Apple orchards feed and contaminate bees during, but even more so after bloom

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Scientific Abstract

Honey bees, *Apis mellifera* Linn., provide vital economic and ecological services via pollination while concurrently facing multiple interconnected stressors impacting their health. Many crops like apples, peaches, and cherries that add diversity and nutrition to our diet are wholly or partially dependent upon the pollination services of insects. Orchard crops are self-incompatible and commonly regarded as crops reliant on the pollination services of insects, and while previous studies have focused on the impact of bees to orchard crops during bloom, fewer studies have examined the reciprocal relationship of the orchards on honey bees, particularly across the entire foraging season. Here we investigated the foraging dynamics of honey bees in an orchard crop environment in Northern Virginia, United States. We decoded, mapped, and analyzed 3,710 waggle dances, which communicate the location of a valuable resource in the environment, for two full foraging seasons (April-October, 2018-2019), and, concurrent to the dance filming, collected pollen from returning foragers. We found that bees forage locally the majority of the time (< 2 km) throughout the season, with some long-range distances occurring in May after bloom (both 2018 and 2019) and in fall (2019). The shortest communicated median distances (0.50 km and 0.53 km), indicating abundant food availability, occurred during September in both years, paralleling the bloom of an important late season resource, goldenrod (*Solidago*). We determined, through plotting and analyzing the communicated forage locations and from the collected pollen from returning foragers, that honey bees forage more within apple orchards after the bloom (29.4% and 28.5% foraging) compared to during bloom (18.6% and 21.4% foraging) on the understory of clover and plantain. This post bloom foraging also exposes honey bees to the highest concentration of pesticides across the entire foraging season (2322.89 ppb pesticides versus 181.8 during bloom, 569.84 in late summer, and 246.24 in fall). Therefore, post bloom apple orchards supply an abundance of forage, but also the highest risk of pesticide exposure, which may have important implications for management decisions of bees in orchards.

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General Audience Abstract

Honey bee hives have been declining significantly in the United States, driven by a multitude of issues and stressors including pesticide exposure, disease, pests such as varroa mites, and poor nutrition caused by natural land being converted into development or agriculture. Agricultural landscapes, in particular, are often monocultures are saturated with pesticides creating a potentially hazardous environment, yet reliant on bees to provide pollination for crops. Because of this interconnected relationship between bees and flowers and the effects of stressors agricultural systems cause have with pollinators, it is necessary to understand how honey bees forage in these environments and what potential health risks they face. We investigated honey bees foraging dynamics in an apple orchard in Northern Virginia, United States by observing honey bee waggle dance behavior, where bees literally waggle back and forth for a certain time and at a certain angle telling their nestmates where a resource is, and collecting pollen from returning forager bees to better understand when, where, and upon what honey bees forage throughout the foraging season, which is when flowers are available and the weather warm enough (April – October). We found that bees mostly forage locally near the hive throughout the season, indicating that sufficient amount of food was available even after short bloom time of the apple (April to mid-May). We determined, through plotting and analyzing the waggle dance locations, that honey bees forage more within apple orchards after the bloom on mostly clover and plantain. This abundance of post bloom foraging also exposes honey bees to the highest amounts of pesticides across the entire foraging season. Post bloom apple orchards supply an abundance of forage, but also the highest risk of pesticide exposure to honey bees.

This thesis is dedicated to BJ, my family, friends and advisors. Without your support and encouragement completing this research would not have been possible.

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CHAPTER 1: BACKGROUND, INTRODUCTION AND LITERATURE REVIEW

General honey bee life history and biology

The western honey bee, *Apis mellifera* Linn., is one of about 20,000 known bee species worldwide (1, 2). Based on fossil records, the ancestry of honey bees has been traced to the Oligocene period over 30 million years ago (3). More contemporarily, honey bee colonies have been managed by humans for thousands of years with records of beekeeping dating back to ancient Egypt and the creation of modern agriculture (4). Honey bees are native to Europe, the Middle East, Africa, and were introduced to the Americas, Asia, Australia, and the Pacific Islands (5). Their introduction to the Americas was sometime during the 1600s, and ever since then we have been extensively managing them for use in honey production and pollination services in agriculture (3, 6).

The taxonomy of honey bees classifies them in the Order Hymenoptera, hymen meaning "membrane" + pteron meaning "wing", and the family Apidae, or long-tonged bees. Honey bees are categorized under the genus *Apis*, and are eusocial insects among the Aculeata, a term used to define the subclade of species within Hymenoptera that have modified ovipositors that function as stingers. Eusociality is an evolutionarily fascinating phenomenon that some species have used to adapt and survive. Eusociality generally follows a few criteria: overlapping generations, reproductive division of labor, and cooperative brood care.

Honey bees are an example of eusocial insects, where a social structure of a colony is composed of three distinct castes: workers (females), drones (males), and a single reproductive queen (female). The workers make up the majority of the colony, are non-reproductive, and responsible for the functions of security, grooming, cleaning, maintenance/ construction, thermoregulation, nursing brood, and foraging for the hive. The life span of the worker bee ranges from five weeks to six months, depending on the season. The queen mates once in her lifetime and her sole responsibility is to reproduce and colony communication, laying up to 2,000 eggs in a summer day. Drones' sole purpose is to mate with a virgin queen. During development, all castes undergo six molts before reaching adulthood, a process lasting between 16-24 days dependent on caste (4). Colony size can range from 20,000 to over 80,000 honey bees. In part, due to their large colony size, communication is fundamental in accomplishing the tasks for the success of the hive, which includes successful exploitation of forage resources in an ephemeral environment.

The communication of honey bees is complex and dynamic. They utilize a combination of olfactory, gustatory, visual and tactile physical cues such as pheromones, trophallactic behavior, vibrations and "dancing" to communicate statuses such as health, alarms, and foraging locations. Their ability to process information and make informed decisions has been the topic of research of everything from leadership to aircraft path planning (7, 8). One of their unique communications, known as the waggle dance, is used to communicate to their nest mates the foraging locations of quality food resources in their surrounding environment (9, 10).

The waggle dance is a behavior unique to *Apis* bees, and it communicates a distance and direction from the hive to the resource location via the "waggle run" portion of the dance (10, 11). Distance is communicated by duration of the waggle run, while direction is communicated by angle in relation to the solar azimuth. A dance consists of multiple repeated waggle runs, with each waggle run beginning at the end of the return phase. This information allows researchers to understand where honey bees are foraging in their environment (5, 12-14).

Honey Bee Foraging and Waggle Dance Behavior

Honey bees are general and economical foragers, meaning they forage freely on a variety of flowering plant species and consider the benefit (quality and quantity) of the resource against its associated cost (i.e., flight distance) (15, 16). In other words, if all else is equal, honey bees place higher value on resources located closer to the hive because collecting the same quality and quantity resources further away is calorically costly and risky due to energy expenditure and exposure to predation during flight. Distance, therefore, is a particular consideration in honey bee foraging work because (1) foraging profit is inversely related to increased flight distance (12) and therefore (2) the distance at which a bee forages can serve as a proxy for food availability. Bees are exceptionally good at finding resources (17), so if a bee is foraging further from the hive, this indicates that food is less available closer by. Using distance as a proxy for food availability becomes even more topical when one considers recruitment.

The evolution of the waggle dance allowed honey bees to better exploit highly profitable resources that might be located further away from the hive (18). Honey bees scout out resources in their surrounding landscape based on the needs and local cues of the colony and convey to their nestmates the location of quality resources via the waggle dance (9, 19). Only foragers working the most profitable resources, usually nectar and pollen, perform waggle dances; therefore, using waggle dances to understand foraging tells us not about all the forage that is available in the landscape, but only the better resources at any given time (12, 14, 20, 21). Foraging bees will follow the dances of successful foragers and use the information contained within the dances to find high quality resources in the landscape (19, 22).

Significance of the honey bee's pollination services and the perils they face

Over half of the crops produced for human consumption are dependent on insect pollination (23). In the United States (US), honey bees contribute more than $17 billion annually to the US economy (23). Worldwide, honey bees have been in decline over the past decade; meanwhile, the demand of crops requiring pollination is increasing (24-28). The ongoing pollinator crisis has drawn international awareness and concern for bees, causing increased demand for solutions to "save the bees" since they are important pollinators of wildflowers and crops. This has led to recommendations and policies that have great intentions, but without proper and thorough research, it is difficult to determine the most beneficial way to help our pollinators and ensure our well-intentioned efforts are having their intended effect. To address this gap, we must better understand the factors contributing to pollinator decline and develop solutions addressing the most significant stressors in both an accessible and cost-effective way.

The global decline to both native and managed pollinators population is caused by distinct and interconnected stressors attributed to increased reliance upon agrochemical inputs, the proliferation of pollinator pests and parasites, climate change, and habitat loss and fragmentation (29). One of those contributing stressors on which my research is focused is lack of available forage (30), causing both direct and indirect negative effects on honey bee health. Lack of forage directly leads to starvation and nutritional stress, which can then indirectly cause a reduced ability to cope with other external pressures such as pesticides, pests, and pathogens (31). In general, the quality of nutrition is directly linked to the pollinator's ability to overcome or survive the myriad of stressors they face (32).

Corporate efforts, like General Mills' Honey Nut Cheerios' bee-friendly wildflower campaign in 2017, brought awareness to the importance of feeding the bees; however, the movement is also a prime example of how the "one size fits all" model to help bees might do more harm than good: the seed distribution resulted in non-native wildflowers being sown in areas where they are invasive (33, 34). Overall there is a critical need for targeted, regionally specific information about how honey bees collect food in the existing landscape. We possess knowledge gaps in two crucial areas. First, we do not fully understand when (temporally/seasonally) or where (spatially) bees are lacking forage. Even though planting "pollinator friendly" flowers indiscriminately may arguably be better than nothing, these time- and money-consuming efforts could be better informed and more efficient if they directly addressed the needs on a landscape-by-landscape basis (30, 35, 36). Secondly, nutritional stress and foraging behavior has been previously studied in honey bees, but seldom over the course of an entire foraging season within the same agricultural environment. One such important environment that is currently understudied is fruit orchards, especially something like apples that are traditionally considered to benefit from honey bee pollination.

Apple Orchards and Honey Bee Pollination

Pollinators are important to the success of orchard crops. Apples (*Malus* spp.), in particular, require bees for reproduction (37-41). Apples are monoecious and have "perfect" or hermaphroditic flowers, which means that apple trees have both male (stamens) and female (carpels) parts on the same flower (42). The most common insect pollinator of orchard crops is the honey bee (42), though other species, such as other Hymenopterans, like *Osmia* spp. and *Bombus* spp., and some Dipterans and Coleopterans might be more effective at successful pollination (40, 42, 43).

Successful apple pollination is complicated and multi-faceted. Most apple varieties require cross pollination, or pollen from a different variety of apple tree, for successful reproduction. Growers typically plant a pollinizer (commonly a compatible crab apple variety) in their orchards to promote cross pollination (42, 44). Pollinizer density, bloom period and inter-planting distance are very important to successful cross pollination and ultimately to the fruit set. Selection of pollinizer is based on compatibility and overlapping bloom times. Lastly, apple pollen is tricolpate with three germinal furrows, typically 40 micrometers in length and 20 micrometers in width (42). This size makes the pollen dense and heavy and not easily carried by the wind, making wind pollination inefficient.

There are other factors that affect apples and pollination. Growers will thin their blossoms in early spring to reduce the plant's energy consumption, causing the production of fewer, but larger apples that will not weigh down the tree and lead to limb breakage. From a pollinator standpoint, this reduces the number of resources attainable in an area during that time, but allows the resource to be more reliable year to year as the apple tree regulates the flower/ fruit production, known as biennial bearing. (45, 46) Additionally, environmental conditions play a vital role: frost, precipitation and temperature all impact apple pollination. For example, if temperature is too low in the winter, this can decrease the number and viability of pollen grains produced the following spring. Low temperature (13 degrees Celsius or below) will also impact bee activity, decreasing visitation and distance travelled. Conversely, if temperature is too high, this will inhibit flower growth, and subsequently, pollen production (42).

Previous Research with Apple Orchards and Honey Bees

Previous research of pollination by honey bees within apple orchards has been mostly restricted to during apple bloom and varied, with some reports suggesting that honey bees do not maximize the pollination services in orchard crops and are likely, if not more likely, to visit other blooming flowers (47). Other reports suggest that only honey bees proved to be practical pollinators of a majority of apple varieties (39, 48). Therefore, the consensus seems to be that honey bees do visit apple blooms, but there is evidence that they might not be the most effective pollinators of apple flowers (35, 44, 49-53), although see Stern 2001 and Khan 2004, who argue that honey bees are amongst the most effective pollinators of apple orchards. (39, 48). It may be that this apparent contradiction can be explained by numbers: whereas a native bee might be better at pollinating apples in a one-to-one comparison with honey bees, the sheer volume of workers from a single hive (c. 20,000-60,000 workers, (54)) covers this deficiency. Additionally, a honey bee worker might also specialize as a nectar gatherer (53, 55), which means that even though a honey bee is foraging on apples, she might only be collecting nectar. For example, a previous study found that only three percent of honey bee workers were gathering pollen in apple orchards (56). This might be problematic to providing economically important pollination services because these bees are often side-working, collecting nectar without making total contact with the stamen of the flower (35, 44, 48, 53). One study found that honey bees were side working in apples 38% of the time (35). Honey bees also have a habit of visiting the same flowers frequently, also known as flower constancy, not moving between trees in an orchard to provide effective pollination between pollinizer tree and varietal (53). Interestingly though, some research has found in orchards with a presence of non-*Apis* bees, such as bumble bees, pollination services by *Apis* bees was improved (44, 57).

Another topic relating to honey bees and apples is the effect of semi-natural lands, marginal/understory forage, or the addition of flowering strips within and around agricultural lands. What has been found is that marginal forage and flower strips enhance pollinator species richness and diversity, but do not necessarily enhance successful pollination services in orchards (52). Lastly, Földesi and collaborators found that honey bees were more abundant in apple orchards when undergrowth bloom was also present (58).

It is clear, however, that with rapid pollinator decline, the addition to nearby semi-natural lands will not only improve nutrition of bees, but also diversity and richness of pollinator populations, allowing for potentially better pollination services in agricultural land (26, 38, 49, 52, 58, 59). Studies looking at landscape level effects surrounding orchards have found that the presence of surrounding meadow and forests areas around agricultural lands can complement seasonal provisions of foraging resources for bees (35). Also, the majority of alternative pollen sources, other than apple during early spring, are the early blooming tree species in surrounding woodlands and forests (60). Overall, while previous research has investigated many aspects of the relationship between honey bees and orchards, few, if any, have also looked at the effect of the orchard on the bees, especially across the entire foraging season. My research will seek to fill in these gaps, specifically we test the percent foraging in apple orchards during and after apple bloom in Chapter 2, and where and what (nectar or pollen) honey bees are foraging on in an orchard landscape in Chapter 3.

