae, Scrophulariaceae
Yellow	4	4	Brassicaceae spp., *Limnanthes alba*, *Prunus* sp., Rosaceae spp.	Brassicaceae, Limnanthaceae, Rosaceae
Violet	1	1	*Aesculus hippocastanum*	Hippocastanaceae
Ash	1	1	*Limnanthes alba*	Limnanthaceae
Gold	1	1	*Limnanthes alba*	Limnanthaceae
Yellow Green	1	1	*Limnanthes alba*	Limnanthaceae

Clover (Table A.2 continued)				
Color pellet label	*n* sites detected	*n* Taxa	Listed taxa	Plant family
Ash	2	2	Anacardiaceae sp., *Pedicularis* sp.	Anacardiaceae, Scrophulariaceae
Orange	2	1	*Taraxacum officinale*	Asteraceae
Purple	1	1	*Cirsium* sp.	Asteraceae
Yellow Gold	2	4	*Taraxacum officinale,* Brassicaceae spp., *Vicia* sp., *Verbascum thapsus*	Asteraceae, Brassicaceae, Fabaceae, Scrophulariaceae
Yellow	1	1	Brassicaceae sp.	Brassicaceae
Black	1	3	*Cornus stolonifera, Medicago sativa,* Poaceae sp.	Cornaceae, Fabaceae, Poaceae
Army green	2	1	*Trifolium repens*	Fabaceae
Brown	1	1	*Trifolium repens*	Fabaceae
Grey	2	1	*Medicago* sp.	Fabaceae
White	1	2	*Trifolium arvense,* Poaceae sp.	Fabaceae, Poaceae

Radish (Table A.2 continued)				
Color pellet label	*n* sites detected	*n* Taxa	Listed taxa	Plant family
Ash	2	1	Anacardiaceae sp.	Anacardiaceae
Orange	2	3	*Toxicodendron rydbergii*, *Cichorea* sp., *Trifolium arvense*	Anacardiaceae, Asteraceae, Fabaceae
Reddish Orange	1	2	Brassicaceae sp., *Trifolium arvense*	Brassicaceae, Fabaceae
Yellow	2	1	Brassicaceae sp.	Brassicaceae
Yellow Gold	1	1	Brassicaceae sp.	Brassicaceae
Army green	2	1	*Trifolium repens*	Fabaceae
Grey	2	1	*Medicago* sp.	Fabaceae
Brown	1	1	*Limnanthes alba*	Limnanthaceae

Carrot (Table A.2 continued)				
Color pellet label	*n* sites detected	*n* Taxa	Listed taxa	Plant family
Black	2	4	*Cirsium* sp., Cheno-AM sp.[1], *Trifolium incarnatum*, *Fragaria* sp.	Asteraceae, Cheno-AM[1], Fabaceae, Rosaceae
Light Brown	1	1	Cheno-AM spp.[1]	Cheno-AM[1]
Orange	2	3	*Anethum graveolens*, Asteraceae sp., *Ratibida columnifera*	Apiaceae, Asteraceae
Reddish Orange	1	5	*Anethum graveolens*, *Cirsium* sp., *Ratibida columnifera*, Cheno-AM spp.[1], *Plantago* sp.	Apiaceae, Asteraceae, Cheno-AM[1], Polygonaceae
Yellow Gold	2	3	*Anethum graveolens*, Asteraceae sp., Cheno-AM sp.[1]	Apiaceae, Asteraceae, Cheno-AM[1]
Army Green	2	1	Cheno-AM sp.[1]	Cheno-AM[1]

[1]Cheno-AM refers to pollen samples within Chenopodiaceae or *Amaranthus* of Amaranthaceae. Due to the nature of these pollen grains, they are nearly impossible to differentiate (Martin, 1963).

Appendix B: Chapter 3 Supporting Information

Table B.1 Pesticide residue analysis of 10-gram sample of pollen per cropping system.

Pesticide	Pesticide class	Quantity (ppb)
Almond		
2,4 Dimethylphenyl formamide (DMPF)	Acaricide	5
Azoxystrobin	Fungicide	91.8
Boscalid	Fungicide	64.9
Carbendazim (MBC)	Fungicide	5.6
Cyprodinil	Fungicide	169
Fenbuconazole	Fungicide	23.5
Methoxyfenozide	Insect growth regulator	585
Oxyfluorfen	Herbicide	15.3
Pendimethalin	Herbicide	310
Pyraclostrobin	Fungicide	25
Pyrimethanil	Fungicide	14.1
Cherry		
Azoxystrobin	Fungicide	254
Chlorpyrifos	Organophosphate	32.7
Fluvalinate	Pyrethroid	5.4
Myclobutanil	Fungicide	55
Pendimethalin	Herbicide	13.3
Tebuconazole	Fungicide	36
Meadowfoam		
2,4 Dimethylphenyl formamide (DMPF)	Acaricide	6.5
Chlorpyrifos	Organophosphate	7
Fluvalinate	Pyrethroid	18.4
Blueberry		
Azoxystrobin	Fungicide	9.5
Bifenthrin	Pyrethroid	20.7
Captan	Fungicide	65
Chlorpyrifos	Organophosphate	15.7
Pendimethalin	Herbicide	22
Pyraclostrobin	Fungicide	25.8
Trifluralin	Herbicide	2.1