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CHAPTER 2: APPLE ORCHARDS FEED BEES DURING, BUT EVEN MORE SO, AFTER BLOOM

Introduction

Pollinators have coevolved with flowering plants in a relationship that is essential to the health of both our ecosystems and agriculture. Pollinators, especially insects, contribute an estimated $24 billion annually to the United States economy by boosting yields. (1, 2). Many crops like apples, peaches, and cherries that provide diversity and nutrition to our diets are wholly or partially dependent upon insect pollination services (3-7). However, flower-visiting insects are declining worldwide. Despite increasing demands for their services, over 40% of insect pollinator species are currently threatened (8-12).

These widely publicized declines have been associated with many interconnected stressors, including lack of available nectar and pollen (9, 13). This stressor directly and indirectly affects insect pollinators, causing both malnutrition and a reduced ability to cope with other stressors like diseases, pests and pesticides (13-15).

Forage availability is declining partly because of human-induced changes to the landscape. Natural or semi-natural lands, like hay meadows in parts of Europe or prairies across the United States (US), have been largely converted to monocultures in response to the food and fuel demands of a steadily increasing global population (16-19). Monocultures, necessary to our current way of life, may cause substantial, damaging effects on the environment, including reduced availability of flowers providing food for insect pollinators, which then contributes to declines in their populations (11, 13, 20-22). To complicate the issue further, temporal variation may exist within the landscape's capacity to feed pollinators, especially for insects with foraging seasons that span many months (23).

One such insect that requires nectar and pollen from early spring until autumn is the honey bee (*Apis mellifera*). As the primary source of pollination for many crops, honey bees are immensely important, contributing more than $17 billion annually to the US economy (24). The number of managed honey bee colonies has declined globally, with an approximate 40-50% decline in the US since 1940s (5, 10, 20, 21, 25). In response, with fewer hives available to provide pollination services, farmers have increased their reliance on commercial/ migratory beekeeping, which is the process of moving hives into a landscape when a crop is in bloom and then removing the hives, often to another location or crop, when the bloom time is over (26).

Migratory honey bees are frequently utilized in fruit crop systems, like apple orchards. Managed honey bees, native and wild bee's pollination efficacy of apple orchards has been the subject of extensive research finding that wild and native bees may improve fruit set due to foraging strategy (7, 27, 28). Either way, fruit crop systems rely on pollination services for successful seed set, and as a mass flowering crop, they demonstrate a fairly predictable but limited bloom time. Thus, fruit orchards may epitomize a "feast or famine" or "boom and bust" situation: forage is abundantly available for a short period of time when the crop is blooming, but after bloom, the same location could represent a food desert, contributing to poor health outcomes due to insufficient nutrition. However, because honey bee hives are often moved out after bloom, little is known about the foraging dynamics of hives located in a fruit orchard for an entire foraging season, long after the apples stop blooming.

To investigate honey bee foraging in fruit-producing orchards, we utilized a unique honey bee recruitment and communication behavior known as the waggle dance. Honey bee foragers communicate the location of quality resources like pollen or nectar with the waggle dance, which encodes a vector (distance + direction) (29, 30). A waggle dance is composed of two parts: the waggle run phase and the return phase. During the waggle run phase, the forager oscillates her abdomen at a specific angle relative to vertical and for a particular duration, indicating direction and distance, respectively, then stops, loops around to the left or the right (return phase) and repeats the waggle run + return phase combination for a variable number of times (1- >100), depending on resource quality (29-31). This behavior is visible to the eye, so researchers can decode the information and determine where in the landscape the bees are collecting food. Therefore, waggle dances provide a biologically-relevant way to explore reciprocal relationships between bees and pollination-reliant crops.

Here, for the first time, we decoded, mapped, and analyzed the waggle dances of freely flying bees in a fruit crop system for two entire foraging seasons. In particular, we (1) determined the foraging distance, as communicated by the waggle dance, which serves as a proxy for food availability. We also (2) calculated and analyzed the percent foraging, as communicated by the dance, within apple fields. Lastly, we (3) conducted ground truthing surveys to assess available forage that might contribute to the honey bee diet. Together these data will allow us to understand the dynamics of how locating a hive within the fruit crop environment might impact bee food collection, with a particular focus on the bees' use of the orchards.

Materials and Methods

Study organism and experimental set-up

We studied three queenright honey bee (*Apis mellifera ligustica*) colonies, each with brood and approximately 5,000-8,000 workers. The colonies were housed in glass-walled observation hives inside an eight by 14-foot insulated shed. We outfitted the shed with electricity to provide diffuse light and temperature control with heat and A/C. The hives were each composed of three American Standard Deep frames and were connected to the outside via an approximately three cm by 30 cm plastic tube, which allowed the bees to forage freely in the landscape. We hung plumb lines, made of fishing line with a metal washer at the end, on the wooden frame outside of the glass. The fishing line is visible in the videos (see below) and provides a vertical reference. When needed, the hives were provided with supplemental sucrose solution following data collection for the day. The hives were managed using standard beekeeping techniques to prevent swarming.

Study location

Our study site was located in rural Northern Virginia in Frederick County near the city of Winchester at the Alson H. Smith Jr. Agricultural Research and Extension Center (WAREC). This location was selected because of its connection to Virginia Tech and land-use characteristics. In particular, WAREC consists of 124 acres of commercial fruit and value-added horticultural food crops, such as apples. Fredrick County is the leading producer of apples in Virginia. The surrounding landscape within 95th percentile of median foraging range of honey bees (c. two km radius) is composed of apple orchards (c. 16%), forests (c. 25%) (deciduous, evergreen, and mixed combined), and other landcover (c. 59%) which includes types such as grass/pasture, non-alfalfa hay, corn, soybean, developed/open space, and other crops.

Data collection – waggle dance filming and data storage

We filmed the observation hives for the entire foraging season, from April to October, in both 2018 and 2019. We used three Canon Vixia HF R82 cameras with SD cards, one for each observation hive, on tripods placed one meter from the hives to decrease parallax. We pointed the video cameras at the bottom frame where dancing mostly occurred. The plumb lines, spaced five cm apart, provided a spatial reference so we could zoom the cameras to a "dance floor" of approximately 25 x 20cm, as well as providing a vertical reference for dance decoding (see below). Additionally, we set each camera to display the date and time, necessary for calculating solar azimuth, and the videos were recorded at 30 frames per second. We filmed the observation hives between 0900 and 1500 Eastern Standard Time, but usually between 1000 and 1100 on days with good foraging weather when the bees were actively flying. Filming occurred approximately three to four times per week, and we did not allow more than three to four consecutive days to pass between filming. By the end of the study, we had generated one-hour video per day * three WAREC observation hives * four days per week * four weeks per month * six and a half months per year of foraging * two years = approximately 625 hours of video data.

We recorded to SanDisk Extreme SD cards (64-256 GB), which were labelled, locked, and transported back to the main campus. We then backed up the SD card to a Google Team Drive as a permanent, cloud-based data repository. The SD cards are stored permanently in the campus lab.

Data collection – waggle dance decoding

We decoded dances via frame by frame playback using a modified protocol of Couvillon (2012) (32). We used the Image J 1.52i application to decode four, consecutive, non-first, non-last waggle runs per dance. We measured dance duration, which communicates distance, by determining the start and stop frame of each of the four waggle runs within the dance. We measured dance angle, which communicates direction, by drawing a line using the ImageJ tools from the central portion of the thorax during the waggle run and measuring the angle relative to the vertical plumb line angle. These measurements were entered immediately into Excel. At the end of each day each dance decoder uploaded their file to Google Team Drive for archiving and version control.

Since the dance followers average multiple waggle runs for both components of the dance, duration and direction, (33, 34), we calculated the average vector (mean duration and mean angle) to give aggregated dance outcomes. Durations were converted to distance using the Virginian calibration reported by Schürch, Zwirner (35).

Additionally, since multiple bees usually were dancing simultaneously, we decoded all the bees that we saw dancing at time = 0. We then advanced the video six minutes (approx.10,800 frames) allowing the dancer(s) to finish their dance, providing time for a new cohort of dancers to enter the frame (32), which minimizes the opportunities of pseudo-replication. Lastly, we noted whether a forager was recruiting for nectar or pollen, determined by the presence (or absence) of pollen in the corbiculae of the dancer.

Data collection – digital and observational ground truthing

Ground truthing was necessary to accurately define and to understand the landscape of our research. This process allowed us to close information gaps relating to blooms and seasonal changes in the landscape. There were two types of ground truthing we used to understand our environment. Firstly, we conducted digital ground truthing to validate our landscape analysis. Digital ground truthing was based on observational ground truthing, aerial imagery, existing maps provided by WAREC, and the United States Department of Agriculture (USDA) National

Agricultural Statistics Service (NASS) Cropscape and Crop Data Layer for Frederick County Virginia. Initially, we downloaded the Cropscape raster data and added it as a layer into ArcGIS Pro 2.4.1. Next, we converted the Cropscape layer to polygons. At this stage, we discovered the downloaded Cropscape layer did not accurately capture the correct extent of the apple orchards and forest. Therefore, we corrected the polygons using the edit vertices tool in ArcGIS by comparing the layer with the maps provided by the WAREC staff, who possess first-hand, intimate knowledge of the surrounding landscape, including the orchard locations. Once all polygons accurately reflected the composition of the landscape, the shapefile was exported as a keyhole markup language (kml) file for use in our R project allowing us to select the fields of interest (apple orchards and forest) using the crop data layer code (i.e., 68 = Apple).

Additionally, we conducted some observational ground truthing in 2020. For this process, we used the same resources as digital ground truthing and an early, preliminary waggle dance data set to identify "hotspot" areas within periods of interest: we roughly determined the foraging areas the bees were utilizing and defined post bloom, late summer, and fall periods of time based on flowering phenology, including apple bloom (see below). Then we visited the areas and surveyed and recorded the flowering plants, including their abundance and location by date. Relative abundance was assigned on a scale of one to three based on observation, with three representing at least 50% of the flowering land cover or higher, two representing between 25-50% and one representing below 25%. Most of our observational ground truthing took place within apple orchards and forests, although there are developed mixed use areas that the bees foraged within. Unfortunately, we were prevented from conducting observational ground truthing during bloom time because of COVID-19 travel restrictions. Lastly, our observational ground truthing included only flowering plants and trees, all vegetation within the landscape.

Data validation

Prior to analysis and after dances were decoded, we examined our data for any missing values and errors. Then we performed a data validation by calculating for both the duration and direction component the mean for each waggle dance, which is made up of the four decoded waggle runs, and the standard deviation. We then determined the 10 dances possessing the greatest outlying values for both the waggle run duration and waggle run angular (directional) intra-dance standard deviation. We located those 20 dances on the original videos. If the high standard deviation was because of a human error (typo or dance decoding error), we re-decoded the dances and re-entered them into Excel and re-uploaded to Google Drive. We repeated the process until all 10 of the dances were found to possess no human error and, instead, were merely "noisy" dances (i.e., the dancer was imprecise between successive waggle runs within the dance). This involved going through the process once for the duration component (10 dances checked) and six times (60 dances checked) for the direction component until no more errors were found. All in all, our dance data set was comprised of 3,710 individual waggle dances, with each dance being made of four waggle runs, for a total of 14,840 decoded waggle runs from honey bees foraging around WAREC across the two years.

Data analysis – distance and apple bloom time

Because bees are economic foragers (23, 30, 36, 37), communicated foraging distance is a proxy for forage availability because honey bees are not expected to forage and to recruit further away if the food is available closer to the hive (23). For this reason and to determine seasonal fluctuations in forage availability, we analyzed the communicated foraging distance as a function of time. We calculated foraging distance as the median predicted distance for each observed (decoded) dance, composed of four waggle runs, from dances simulated 1,000 times. We then analyzed distance across different timescales: first we analyzed the raw data by plotting distance over time with a LOESS regression to visualize the distance foragers were travelling from the hive.

Next, we analyzed time for each month to conceptualize and help understanding broad changes in distance and also allows us to compare median foraging distance by month to previous studies (23). Lastly, because our study was in an agricultural setting with predictable and short bloom times, we also wanted to examine foraging distance as a function of bloom, with bloom, post bloom, late summer, and fall (note: our study did not begin early enough to include pre-bloom). These categories were selected because flower bloom can overlap through months and vary by year. Therefore, we determined apple bloom season (April 13- May 24, 2018, and April 18- May 29, 2019, or 41 days) from pollen samples and observational information provided by

AREC staff. We defined post apple bloom (May 25- July 5, 2018, and May 30 – July 10, 2019) by applying a similar time period (41 days) as had been used for bloom. Next, from our personal observations and from previous work (23, 38, 39), we knew that in temperate areas, there is commonly an autumnal forage source that provides an abundant, ubiquitous, late season food (e.g., ivy in England, goldenrod in the Eastern and Midwestern US). In our study area, we defined autumn as the start of the goldenrod bloom until the end of the foraging season (August 23 – October 3, 2018, and August 29 – October 15, 2019, 41 and 47 days respectively) and then, for comparison, applied the same time period in days as the autumn to ahead of autumn bloom, which we called late-summer (July 6 – August 22, 2018 and July 11 – August 28, 2019, with 47 and 48 days respectively). Lastly, statistical analyses were performed using R (version 3.5.1). To determine statistical differences between foraging distances by month and season, the Kruskal-Wallis rank sum test was used. We then performed a Dunn test (from the FSA package version 0.8.30) to perform pairwise comparison. P-values for the pairwise comparisons were adjusted using the Bonferroni method.

Data analysis – percent foraging in apple orchards and forests and relative attractiveness of orchards

Since our study location is defined by apple orchards, we were primarily interested in the reciprocal relationship between bee hives and apple fruit orchards over two foraging years. We not only wanted to know how honey bees might be visiting and potentially pollinating apples during bloom (April – May), but we additionally were interested in how orchards might play host to honey bee hives and provide (or not provide) forage for the rest of the time (June – October). Therefore, we calculated the percent foraging that occurs within the apple orchards as a function of time. Additionally, percent foraging was calculated within forests as a contrast to apples because we would predict honey bee use of forests to be less affected by the transition from (apple) bloom to post (apple) bloom.

Due to the inherent error within both components of the honey bee’s waggle dance communication (40-42), it is not possible to precisely map exact foraging locations from decoded waggle dances. Therefore, we utilized a developed analysis method that takes this error into account to simulate individual dances and then map them as a probability cloud (23). We based our percent foraging calculations on this methodology (41). Briefly, we downloaded the Cropscape raster data for Frederick County, Virginia and added it as a layer in to ArcGIS Pro 2.4.1. Next, we converted the Cropscape layer to polygons, digitally corrected polygons based on our knowledge of the area and other digital tools (see above). The polygon layers allowed us to select the fields of interest (apple orchards) using the crop data layer code (i.e. 68 = Apple) when the layer was exported into our R project.

To calculate the percent foraging in fields of interest, we predicted each observed dance's advertised location 1000 times, factoring in uncertainty (35). Then, for each dance, we picked one prediction and calculated the percentage of dances in fields of interest. This process was repeated 10,000 times, each time with different combinations of predictions from each dance, resulting in a distribution of 10,000 percentages. From the distribution of percentages, we took the median percentage as the point estimate and the 5th and 95th percentile for our confidence interval to calculate the percent foraging during and post bloom for both apple and forests to understand how foraging in our fields of interest fluctuates as a function of bloom and throughout the rest of the foraging season.

Lastly, we wanted to determine the attractiveness of apple orchards and forests relative to other land use types more formally by performing a multinomial logistic regression. For this analysis, each (simulated) dance was either pointing to apples, forests, or other (dependent variable, with reference category "other"). We wanted to see if the relative odds of visiting apple or forests (relative to other) would change over the months (predictor variable, reference month April). Because distance will affect if a certain spot will be visited and advertised, we adjusted for distance in our model. We used the R package mclogit version 0.8.7.3 (43) to run the model 1000 times, each time randomly picking only one simulated foraging location for each of the observed dances. Point estimates and confidence intervals were calculated from estimated log-odds from the 1000 model results and then inverse-logit transformed to arrive at the odds ratio (+/- 95 % confidence intervals). All data and code will be made available through Virginia Tech's data repository after the conclusion of the larger, on-going project in the lab.

Results

Honey bees in orchards forage nearby with some extremely long-range foraging events

While honey bees in our study foraged up to 11.2 km, the majority of foraging occurred within short distances from the hive: 95% of the mean predicted distances were within 2.2 km. As shown in Figure 2.1, the median distance travelled for food in an orchard crop landscape during the combined 2018 and 2019 foraging seasons (April through October) was 0.76 km (0.05 - 11.18km, n = 3710). In 2018, the median distance was 0.78 km (0.053 km - 7.42 km, n = 2078). In 2019, the median distance was 0.74 km (0.050 - 11.18 km, n = 1632). In general, 2019 was marked by more longer-range foraging events: in 2018, there were seven decoded dances > 5 km, and in 2019, there were 21. Additionally, the maximum recruited distance in 2019 was nearly four km greater, making the entire foraging range much larger in the second year. However, despite these details, the upper quartile for both 2018 and 2019 remained remarkably similar (1.112 km and 1.109km, respectively), suggesting that overall food availability was comparable across the two years.

Communicated foraging distance varies significantly with month and year

As shown in Figure 2.2, foraging distance varied significantly by month in both 2018 and 2019 (Kruskal-Wallis tests 2018: $\chi 2 = 69.61$, df = 6, p = 4.916e-13; 2019: $\chi 2 = 135.96$, df = 6, p < 2.2e-16). In 2018, the months with the highest median foraging distance were in October (0.855 km) and July (0.838 km, Figure 2.2), although the October sample size (n= 36) was too small to make strong inferences (see discussion). In 2019, the months with the highest median foraging distances were in September (1.003 km) and August (0.966 km, Figure 2.2). September 2019 had the highest median foraging distance over the entire two foraging seasons, likely due to drought (see discussion). In general, communicated foraging distance increased from early to late summer each year, suggesting food becomes less available across that time. Foraging distance also varied significantly by year between 2018 and 2019 ($\chi 2 = 12.666$, df = 1, p = 0.0003724).

Communicated foraging distance was not driven by apple bloom

Before conducting our study, we expected communicated foraging distances to vary significantly relative to apple bloom. In particular, we predicted foraging distances would be lowest during bloom and then increase in post bloom because the foragers would suddenly be without a nearby, abundant food source (apple tree blooms). In contrast, as shown in Figure 2.3, the median foraging distance did not vary with bloom time. In fact, in 2019, the post bloom distance decreased significantly compared to bloom, suggesting something other than apple blooms is driving foraging distances because the bees are actually able to forage and recruit closer by after the apples ceased blooming. Late summer indicates relatively higher foraging distances in both years, which matches our monthly analysis above and could be indicative of a late summer foraging gap, with less food available locally. The presence of such a gap would agree with previous studies (23, 39, 44, 45).

Honey bees forage in apple orchards during and more so after bloom but consistently and at a higher level in forests

Since apple orchards are 16.1% of the landscape within the two km foraging area around WAREC, we would predict, if all land types are equally attractive, honey bees to forage in orchards c. 16% of the time. We found, on average, that 20% of honey bee waggle dances indicated apple orchards (Table 2.1, Figure 2.4) during the season when the orchards are blooming (18.6% in 2018, 21.4% in 2019). Even more surprisingly, honey bees increased their recruitment to an average of 28.9% (29.4% in 2018, 28.5% in 2019) in the same orchards in the period immediately after bloom, indicating that attractive forage became more available in the same fields after apple bloom and/or previously attractive forage elsewhere became less available.

To contrast, we also examined honey bee foraging in forests. Forests comprise c. 25.3% of the landscape within the two km foraging area around WAREC. Therefore, we predicted honey bees would indicate forests with their waggle dances c. 25% both during and after the apple bloom because apple bloom should not affect forest foraging, even though honey bees are generally flower constant. In contrast, the data show foraging in forests was generally higher than expected and did not follow a consistent pattern: in 2018, foraging within forests during bloom was significantly higher than expected (36.9%) but decreased during post bloom (25.7%, Table 2.1, Figure 2.4), concurrent with the increased recruitment to apple orchards. In other words, the honey bees were using the forests more than expected during the time when the orchards were blooming, but this use decreased to a percentage that more closely matched the forest's representation in the landscape while the bees turned their attention to the post bloom orchards. In 2019, foraging within forests during bloom was also significantly higher than expected (32.6%) and then remained similar during post bloom (32.9%, Table 2.1 Figure 2.4), indicating that the forests were and remained attractive to foragers across that time period.

Post bloom, not bloom, orchards are extremely attractive to foraging honey bees

We found the highest odds ratios for apple orchards in the post bloom month of June (relative to the preference of these fields in April; OR (95% CI): 1.9 (1.3 to 2.3)). In other words, bees are approximately twice as likely to forage in apple orchards in post bloom June compared to other fields and compared to April when apples were in bloom. In contrast, the odds ratio for May, which is during bloom, was no different from April (also during bloom) in honey bee preferences for apple (OR (95% CI): 1.2 (0.8 to 1.4)). Overall, these data indicate that the apple orchards were advertised by dancing honey bees in the post bloom period of June more than at any other time of year, including during the apple blooms in April and May.

Orchards provide post bloom forage with flowering understory plants

We catalogued 40 species of plants in apple orchards during our observational ground truthing, with 23 during post bloom, 12 during late summer and five during fall (Table 2.2). Therefore, the most numerous records of species occurred during post bloom, both in terms of ground cover and number of unique species. In particular, our observations in apple orchards during the post bloom period determined the abundant presence of red and white clover (*Trifolium*) and plantain (*Plantago*, Table 2.2). The presence of these attractive foraging opportunities coincides with our proportion foraging results (i.e., the increased use of orchards after the apple bloom) and what we know about the prolific nature of spring and late spring, where many abundant blooms are available, including in trees in forests, which simultaneously provides abundant and overlapping foraging opportunities (see discussion).

Discussion

Here we decoded, mapped, and analyzed the waggle dances of freely flying bees in a fruit crop system across two entire foraging seasons. Our main outcomes from this study not only reflected but also built, in unexpected ways, upon previous research. Contrary to our expectations, apple blooms did not drive foraging behaviors. In fact, although we found honey bees forage in apple orchards during bloom, they foraged more so in the same orchards after the apple bloom. Concurrent with this increased use of the orchards, the honey bees decreased their recruitment to other, previously attractive areas, like forests, especially in 2018. Our on-the-ground observations suggest that the abundant presence of wildflowers might be the attractant force to orchards during the post bloom period. Indeed, our analysis also indicates that the post bloom orchards are twice as likely to be indicated in post bloom June than in any other time of year, including bloom time. Apple orchards therefore provide forage to bees, but in a surprising and alternative manner, with the bees utilizing wildflowers, understory and edge plants.

Additionally, we found honey bees in orchards mostly forage nearby, with some extremely long-range foraging events. Communicated foraging distance varied significantly with season and year. Lastly, we found in our study that honey bees foraged further afield in late summer, suggesting a decrease in available forage, especially compared to spring and early summer.

In general, honey bees will opt to forage and recruit for resources maximizing the benefit to the cost of that foraging decision (37, 46). Some benefits might include the sweetness (sugar content, or Brix) of the nectar, its quantity, and its availability, whereas the costs might include distance to the resource, if there are any dangers (predators) nearby, and efficiency of flower handling. That honey bees indicate apple orchards more than we would expect (18 and 21% for 2018 and 2019, respectively), given their representation in the landscape (c. 16%), suggests that apple blooms present a beneficial foraging opportunity (Table 2.1, Figure 2.4). However, the increase in use of the orchards after the bloom further suggests that either another, alternative forage within the orchards themselves is more attractive and becomes available or other, concurrent options in other locations have become less available. Although we did not specifically test between these two hypotheses, we do have some evidence supporting that additional forage became available within the orchard. As we saw in Table 2.2, we identified 29 species of flowering plants, of which 23 were during the post bloom period. In particular, abundant red and white clover (*Trifolium*) and plantain (*Plantago*) were observed in between the rows of apple trees and field edges. These plants are known forage for honey bees (47-51) and are likely very attractive in the period after apple bloom, attracting recruiting foragers in even greater numbers than were visiting the same orchards during apple bloom. Additionally, we observed persimmons (*Diospyros*) and chestnut (*Castanea*) in adjacent forests. However, unlike apple orchards, the visitation to the forests decreased from bloom to post bloom in 2018, and remained consistent between bloom and post bloom in 2019. One scenario that explains this occurrence is the bees are using orchards and forests with abundant spring tree blooms, but then, in late May, there are fewer tree blooms. With orchards and trees no longer in bloom, wildflowers like *Trifolium* and *Plantago*, which are available, become relatively more attractive and potentially fill in the gap, pulling foragers away from forests.

These data are also supported by a previous study conducted in the United Kingdom that showed that another mass flowering crop, oilseed rape, which is concurrently blooming, competes with apple blossoms (52). In that instance, foraging honey bees waggle dances indicated foraging in orchards 23.5%, compared to 12.7% with oilseed rape. However, when distance is factored in (i.e., corrected for statistically), the story reversed: oilseed rape fields are much more attractive than apple orchards (52). Therefore, it might be that apple blossoms, although attractive, are less attractive than other springtime resources, including other crops like oilseed rape, available at the same time (53). Overall, these data demonstrate the increasing importance for landscape management and agroecology decisions to be made with the appropriate foraging scale in mind, which in this case might include landscapes further afield than just the orchards.

In contrast to the use of the apple orchards, honey bee foraging within forests is more consistent, with a decreasing (36% to 25%) or consistent (32.6% to 32.9%) use of forests as we moved from bloom to post bloom in 2018 and 2019 respectively. Although both apple orchards and forests presented foraging opportunities in the post bloom period, it appears orchards were more attractive, perhaps because of the higher number of flowering plant species observed (Table 2.2).

Our honey bees located within fruit orchards foraged mostly locally, with a median foraging distance of 0.76 km from the two years (Figure 2.1). We did see some extremely long range (>10km) recruitment events in 2019. Although honey bees can and will fly several kilometers or more, these events typically are rare or represent an unusual situation of a very valuable resource coming into bloom while other resources concurrently waned (54). Our usual median foraging of under one kilometer agrees with previous work's finding that most recruitment occurs within two kilometers (23, 55, 56).

Foraging distance remained low in the spring time, a season where flowers are generally available, which was also found in previous studies (23, 39, 44). Foraging distances then increased through summer, suggesting a decrease in forage availability. The presence of a late summer forage gap has also been previously described (23, 39, 44, 45) and might represent a general finding for temperate regions, where there is a lack of available forage after spring and summer flowers and trees bloom but before the autumnal resources become available. With this in mind, we were not surprised to see a decrease in foraging distance in the autumn, as the landscape at that time was characterized by the presence of goldenrod (*Solidago*, Table 2.2), an important autumnal bloom for pollinators in the eastern United States. The late seasonal importance of a bloom like goldenrod is also consistent with previous studies in the United Kingdom that found ivy, a ubiquitous autumnal bloom with abundant nectar and pollen, likewise allowed honey bees to forage more locally (23, 38) in the last months of the foraging season.

The extremely high median foraging distance in September 2019 might have been driven by drought: that location in September typically sees close to four inches of rainfall; however, for that year, only 0.63 inches fell within September. Dry conditions will affect some plant's nectar production, making it necessary for flower visiting insects to seek out more drought tolerant plants (57). Here we see that such a circumstance might have a big impact on honey bee forage availability, indicating the potential impacts of a warming, drying climate on food availability for bees. Lastly, the unexpected increase in foraging distance in October 2018 is likely due to our small sample size: we were only able to decode dances from one day for that month, making the distance a non-representative sample of a larger month. Since goldenrod begins to bloom in late August in our study site, the decrease in communicated foraging distance in the previous month (September) is likely more reflective of the actual forage availability.

Here we have shown apple orchards do indeed provide forage for honey bees, but apple bloom is simultaneously occurring during the time of year when many other attractive blooms in other areas are competing for pollinators' services. Although orchards may be a contributor to the honey bee's diet, and honey bees may be important to the success of orchard crop, they do not appear to be highly representative to each other because of the interaction of foraging dynamics seasonality. Although my research did not specifically look at non-*Apis* and native bees, my results could have implications towards all pollinators. Honey bees foraging in apple orchards more after apple bloom means there could be an important foraging resource(s) available after the mass bloom of apples. This could represent a nutritionally important resource for diverse species of pollinators present in this landscape. Future studies should further investigate the comparative pollinating efficiency of other bees versus honey bees to fruit set as Brittain, Williams (28), Mallinger and Gratton (7) and Russo, Park (27) have previously explored in California almonds, and Wisconsin and New York apples. It might be possible for apple production to be maximized by a judicious timing of honey bee exposure versus other bees, a timing that also should take into account the flowering phenology of alternative forage sources for honey bees in the surrounding landscape.

Tables and Figures

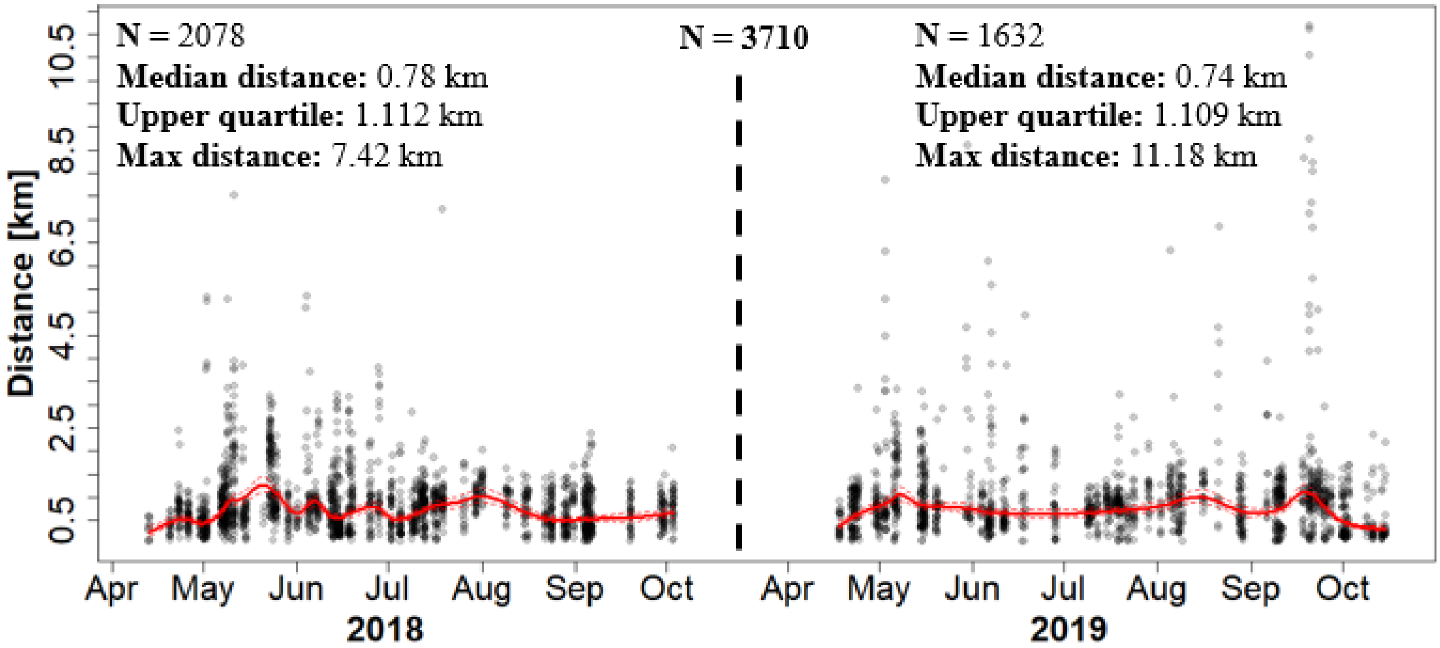

Figure 2.1. Distance as communicated by dancing honey bees across the foraging seasons in both 2018 and 2019. We fitted the raw data with a LOESS regression (span = 0.1). Each point represents a decoded dance, composed of the average duration (encoding distance) of four, mid-dance waggle runs. Duration was then converted to distance (kilometers). Both 2018 and 2019 show an increase in communicated foraging distance in between late spring and early summer and another slight spike in foraging distance in high summer before distances decrease in autumn.

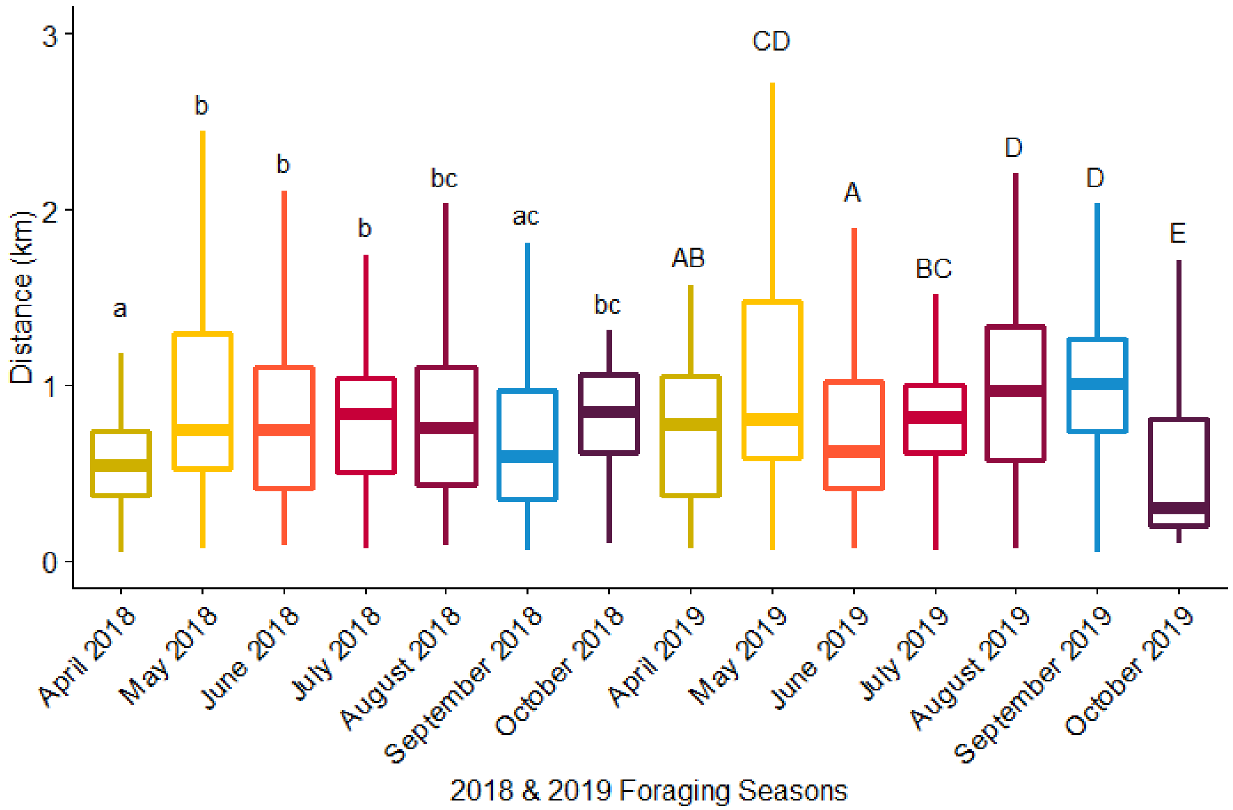

Figure 2.2. Communicated median foraging distance (kilometers) by month in 2018 and 2019 (n = 3710). Letter assignments indicate significant differences between months ($p < 0.05$) for post hoc comparison (Dunn test after Bonferroni correction), while months with the same letters are not statistically different from each other. Post hoc results are lower case for 2018 data and capitalized for 2019 data. Rainbow colors correspond by month (April-October) per year. The lower-case letters indicate significance for 2018 data, while the upper-case letters indicate significance in 2019 data. Overall foraging distance varied by month and increased from early to late summer.

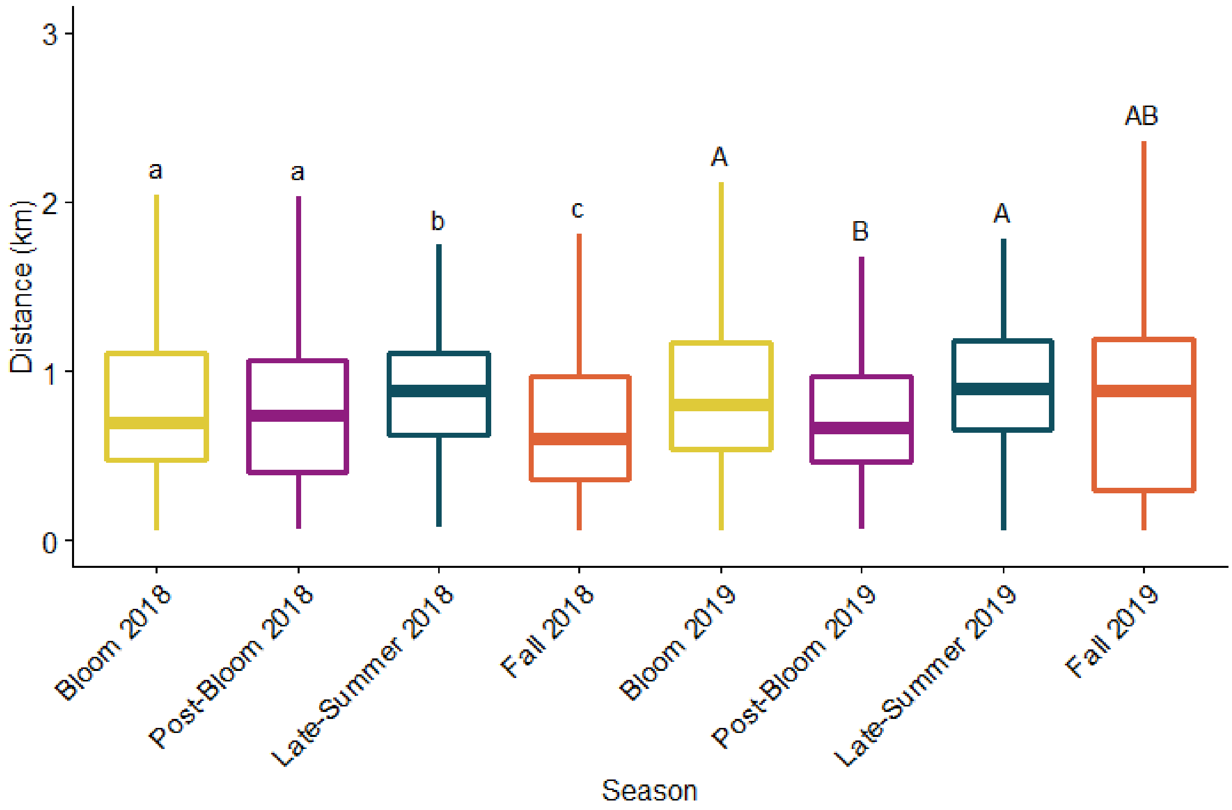

Figure 2.3. Median foraging distance travelled (kilometers) across seasons in 2018 and 2019 (n = 3710). Dates for calculating bloom, post bloom, late summer and fall are based on apple and golden rod bloom period in Northern Virginia. Post hoc results (Kruskal-Wallis with Dunn test and p-values corrected with the Bonferroni method) are lower case for 2018 data and capitalized for 2019 data. Colors correspond with season. Both years show that late summer might represent a foraging gap, where honey bees must travel further for food.

Field of Interest	Year	During Apple Bloom (95% CI)	Post- Apple Bloom (95% CI)
Apple (16.1%)	2018	18.6% (16.3% to 21.2%)	29.4% (25.4% to 32.9%)
	2019	21.4% (18.1% to 24.6%)	28.5% (23.4% to 33.5%)
Forest (25.3%)	2018	36.9% (33.4% to 40.1%)	25.7% (22.3% to 29.3%)
	2019	32.6% (28.6% to 36.1%)	32.9% (27.6% to 38.0%)

Table 2.1. Percent foraging in apple orchards, which comprise 16.1% of the landscape, and forests, which comprise 25.3% of the landscape, during and post apple orchard bloom period.

Season	**Common Name**	**Genus**	**Location**	**Abundance**
Post Bloom	Plantain	*Plantago*	Orchard	3
	Red Clover	*Trifolium*	Orchard	3
	White Clover	*Trifolium*	Orchard	3
	Chestnut	*Castanea*	Forest	2
	Annual fleabane	*Erigeron*	Orchard	1
	Carolina horsenettle	*Solanum*	Orchard	1
	Chicory	*Cichorium*	Orchard	1
	Cinquefoil	*Potentilla*	Orchard	1
	Common hedge parsley	*Torilis*	Orchard	1
	Dotty Peas	*Otholobium*	Orchard	1
	European field pansy	*Viola*	Orchard	1
	Field bindweed	*Convolvulus*	Orchard	1
	Hop Trefoil	*Trifolium*	Orchard	1
	Japanese honeysuckle	*Lonicera*	Orchard	1
	Lady's Bedstraw	*Galium*	Orchard	1
	Meadow salsify	*Tragopogon*	Orchard	1
	Mullein	*Verbascum*	Orchard	1
	Purple crownvetch	*Securigera*	Orchard	1
	Sweet peas	*Lathyrus*	Orchard	1

	Vipers-bugloss	*Echium*	Orchard	1
	Yellow sweetclover	*Melilotus*	Orchard	1
	Daylilies	*Hemerocallis*	Development	1
	Persimmon	*Diospyros*	Forest	1
Late Summer	Red Clover	*Trifolium*	Orchard	3
	White Clover	*Trifolium*	Orchard	3
	Wild Carrot	*Daucus*	Orchard	3
	Bladder Campion	*Silene*	Orchard	1
	Bull Thistle	*Cirsium*	Orchard	1
	Carolina horsenettle	*Solanum*	Orchard	1
	Common Selfheal	*Prunella*	Orchard	1
	Daisy Fleabane	*Erigeron*	Orchard	1
	Lady Bedstraw	*Galium*	Orchard	1
	Mullein	*Verbascum*	Orchard	1
	Alfalfa	*Medicago*	Field	1
	Surprise Lily	*Lycoris*	Development	1
Fall	Goldenrod	*Solidago*	Orchard	3
	White Clover	*Trifolium*	Orchard	3
	Wild Carrot	*Daucus*	Orchard	3
	Chicory	*Cichorium*	Orchard	2
	Plumeless Thistle	*Carduus*	Orchard	2
	American Aster	*Symphyotrichum*	Orchard	1
	Common Toadflax	*Linaria*	Orchard	1
	Foxtail/Bristlegrasses	*Setaria*	Orchard	1
	Woodsorrel	*Oxalis*	Orchard	1

Table 2.2. Plant species identified during observational ground truthing events in the post apple bloom, late summer, and fall seasons. We included their locations and relative abundance on a scale of one to three, with three representing 50% or higher of the bloom during that season, two between 25-50% and one below 25%.

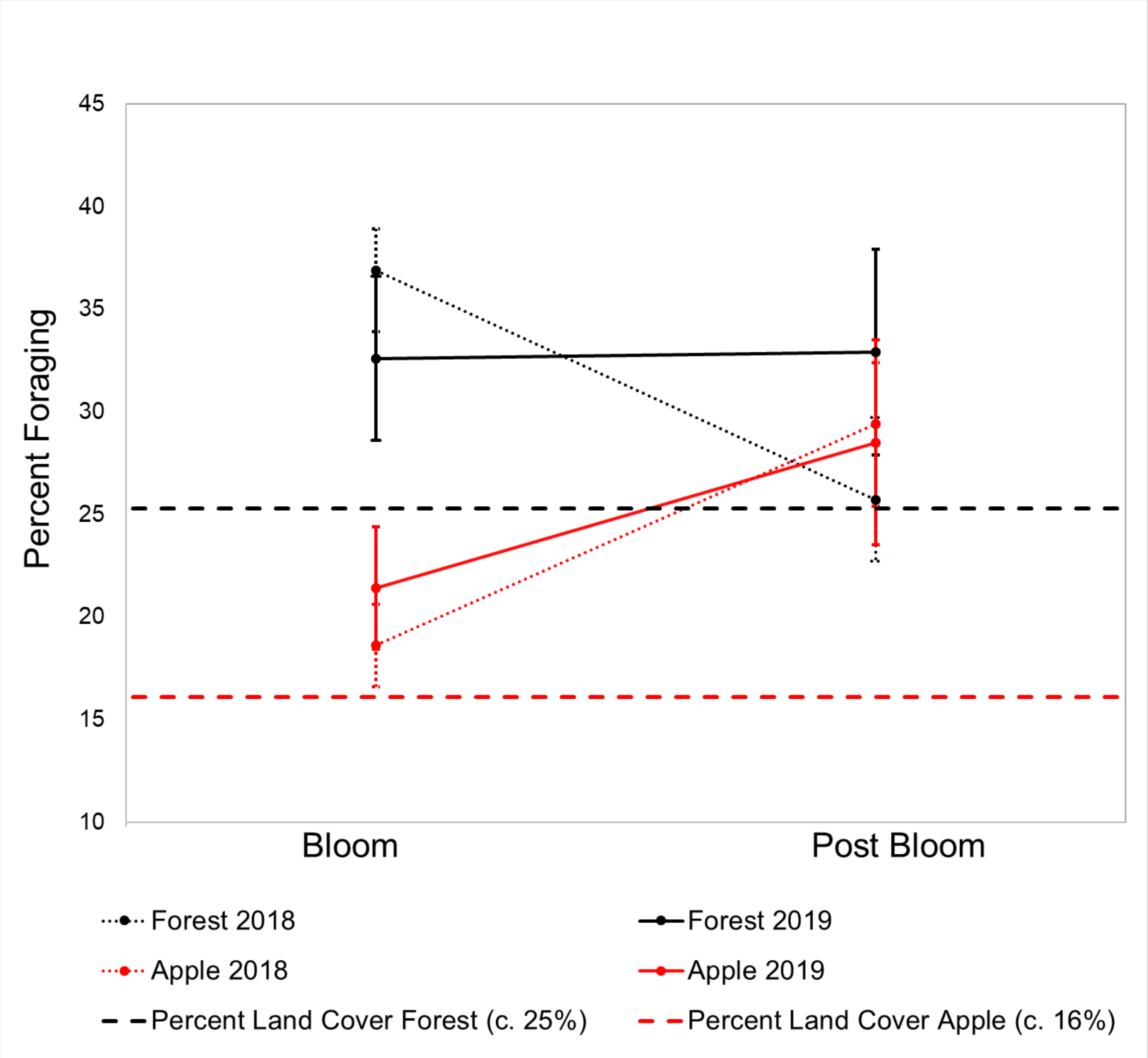

Figure 2.4. Percent foraging in apple orchards and forests as a function of bloom, calculated during and after apple bloom. Foraging to apple orchards (red) increased from bloom to post bloom for both 2018 (18% to 21%, small red dash) and 2019 (21% to 28%, solid red line), with the apple orchards representing 16% of the landscape (larger dashed horizontal red line). In contrast, percent foraging to forests (black) decreased from bloom to post bloom for 2018 (36.9 to 25.7%, small dash) but remained consistent for 2019 (32.6% to 32.9%, solid black line), with forests representing 25% of the landscape (larger dashed horizontal black line).

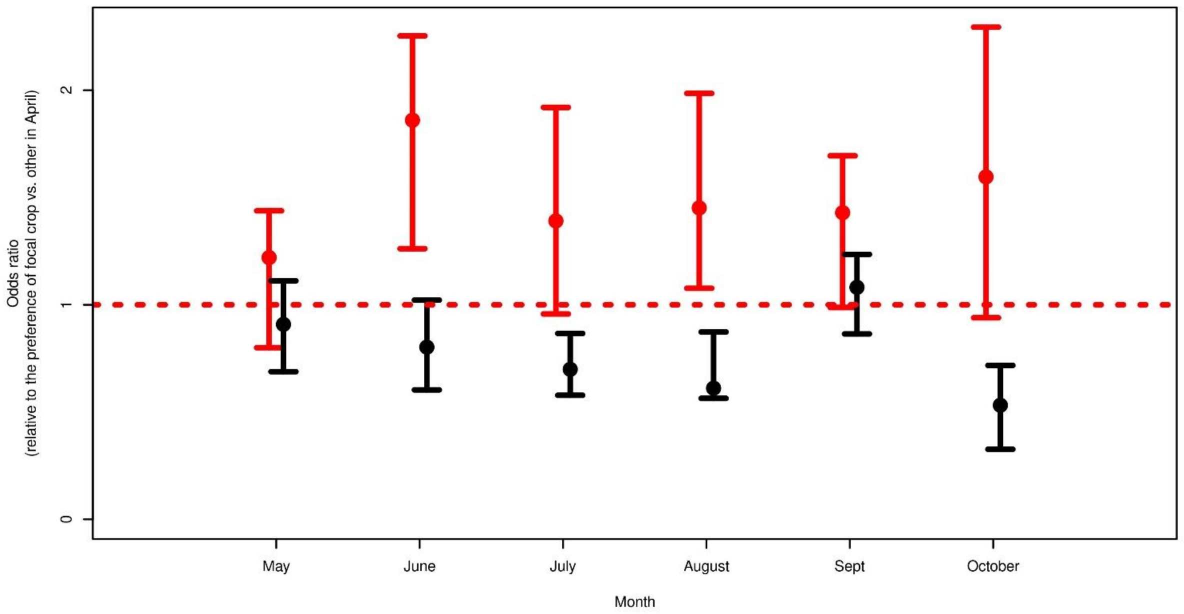

Figure 2.5. Honey bee foraging in apple orchards (red) was nearly twice as likely post apple bloom (June) compared to apple bloom (April). Forest (black), in comparison, has lower odds ratio when compared to apple orchards regardless of time of year. Therefore, apple orchards are most attractive in to foraging and recruiting honey bees in the post bloom June, not the bloom May.

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CHAPTER 3: POST BLOOM APPLE ORCHARDS PROVIDE SUPRISINGLY ABUNDANT FORAGING AND CONTAMINATION OPPORTUNITIES FOR HONEY BEES

Introduction

The production of many fruits, nuts, and vegetables is dependent on pollination by honey bees (*Apis mellifera*) (1). These insects yield an immense economic impact: in 2019-2020 in the United States (US), farmers paid an estimated $563 million for crop pollination services (2). One of the crops requiring pollination by bees are apples, one of the most valuable fruit commodities in the US (3). Apples are produced across the country, but the most productive states are Washington, New York, Michigan, Pennsylvania, California, Virginia, North Carolina, Oregon, Ohio and Idaho (4). Apple production in the US comprises approximately 322,000 acres of land (4).

Tree fruit and nut orchards are part of a larger agricultural network, which comprises 2% of the US rural landscape (5). In 2018, the total US acreage of major non-citrus fruits, including apple, was an estimated 1.5 million acres (6). For this reason and also because these lands are often where flower-visiting insects are foraging and pollinating, it is important to explore the interaction between these modified ecosystems and pollinators. In particular, we still do not fully understand either the benefits or the risks associated with bees foraging in these systems. For example, when honey bees are located within apple orchards for a foraging season, where and upon what do the bees forage, and in doing so, what pesticide exposure will they experience?

To fill these information gaps, we monitored honey bee pollen collection in a northeastern Virginian apple orchard landscape over the course of two foraging seasons (April-October, 2018-2019). Additionally, we utilized the honey bee waggle dances, whereby returning foragers communicate the distance and direction from the hive to the forage, usually nectar or pollen (7, 8). These visible behaviors allowed us to analyze not just where the forager collected pollen, but, since the pollen itself can be sampled, we are also able to determine colony-level plant identification and potential pesticide exposure. Our research objectives were (1) to determine where honey bees are foraging for pollen within an orchard crop landscape using waggle dance decoding with a particular focus during and immediately after apple bloom and, within an orchard environment based on forager collected pollen, (2) to identify what flora honey bees forage upon and (3) to determine to what pesticide residues honey bees are exposed during and after apple bloom.

Materials and Methods

Study organism and experimental set-up

We studied three queenright honey bee (*Apis mellifera ligustica*) colonies, each with brood and approximately 5,000-8,000 workers. The colonies were housed in glass-walled observation hives inside an eight by 14-foot insulated shed. We outfitted the shed with electricity to provide diffuse light and temperature control with heat and A/C. The hives were each composed of three American Standard Deep frames and were connected to the outside via an approximately three cm by 30 cm plastic tube, which allowed the bees to forage freely in the landscape. We hung plumb lines, made of fishing line with a metal washer at the end, on the wooden frame outside of the glass. The fishing line is visible in the videos and provides a vertical reference. When needed, the hives were provided with supplemental sucrose solution following data collection for the day. The hives were managed using standard beekeeping techniques to prevent swarming.

Study location

Our study site was located in rural Northern Virginia in Frederick County near the city of Winchester at the Alson H. Smith Jr. Agricultural Research and Extension Center (WAREC). This location was selected because of its connection to Virginia Tech and land-use characteristics. In particular, WAREC consists of 50.2 hectares of commercial fruit and value-added horticultural food crops, such as apples. Fredrick County is the highest producer of apples in the state. The surrounding landscape within 95th percentile of median foraging range of honey bees (c. two km radius) is composed of apple orchards (c. 16%), forests (c. 25%) (deciduous, evergreen, and mixed combined), and other landcover (c. 59%) which includes types such as grass/pasture, non-alfalfa hay, corn, soybean, developed/open space, and other crops such as peaches, cherries, tomatoes, melons, beans, cucumbers, etc.

Data collection – waggle dance filming

As part of a larger project, we filmed the observation hives for the entire foraging season, from April to October, in both 2018 and 2019. We used three Canon Vixia HF R82 cameras with SD cards, one for each observation hive, on tripods placed one meter from the hives to decrease parallax. We pointed the video cameras at the bottom frame where most dancing occurred. The plumb lines, spaced five cm apart, provided a spatial reference allowing us to zoom the cameras to a "dance floor" of approximately 25 x 20cm, as well as providing a vertical reference for dance decoding. Additionally, we set each camera to display the date and time, necessary for calculating solar azimuth, and the videos were recorded at 30 frames per second. We filmed the observation hives between 0900 and 1500 Eastern Standard Time, but usually between 1000 and 1100 on days with good foraging weather when the bees were actively flying. Filming occurred approximately three to four times per week, and we did not allow more than three to four consecutive days to pass between filming. By the end of the study, we had generated one-hour video per day * three WAREC observation hives * four days per week * four weeks per month * six and a half months per year of foraging * two years = approximately 625 hours of video data. For the purposes of this study, we focused our waggle dance analysis on the time periods of bloom and post bloom, specifically the subset of pollen dances.

We recorded to SanDisk Extreme SD cards (64-256 GB), which were labelled, locked, and transported back to the main campus. We then backed up the SD card to a Google Team Drive as a permanent, cloud-based data repository. The SD cards are stored permanently in the campus lab.

Data collection – waggle dance decoding

Briefly, we decoded dances via frame-by-frame playback using a modified protocol of Couvillon 2012 (9). We used the Image J 1.52i application to decode four, consecutive, non-first, non-last waggle runs per dance. We measured dance duration, which communicates distance, by determining the start and stop frame of each of the four waggle runs within the dance. We measured dance angle, which communicates direction, by drawing a line using the ImageJ “straight, segmented or freehand lines” tool from the central portion of the thorax during the waggle run and measuring the angle relative to the vertical plumb line angle. These measurements were entered immediately into Excel.

Since the dance followers average multiple waggle runs for both components of the dance, duration and direction (10, 11), we calculated the average vector (mean duration and mean angle) to give aggregated dance outcomes. Durations were converted to distance using the Virginian calibration reported by Schürch, Zwirner (12).

Additionally, since multiple bees usually were dancing simultaneously, we decoded all the bees that we saw dancing at time = 0. We then advanced the video six minutes (approx.10,800 frames) allowing the dancer(s) to finish their dance, providing time for a new cohort of dancers to enter the frame (9), which minimizes the opportunities of pseudo-replication. Lastly, we noted whether a forager was recruiting for nectar or pollen (nectar = 0, pollen = 1), determined by the presence (or absence) of pollen in the corbiculae of the dancer.

Data collection – pollen collection, sorting, preparation, and analyses

Twice a week, concurrently with waggle dance filming, we collected pollen from returning foragers using wire mesh pollen traps placed on the circular entrance tube of the hive. The mesh size allowed the forager to return inside the hive, but it removed the pollen from their corbiculae. We also placed a cookie sheet under each hive entrance because the pollen sometimes fell out of the trap onto the ground below. At the end of the one hour of filming, we collected the pollen from both the pollen trap and the cookie sheet and stored it in Eppendorf tubes labelled with the hive identifier (A, B, or C) and the date. We then placed the Eppendorf tubes into the freezer until we were able to transport them back to the main campus, where we stored them for later identification. A few times on pollen collecting days, we would find no pollen in either the pollen trap or on the cookie sheet, indicating that the hives were either not actively foraging or were foraging exclusively on non-pollen, usually nectar, sources. This occurs sometimes if the hive is not rearing brood (13).

After we were finished with the dance filming in October 2019, we took the pollen collected over the two years and sorted the tubes by date, where each tube represented the pollen collected from one hive on one day (herein, referred to as sample). We ended up with a total of 144 samples, 84 for 2018 and 60 for 2019, respectively. For the overall project, we planned to use the pollen for three objectives: (1) plant identification through color sorting & palynology, (2) DNA metabarcoding, and (3) organic contaminant analyses. This study will focus on objectives (1) and (3), which involves plant identification through color sorting and palynology and also organic contaminant analyses.

We sorted the samples into three groups for the three overall objectives. We ensured that each group possessed samples ranging from April – October: in this way, each pollen analysis objective would demonstrate good representation across the foraging season. We also made sure to have at least two samples for each analysis objective during each bloom period/ critical season (bloom, post bloom, late summer, and fall). Five samples from each year were too small for some analyses so those were combined (Hive A + B + C from the same day) to have enough pollen for the analyses to succeed. These instances were rare and were noted. Additionally, 23 samples (nine in 2018 and 13 in 2019) were the only samples available across a two-week period and were large enough that we could split them equally between each analysis objective. With these data, we are able to correlate where the bees have foraged (from waggle dance decoding) with what they are foraging on (from pollen color sorting / palynology).

For the samples designated for palynology, we first ordered them by date, and then we sorted the pollen pellets by color visually and then weighed the piles in grams. We selected a representative pellet of each color morph and stored it individually in a 5mL tube for palynological confirmation. Our working hypothesis was that pellets of identical color morph within the same sample (i.e., collected by foragers from the same hive on the same day) would mostly represent a single floral resource that was abundantly available. We could usually list a few candidate species based on pollen color identification charts, our knowledge of local flowering phenology, and what resources are typically used by bees at particular times. The representative pellet would then give species identification, which we could apply to the weighed piles of identical morphs per sample (see Table 3.3, subheading “Percent pollen by weight (g)”). This is a common technique used in honey bee pollen identification (14-16). Ultimately, we sent off 60 pollen samples (April – September 2018, May – September 2019) for palynological analysis.

We used the palynology processing technique developed by Dr. Vaughn M. Bryant at Texas A&M University and conducted by Global Geolab Limited (Alberta, Canada). Samples were prepared on 75 x 25 millimeter (mm) microscope slides with 18 x 18 mm number one thickness glass coverslips using an acetolysis procedure without Safarin-O to dye. After slide preparation, slides were sent to the Center for Excellence in Palynology (CENEX) lab at Louisiana State University under the direction of Dr. Sophie Warny for palynological analysis. The dried samples were examined at 600x and 1000x magnification with an Olympus BX41 to identify the pollen types present. To establish statistical validity (i.e., relative abundance for each taxon), a minimum of 300 pollen grains were counted for each of the samples using traverses that prevented any duplication of counts (same pollen being counted twice). When the palynological analysis was received back from CENEX, we were able to attribute the identification of the plant to the proportion of the sample based on the weight in grams. Relative abundances (%) are presented in Table 3 with samples organized by date.

In making quantitative counts, each pollen type was identified to the family and genus, with the exception of Apiaceae, Asteraceae and Mentheae due to the large size of the family deeming it nearly impossible to distinguish between genus and species. In these instances, we used our ground truthing data to work backwards through the process of elimination to arrive upon a plant identification.

Data analysis – percent foraging for nectar and pollen in apple versus forest during bloom and post bloom

Since our study location is defined by apple orchards, we were primarily interested in the reciprocal relationship between bee hives and apple fruit orchards over two foraging years. We not only wanted to know how much honey bees might be visiting and potentially pollinating apples during bloom (April – May), but we additionally wanted to know what resources, nectar or pollen, honey bees are collecting. Therefore, while Chapter 2 discussed percent foraging in orchards and forests for all dancing honey bees (i.e., for nectar and pollen), here we separated nectar and pollen dancers and calculated percent foraging again within orchards and forests for each per year. We once again included forest as a contrast to apples because that land type would not be predicted to be affected by apple blooms, and the exact details of resource foraging (i.e., nectar versus pollen) are unknown. It is important to note that even though we use the label "nectar" (Table 3.1), "nectar" represents any non-pollen resource collected by honey bees including nectar, water or propolis. It should be noted that nectar will represent the vast majority of the non-pollen dances (17, 18). Lastly, it might be that, due to temporary drops in video quality, every now and then a pollen dancer would be incorrectly labelled as a nectar dancer because it might be hard to distinguish the pollen in her corbiculae. These instances are not common, as usually at some point a bee being watched for several minutes (i.e., is being decoded) will come into focus sufficiently to see pollen. Luckily the opposite, where a non-pollen dancer would be incorrectly labelled as a pollen dancer by the decoder, would be extremely rare.

Due to the inherent error within both components of the honey bee's waggle dance communication (7, 19, 20), it is not possible to precisely map exact foraging locations from decoded waggle dances. Therefore, we utilized a developed analysis method that takes this error into account to simulate individual dances and then map them as a probability cloud (18). We based our percent foraging calculations on this methodology (19).

To calculate the percent foraging in a subset of data in landscape of interest and selecting for nectar or pollen foraging, we predicted each observed dance's advertised location 1000 times, factoring in uncertainty (12). Then, for each dance, we picked one prediction and calculated the percentage of dances in the landscape of interest. This process was repeated 10,000 times, each time with different combinations of predictions from each dance, resulting in a distribution of 10,000 percentages. From the distribution of percentages, we took the median percentage as the point estimate and the 5th and 95th percentile for our confidence interval to calculate the percent foraging during and post bloom for both apple and forests to understand how foraging in our fields of interest for nectar or pollen fluctuates as a function of bloom and throughout the rest of the foraging season.

Pesticide Residue Analysis

To analyze pesticide residues in our pollen samples, a subset of pollen samples was sent either to the McArt lab at Cornell University (n=18) or to the United States Department of Agriculture (USDA), National Science Laboratories, Gastonia, NC (n= 2). We decided to use both labs to obtain the most comprehensive evaluation possible because some compounds could only be detected with a specific methodology used at that specific lab. For example, the USDA lab could detect Captan and Avermectin in their samples, a chemical we were specifically concerned about given its prolific use in apple orchards and because of its highly toxic effects on honeybees.

Using known standards, the McArt lab used liquid chromatography-mass spectrometry and the USDA lab used gas chromatography-mass spectrometry. The McArt Lab at Cornell prepared the samples for extraction using a scaled-down version of the EN 15662 QuEChERS procedure and screened for 92 pesticides on the 18 pollen samples sent by liquid chromatography mass spectrometry (LC-MS/MS). Liquid Chromatography and Mass Spectrometry analysis was performed with a Vanquish Flex UHPLC system (Dionex Softron GmbH, Germering, Germany) coupled with a TSQ Quantis mass spectrometer (Thermo Scientific, San Jose, CA). The MS/MS detection was carried out using the Selected Reaction Monitoring (SRM) mode. Two transitions were monitored for each compound: one for quantification and the other for confirmation.

Gas Chromatography and Mass Spectrometry was performed by the USDA lab. To ensure the validity of results, sample size was required to be equal or greater than three grams. They were able to screen for 192 different chemicals potentially in the pollen samples. The chemical analytes of interest are extracted from the samples by high-speed grinding in an acetonitrile and water solution followed by a enhance matrix reduction (EMR) clean-up step to remove some matrix components combined with filtration to remove particulates. Separate aliquots of extract are analyzed for chemical residues by gas chromatography (GC) utilizing mass selective detection systems (MS with negative chemical ionization [NCI] and MS/MS with electron impact [EI]) and/or liquid chromatography (LC) techniques utilizing a tandem mass selective detection system.

After receiving the analysis, we calculated contaminants totals in ppb for insecticides, herbicides, and fungicides detected throughout the foraging seasons in both 2018 and 2019 by adding all samples from that season together for the total.

Lastly, we determined toxicity using the pesticide decision-making guide to protect pollinators in tree fruit orchards (21), Vera Krischik's "2017 Updated Toxicity to Pollinators of Insecticides Used in Greenhouse", and Landscapes and Extension Toxicology Network (EXTOXNET). For each contaminant that was found within the bee pollen, we would search the documents to determine their relative toxicity to bees (Table 3.5).

Data management and validation

Prior to analysis and after dances were decoded, we examined our data for any missing values and errors. Then we performed a data validation by calculating for both the duration and direction component the mean for each waggle dance, which is made up of the four decoded waggle runs, and the standard deviation. We then determined the 10 dances possessing the greatest outlying values for both the waggle run duration and waggle run angular (directional) intra-dance standard deviation. We located those 20 dances on the original videos. If the high standard deviation was because of a human error (typo or dance decoding error), we re-decoded the dances and re-entered it into Excel. We repeated the process until all 10 of the dances were found to possess no human error and, instead, were merely "noisy" dances (i.e., the dancer was imprecise between successive waggle runs within the dance). This involved going through the process once for the duration component (10 dances checked) and six times (60 dances checked) for the direction component until no more errors were found. All in all, our dance data set was comprised of 3,710 individual waggle dances, with each dance being made of four waggle runs, for a total of 14,840 decoded waggle runs from honey bees foraging around WAREC across the two years.

Results

The relative number of pollen dances declines from bloom to post bloom

Of the 3,710 total waggle dances decoded across the two years, 2,460 dances were for nectar and 1,250 were for pollen (Table 3.1). Post bloom pollen dances steeply declined with both years (87 for 2018, 82 for 2019, Table 3.1). In general, there are more nectar dances than pollen in every season, with the exception of bloom 2019, which is only slightly greater than pollen dances.

Pollen foraging in apple orchards is high during bloom but increases even higher in post bloom for both years

During bloom 2018, foragers were collecting pollen (23.8%) in apple orchards more than they were collecting nectar (15.3%, Figure 3.1, Table 3.2), but foraging for both was significantly higher in forests (pollen = 35.0% and nectar = 38.2%) during the same time. Then in post bloom, the honey bees increased their use of the apple orchards for both pollen (32.5%) and nectar (28.6%), with pollen remaining higher, as their use of the forests decreased (pollen = 23.8% and nectar = 26.0%).

During bloom 2019, foragers were collecting pollen (20.3%) in apple orchards less than they were collecting nectar (22.1%), but foraging remained significantly higher in forests for both resources (nectar = 30.9%, pollen = 34.4%, Figure 3.1, Table 3.2). Then in post bloom, the honey bees increased their use of the apple orchards especially for pollen (32.1%) but also for nectar (26.6%), as their use of the forests decreased for pollen (27.3%) but increased for nectar (36.0%).

Overall, we found for both 2018 and 2019, honey bees significantly increase their use of apple orchards from bloom to post bloom for both nectar and especially for pollen. In fact, if one considers that the overall number of pollen dances decreases bloom to post bloom (Table 3.1), this means that the pollen foraging efforts within orchards after the apples finished blooming represents a huge colony-level effort. In 2018, the increase in foraging remained in the same orientation, where pollen foraging is higher for both, but in 2019, the apple nectar foraging is slightly higher than apple pollen foraging during bloom (22.1% for nectar and 20.3% for pollen), but then, although both increase during post bloom, the pollen foraging increases significantly more (Figure 3.1).

Apple is representative of pollen samples during bloom and clover and plantain are among an abundant post bloom pollen selection

Within the 60 samples sent for palynology, 42 were monofloral, meaning at least 90% of the total pollen grains identified from that pollen ball of a bee's corbiculae were from the same plant. For simplicity's sake in our calculations of proportional abundance, we focus here on these samples (Table 3.3). Of these 42 samples, palynology detected 23 monofloral plant taxa from 37 total plant taxa.

With 42 of the 60 samples coming from a monofloral source, the other 18 were polyfloral, or comprised of multiple plant taxa within one sample. Because both monofloral and polyfloral samples are very informative of what honey bees are collecting in their environment throughout the entire foraging season, every taxon that comprised at least 15% of the sample or more is included in Figure 3.2.

Apple (Table 3.3, in bold) was represented in three of the four monofloral samples during bloom (and was present in five of six total bloom samples, including the polyfloral samples, data not shown). Additionally, four of the seven pollen sources identified during bloom included other non-apple tree species including oak, tulip tree, peaches/ cherries and tupelo. The two other species identified during bloom were honeysuckle and sumac / poison oak (Table 3.3). Overall, these data confirm that honey bees do collect apple pollen during apple bloom, but they also collect pollen from the abundantly available alternative resources in the surrounding forest and orchard margins.

During the post bloom period, plantain (*Plantago*) and clover (*Trifolium*, Table 3.3, underlined), were common foraging resources, showing up within five out of five samples for clover (purple) and four of the five for plantain (green) for that time period (Figure 3.2). Additionally, during post bloom, honey bees collected from herbaceous and tree species, including clover, plantain, and chestnut (Table 3.3, Figure 3.2).

In the late summer, plantain and clover remain common foraging resources, but other sources such as corn (yellow), crepe myrtle (light purple) and wild carrot (light blue) are also available (Figure 3.2). Late summer pollen resources are almost exclusively herbaceous, with the exception of crepe myrtle (tree).

Lastly, in the fall, the importance of Asteraceae as a late season pollen source is prevalent with the presence of goldenrod (golden yellow), ragweed (red violet), chicory (sky blue), thistle (grayish purple) and *Symphyotrichum* (light purple). Fall sources of pollen were exclusively herbaceous, and also included plants other than Asteraceae such as clover, grass from the Poaceae family and jewelweed.

Post apple bloom, when honey bees increase their use of apple orchards, also represents the highest pesticide exposure period

The highest detected contaminant totals were found during post bloom in both years (Figure 3.3, Table 3.3), where the honey bees experienced the most exposure to all three categories of tested contaminants: insecticides, fungicides, and herbicides. Overall this suggests that the post bloom period, when honey bees are foraging for pollen in forests and especially in apple orchards, represents the time where pesticide exposure is highest.

Of the 92 pesticides screened by the McArt Lab, 34 were detected in the 18 pollen samples sent (Table 3.5, Figure 3.3). Of the 192 pesticides screened by the USDA lab (as denoted by asterisks), 20 were detected in the two pollen samples sent, although 11 of the pesticides were detected at trace amounts (Table 3.5). Overall, the category of pesticide detected in the 20 samples sent to both labs included 15 fungicides, 15 insecticides, eight herbicides, one insect growth regulator and one synergist (Table 3.5, Figure 3.3). Insect Growth Regulator and synergists were detected in very small amounts (<3 parts per billion (ppb)) and are not included in Table 4.3 and Figure 3.3.

All 41 detected chemicals are listed in alphabetical order in Table 3.5, along with what is known about their use in the environment, the type of pesticide, the detection in parts per billion (ppb) by season and year, the line of detection (lod), and the toxicity to bees. Twelve of these chemicals detected are highly toxic to honey bees, but thirteen (indicated by the caret in Table 3.5) are known to have negative synergistic effects on bees. The 41 detected pesticides belong to 22 different classes including five neonicotinoids, four triazines, four triazoles, three carboxamides, three organophosphates, two aminopyrimidines, two ryanoids, two strobilurins, and one of each of the following acylalanine, aniline, benzamides, benzodioxoles, butanolide, carbamates, chloroacetamide, diacylhydrazine, oomycete, organochlorine, oxadizine, phenylureas, pyrethroids, and spinosyns.

No herbicides were detected in the samples for both years in Fall (Figure 3.3), which is as to be expected as there is less need for late season herbicide application, though late fall application for woody perennial weeds does occur. Additionally, honey bees focus their late season foraging on goldenrods and other late season wildflower blooms, which, by definition, had escaped herbicide application.

Discussion

Here we have shown pollen and nectar foraging occurred within apple orchards during bloom, and recruitment to both increased post bloom, with pollen foraging increasing proportionally more (20.3% to 32.1%, Figure 3.1) in 2019. Additionally, palynology confirmed presence of apple pollen during bloom in 5 of 6 total samples collected during bloom including 2018 and 2019 (Table 3.3). Moreover, during bloom, we also knew based on Chapter 2 that honey bees utilized forests at a high level, and here we report that honey bees visited tree species, including tulip tree, oak, chestnut and tupelo. Of the eight plant taxa identified during bloom period, four (or half) were tree species found in surrounding forests confirming our honey bee foragers frequent use (37% in 2018 and 32.3% in 2019, Table 3) of nearby forests for nectar and pollen foraging during bloom. Importantly, during post bloom when the bees increased their foraging to orchards even though the apple blooms had ended (Chapter 2), here we report that honey bee pollen foragers brought back an abundance of clover and plantain, but also in smaller amounts persimmon, tulip tree, and chestnut (Figure 3.2). Ground truthing discussed in previous chapter shows that clover and plantain were growing in apple orchards in the post bloom period between rows and near orchard edges and margins, strongly suggesting that pollen foragers are collecting from these wildflowers growing in the apple orchards in the post bloom period. Though plantain seems to be an important post-bloom foraging source, problematically, narrowleaf plantain is the summer host of rosy leaf aphid, the most damaging aphid on apple. Managing for pests harmful to apples, but also providing a potentially important foraging resource for pollinators complicates how stakeholders should approach their management strategies. Lastly, during the post bloom period when the honey bees increase their use of the orchards, we found the highest total amounts of pesticide exposure through pollen foraging (Figure 3.3). Taken together, these data indicate that post bloom apple orchards provide a plethora of foraging opportunities for honey bees, but also provide the highest and most probable risk of pesticide contamination. Post bloom orchards therefore might represent an enormous, unmonitored (or less monitored), and unintended route of exposure to non-target beneficial organisms like honey bees.

We found both pollen and nectar foraging occurred within apple orchards during the apple bloom period, and both increase post bloom, with pollen foraging even increasingly proportionally more, 23.8% to 32.5% in 2018, and 20.3% to 32.1% in 2019. Honey bees are always motivated to recruit for nectar, but pollen recruitment is a function of colony need and is most common during brood rearing times (i.e., early spring) (17). According to the palynology during post bloom, the increase in pollen foraging in apple orchards can be attributed to the prevalence of clover and plantain covering the understory of the orchards. This provides an ample nutritional resource to honey bees but also could be leading to a high risk of pesticide exposure in post bloom apple orchards.

Post bloom pesticide exposure was the highest in every category insecticide (609.15 ppb (2018) and 791.66 ppb (2019)), fungicide (416.79 ppb (2018) and 381.44 ppb (2019)) and herbicide (38.23 ppb (2018) and 85.62 ppb (2019)), with the exception of herbicide ppb slightly higher (42.02 ppb detected) in bloom 2018 than in post bloom 2018 (38.23 ppb). Pesticides are known to have negative effects on pollinators and other non-target organisms (22-24). Thirteen of the 41 pesticides detected in our pollen samples are also recorded to have synergistic effects on pollinators potentially increasing mortality. Eighteen of the 41 are known to be applied to pome fruits such as apple (Table 3.5). One study found that pesticides are interacting synergistically to increase bee mortality (25). Therefore, in mass-flowering orchard crops, such as apple, agricultural managers have made recommendations to reduce exposure of pollinators to chemicals such as application outside of crop bloom periods (26, 27).

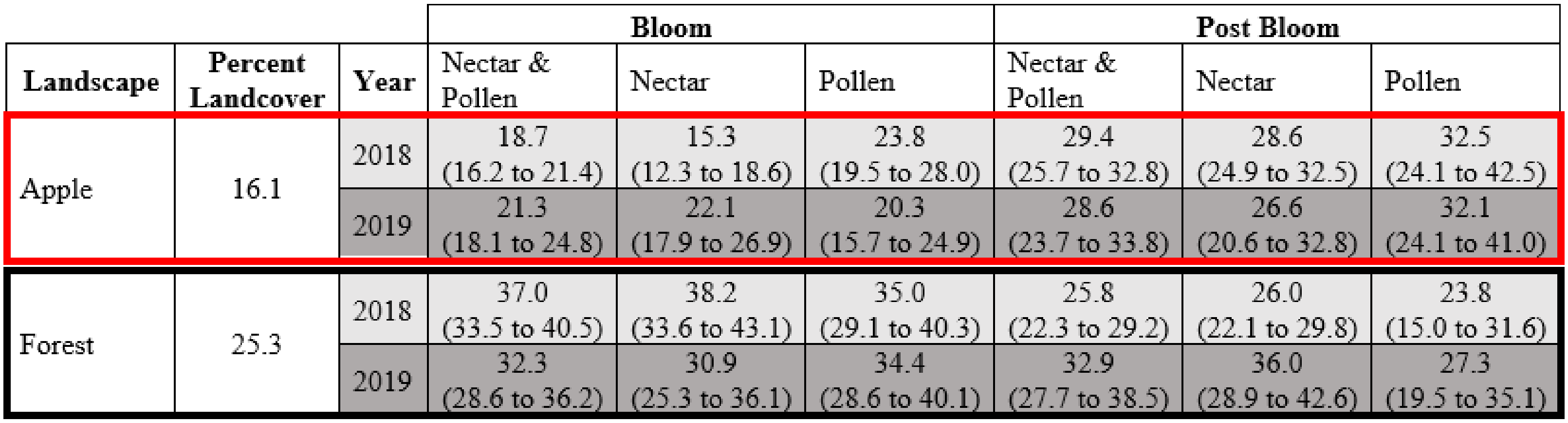
Even with these concessions, the presence of flowering weeds, such as clover and plantain, can massively increase foragers exposure to all categories of chemicals. Our data agree with one previous study that hypothesized that by removing the weedy understory within orchard groundcover, pollinators would not forage as frequently in orchards after the bloom, thus reducing exposure (28). In other words, the removal of understory significantly lowered abundance, richness, diversity, and evenness of pollinators, and understandably brought about an overall reduction in foraging (28). However, it is important to note that, although removing the understory reduces the exposure to chemicals, adopting this practice into the integrated pest management programs in orchards may not be in the best interest of pollinators overall because it may contribute to additional nutritional stress. Our palynology results agree with this cautionary note because clover and plantain are major foraging resources in apple orchards during the post bloom period, with clover present in all five samples collected during post bloom and plantain present in four of the five.

This study demonstrates the complicated nature of balancing management decisions, especially in the assessment of nutritional requirements versus risk of pesticide exposure. Future research should investigate different agricultural management techniques to understand the consequences of balancing these potentially competing issues.

Tables and Figures

		Number of Decoded Dances	
Year	Season	Nectar	Pollen
2018	Bloom	427	243
	Post bloom	452	87
	Late Summer and Fall	601	268
2019	Bloom	252	211
	Post bloom	172	82
	Late Summer and Fall	556	359
	Total	**2,460**	**1,250**

Table 3.1. Pollen and nectar dances decoded for entire project (n=3,710), broken down by bloom, post bloom, Late Summer and Fall. The number of pollen dances declined during post bloom in both years. Total nectar dances are almost double decoded pollen dances, which is to be expected.



			Bloom			**Post Bloom**		
Landscape	**Percent Landcover**	**Year**	Nectar & Pollen	Nectar	Pollen	Nectar & Pollen	Nectar	Pollen
Apple	16.1	2018	18.7 (16.2 to 21.4)	15.3 (12.3 to 18.6)	23.8 (19.5 to 28.0)	29.4 (25.7 to 32.8)	28.6 (24.9 to 32.5)	32.5 (24.1 to 42.5)
		2019	21.3 (18.1 to 24.8)	22.1 (17.9 to 26.9)	20.3 (15.7 to 24.9)	28.6 (23.7 to 33.8)	26.6 (20.6 to 32.8)	32.1 (24.1 to 41.0)
Forest	25.3	2018	37.0 (33.5 to 40.5)	38.2 (33.6 to 43.1)	35.0 (29.1 to 40.3)	25.8 (22.3 to 29.2)	26.0 (22.1 to 29.8)	23.8 (15.0 to 31.6)
		2019	32.3 (28.6 to 36.2)	30.9 (25.3 to 36.1)	34.4 (28.6 to 40.1)	32.9 (27.7 to 38.5)	36.0 (28.9 to 42.6)	27.3 (19.5 to 35.1)

Table 3.2. Percent foraging to orchards and forests for nectar and pollen during and after apple bloom in 2018 and 2019. During bloom in 2018, foragers were collecting pollen in apple orchards more than they were collecting nectar, but foraging for both was significantly higher forests. Post bloom in 2018, honey bees were collecting both pollen and nectar more within apple orchards than in forests. During bloom in 2019, foragers were collecting pollen and nectar in apple orchards less than during post bloom. Post bloom in 2019, honey bees increase their overall foraging, especially for pollen, to the apple orchards, and decrease pollen foraging in forests.

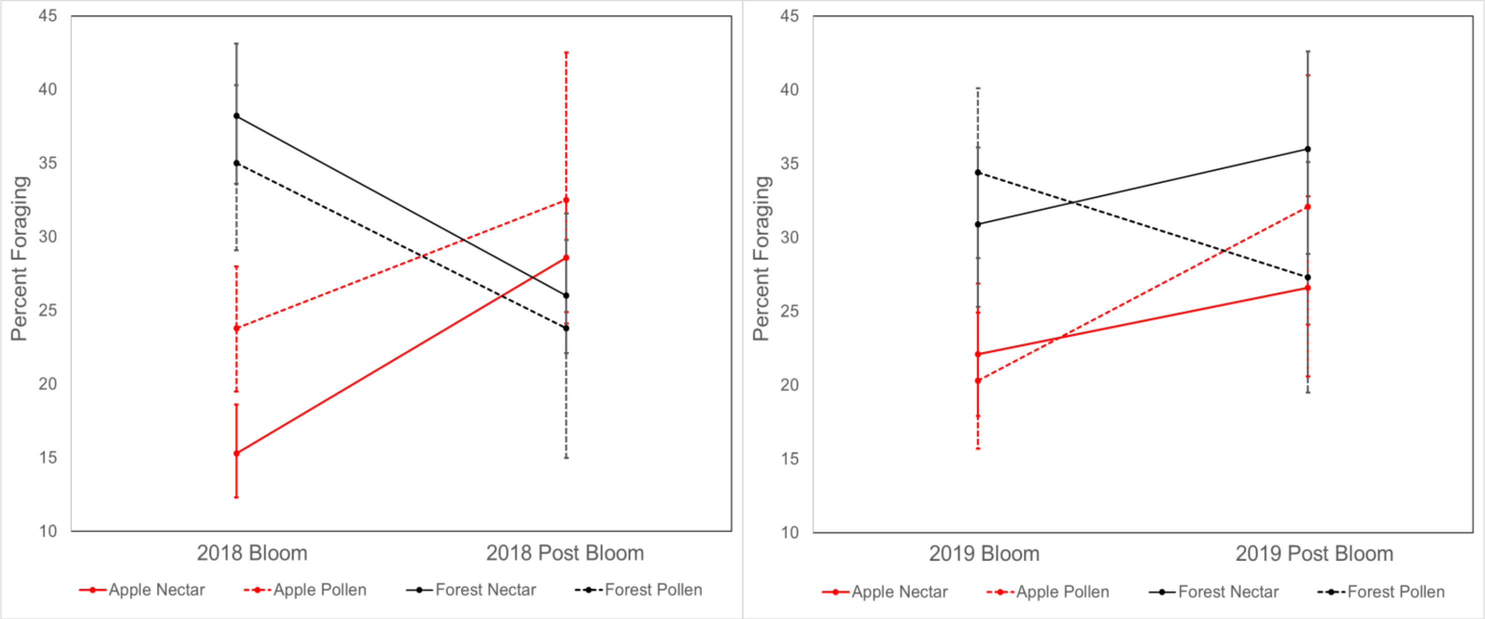


Figure 3.1. Pollen (dashed line) versus nectar (solid line) foraging during and after apple bloom in apple orchards (red) and forest (black) during the 2018 and 2019 foraging seasons. For both 2018 and 2019, honey bees increase their use of apple orchards bloom to post bloom for both nectar and pollen, although in 2018, the increase remains in the same orientation, where pollen foraging is higher for both, whereas in 2019, the apple nectar foraging is higher than apple pollen foraging for bloom, but then although both increase to post bloom, the pollen foraging increases significantly more. Meanwhile, in forests in 2018, foraging decreases bloom to post bloom for both pollen and nectar and remains in the same orientation. In forests in 2019, pollen foraging decreases markedly bloom to post bloom, probably because of the large increase in pollen foraging within apple orchards across the same period. In forests in 2019, nectar foraging increases bloom to post bloom.

Date	**Season**	**Pollen Identity**	**% Pollen Grains via Microscopy**	**Percent Pollen by Weight (g)**
4/14/2018	Bloom	**Apple** (*Malus*)	96.6	100
5/11/2018	Bloom	Honeysuckle (*Lonicera*)	96.8	37
5/11/2018	Bloom	Tupelo (*Nyssa*)	98.6	34
5/11/2018	Bloom	Oak (*Quercus*)	97.9	29
5/24/2018	Bloom	**Apple** (*Malus*)	99.5	76
5/24/2018	Bloom	Sumac/Poison Oak (*Rhus/Toxicodendron*)	100	24
6/1+8/2018	Post bloom	Chestnut (*Castenea*)	99.7	42
6/1+8/2018	Post bloom	Clover (*Trifolium*)	100	22
6/1+8/2018	Post Bloom	Persimmon (*Diospyros*)	96.9	8
6/15+28/2018	Post Bloom	Plantain (*Plantago*)	99.4	37
6/15+28/2018	Post Bloom	Clover (*Trifolium*)	98.7	30
6/15+28/2018	Post Bloom	Unknown (possibly Daffodil (*Narcissus*))	99.4	19
6/15+28/2018	Post Bloom	Tulip Tree (*Liriodendron*)	98.4	14
7/13/2018	Late Summer	Crepe Myrtle (*Lagerstroemia*)	96.5	5
7/13/2018	Late Summer	Thistle (*Cirsium*)	99.1	3
7/13/2018	Late Summer	Goldenrod (*Solidago*)	99.0	1
7/19/2018	Late Summer	Clover (*Trifolium*)	97.0	100
7/30/2018	Late Summer	Wild Carrot (*Daucus*)	99.4	24
7/30/2018	Late Summer	Plantain (*Plantago*)	98.5	3
8/15/2018	Late Summer	Corn (*Zea*)	97.0	100
9/5+6/2018	Fall	Ragweed (*Ambrosia*)	97.5	5
9/5+6/2018	Fall	Jewelweed (*Impatiens*)	100	2
9/5+6/2018	Fall	Wild Carrot (*Daucus*)	100	1
9/19/2018	Fall	Goldenrod (*Solidago*)	100	74
9/19/2018	Fall	Chicory (*Cichorium*)	97.2	26
5/2019	Bloom	Tulip Tree (*Liriodendron*)	100	43
5/2019	Bloom	**Apple** (*Malus*)	100	39
5/2019	Bloom	Peach/Cherry (*Prunus/Rubus*)	91	11
6/6+11/2019	Post Bloom	Clover (*Trifolium*)	94.9	69
6/6+11/2019	Post Bloom	Persimmon (*Diospyros*)	97.4	31
6/27/2019	Post Bloom	Clover (*Trifolium*)	98.0	70
6/27/2019	Post Bloom	Unknown (possibly Daffodil (*Narcissus*))	100	2
7/9/2019	Post Bloom	Plantain (*Plantago*)	100	67
7/9/2019	Post Bloom	Clover (*Trifolium*)	93.9	33
8/2+9/2019	Late Summer	Plantain (*Plantago*)	100	71

9/6/2019	Fall	Ragweed (*Ambrosia*)	99.4	52
9/18+25/2019	Fall	*Symphyotrichum*	99.8	91
9/18+25/2019	Fall	Grass (Poaceae)	98.8	4
9/18+25/2019	Fall	Plumeless thistle (*Carduus*)	96.6	3
9/18+25/2019	Fall	Clover (*Trifolium*)	98.5	2
10/9/2019	Fall	Goldenrod (*Solidago*)	100	86
10/9/2019	Fall	Clover (*Trifolium*)	98.2	14

Table 3.3. Flora as determined by palynology. Some dates were combined because they fell within the same season and because the sample was very small (<0.5g). We only included samples that were determined to be monofloral (90% or more of the pollen count per sample) were included in this table. Apple (in bold) pollen is represented in three of the four monofloral samples during bloom. Plantain (*Plantago*) and clover (*Trifolium*, both underlined) are important sources during the post bloom period. Other pollen sources during bloom included mostly other tree species including Oak, Tulip Tree, Peaches/ Cherries and Tupelo (4 of the 7 monofloral samples). The two species identified during bloom, honeysuckle and Sumac or Poison Oak, could be found within and around the surrounding forests and margins.

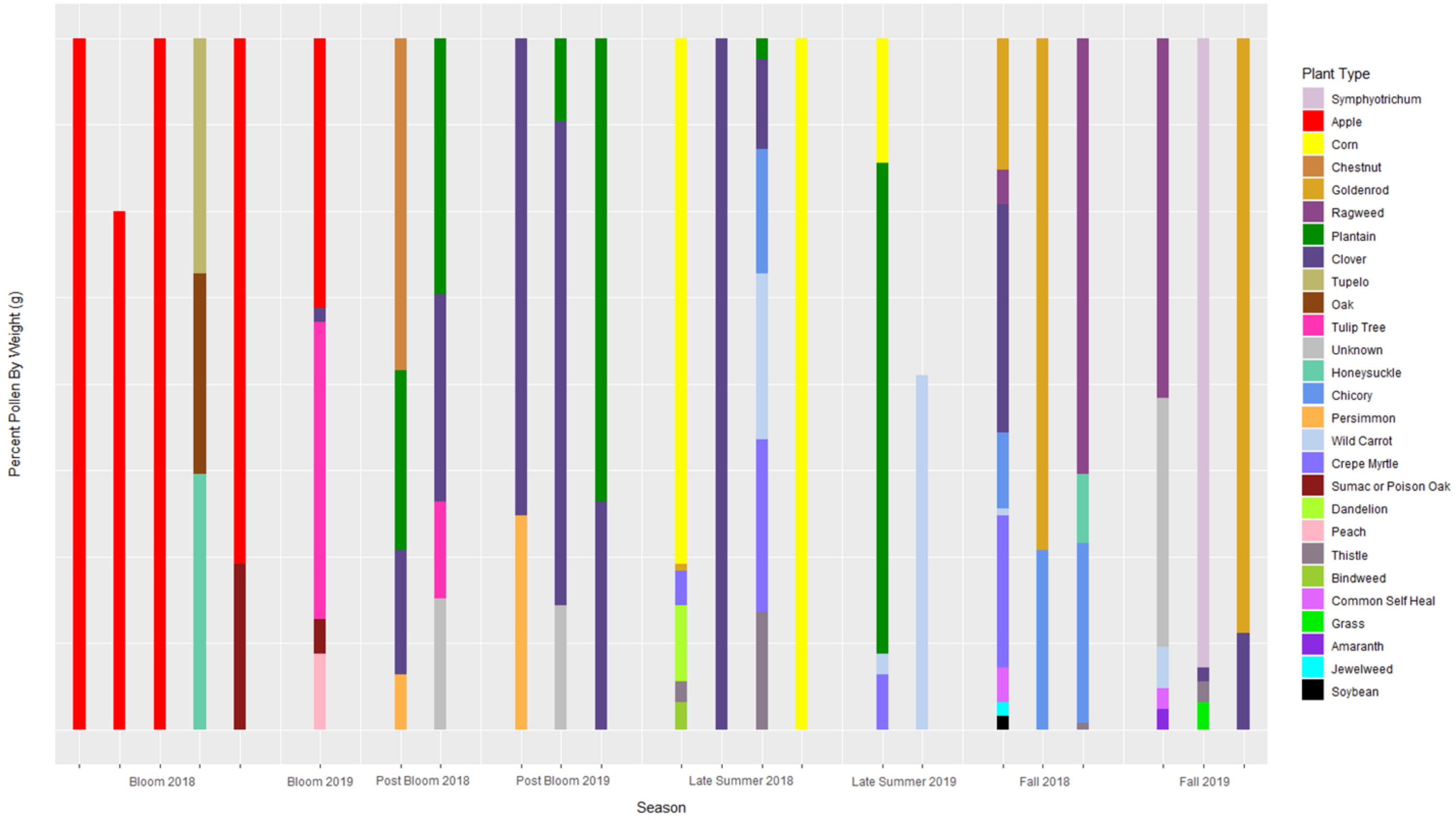


Figure 3.2. Type of flora determined by palynology and percentage of pollen by weight in grams across the entire foraging season in both 2018 and 2019. Of the five samples that were collected during apple bloom in 2018, four of the five had a high detection of apple pollen. One sample is representative of the apple bloom period in 2019. Post bloom in both 2018 (two samples) and 2019 (three samples) had a high presence of clover (*Trifolium*) (dark purple) and plantain (*Plantago*) (green). Late summer 2018 (four samples) had a high prevalence of corn, wild carrot, clover and crepe myrtle, while late summer 2019 (two samples) show a smaller percentage of corn pollen represented, but still a large percentage of wild carrot and plantain. We found three late season blooms: goldenrod, ragweed and chicory in Fall 2018, and in 2019, *Symphyotrichum*, goldenrod, and ragweed. Two samples (one in Bloom 2018, and another in Late Summer 2019) display less than 100%, this is because only pollen grains comprising at least 15% of the sample were included in the data for clarity.

	Bloom 2018	Bloom 2019	Post bloom 2018	Post bloom 2019	Late Summer 2018	Late Summer 2019	Fall 2018	Fall 2019
Insecticide	143.00*/ 85.03	6.00*/ 20.47	609.15	791.66	124.38	343.32	62.69	30.75
Herbicide	17.00*/ 42.02	4.00*/ 2.16	38.23	85.62	4.74	11.36	0	0
Fungicide	56.00*/ 28.60	12.00*/ 3.52	416.79	381.44	51.99	34.05	144.25	8.55

Table 3.4. Contaminants totals in ppb for insecticides, herbicides, and fungicides detected throughout the foraging seasons in both 2018 and 2019. The numbers with asterisks indicate that the sample was analyzed by the USDA lab. The highest detected contaminants were found during post bloom in both years. No herbicides were detected in the samples for both years in Fall, a time of year when honey bees are utilizing the abundant blooming of goldenrod, which isn’t likely to be growing where herbicides are used.

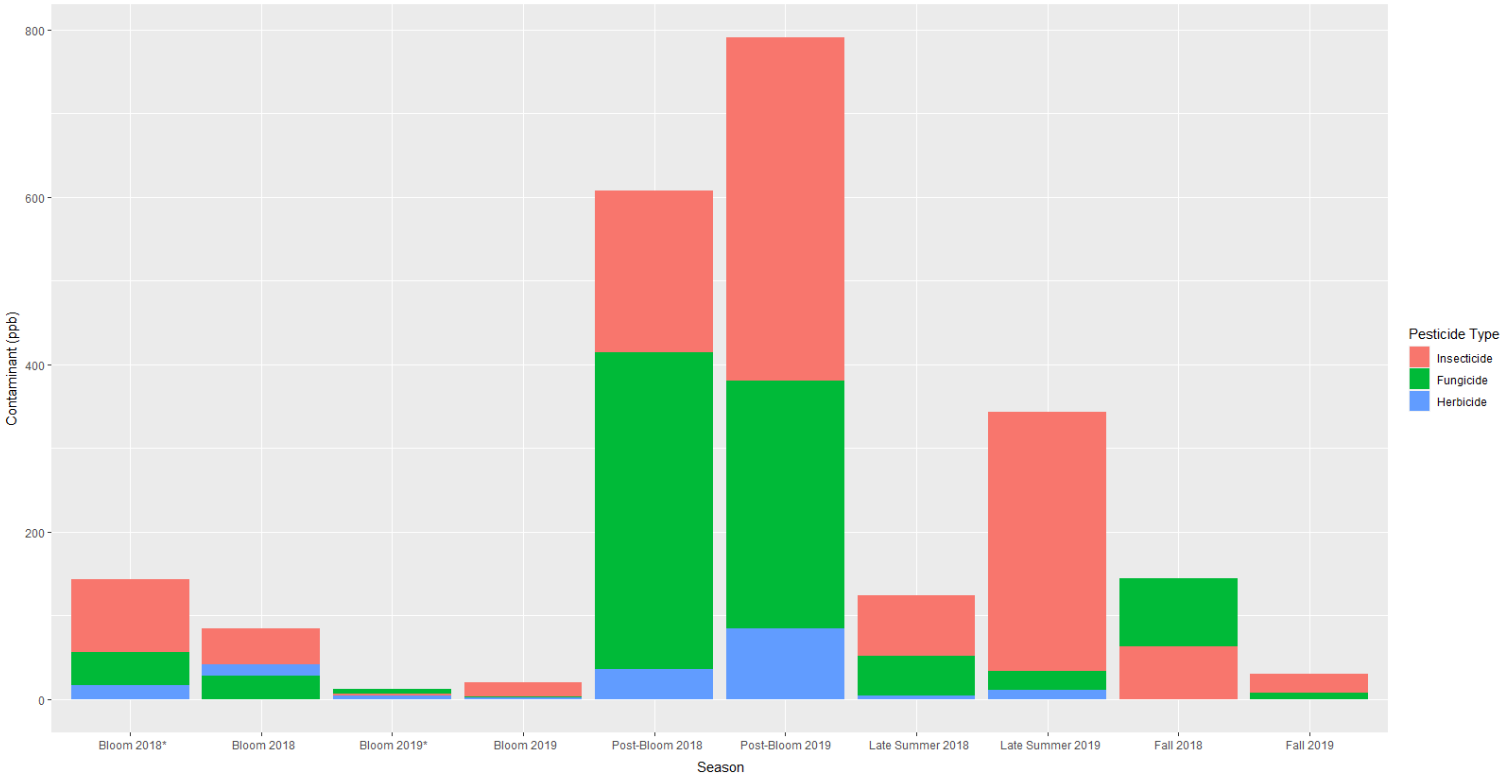


Figure 3.3. Contaminants detected (ppb) in pollen samples collected from returning foragers corbiculae during and after apple bloom in 2018 and 2019. Pesticide are grouped by pesticide type. The season labels with asterisk were analyzed at the United States Department of Agriculture, National Science Laboratory. All other samples were analyzed by McArt Lab at Cornell. The exposure to all types of pesticides was vastly more during post bloom.

Pesticide	Usage	Pesticide Type	Bloom 2018*	Bloom 2018	Bloom 2019*	Bloom 2019	Post bloom 2018	Post bloom 2018	Late Summer 2018	Late Summer 2019	Fall 2018	Fall 2019	USDA LOD (ppb)	Cornell LOD (ppb)	Toxicity to Bees
Acetamiprid	Apple	Insecticide	11	8.26	Trace	8.72	82.93	29.11	9.39	6.53	6.03	4.06	3.2	0.07	Moderate^
Amitraz	Crops/Residential	Insecticide	96										10		low
Alachlor	Weeds	Herbicide	trace										100		non-toxic
Atrazine	Weeds	Herbicide	11	22.51	4	1.59	23.01	42.07	1.32	9.44		0.12	1.2	0.03	non-toxic
Bensulide	Weeds	Herbicide	6										2		high
Boscalid	Grape/Peach	Fungicide					183.57		45.33		9.6			2.8	non-toxic^
Carbaryl	Apple	Insecticide	6	5.51	6	3.41	139.56	1.43	81.49		0.93		2	0.11	high
Chlorantraniliprole	Apple/Peach	Insecticide	30	17.21	trace	7.73	328.91	197.3	10.35	38.93	4.03	22.99	5.2	0.11	non-toxic
Chlorpyrifos	Crops/Residential	Insecticide	trace		trace			18.34					5	4.2	high^
Clothianidin	Crops/Residential	Insecticide		1.35			1.92	7.25	1.41	8.89				0.28	high
Cyantraniliprole	Crops	Insecticide						4.27						0.14	high
cyhalothrin lambda	Apple/Peach	Insecticide	trace										25		high^
Cyprodinil	Apple	Fungicide	44	7.17	6	0.29	20.28	3.76	3.16	3.84		0.1	1.2	0.03	non-toxic^
DDE p,p'		Pesticide	trace		trace								3		unknown
Difenoconazole	Apple	Fungicide	12	15.06		0.72	63.84	176.66	0.66	1.71		1.52	1.6	0.07	non-toxic^
Dinotefuran	Crops/Residential	Insecticide					5.54	0.42			1.4	0.84		0.14	high
Diuron	Weeds	Herbicide	trace	7.43			1.22	25.65	2.86	1.34			2	0.28	non-toxic
Fenbuconazole	Apple/Peach	Fungicide					39.17	5.1		3.04				0.14 (0.28)	non-toxic^
Fluopyram	Apple/Peach	Fungicide		0.12			3.18	0.45		0.07	0.5			0.01	non-toxic
Flupyradifurone	Crops	Insecticide						152.92		1.31				0.28	non-toxic
Flurifol	Apple	Fungicide			6								2		non-toxic^
Fluxapyroxad	Apple	Fungicide	trace	2.63	trace	2.51	32.32	21.58		3.07	133.29	6.76	0.8	0.28	non-toxic^
Imidacloprid	Apple/Peach	Insecticide					14.51	21.91	2.12	1.37	0.48			0.14	high
Indoxacarb	Apple	Insecticide					12.42							0.42	high
Mandipropamid	Grape	Fungicide					57.64	157.08						0.06	non-toxic
Metalaxyl	Crops	Fungicide					0.15	0.17						0.03	non-toxic
Methoxyfenozide	Crops	IGR					0.33	0.61				2.84		0.07	low
Metolachlor	Weeds	Herbicide	trace	11.64		0.57	14	16.91					25	0.07	non-toxic
Myclobutanil	Apple	Fungicide	trace	2.14	trace		1.08	11.57					3.2	0.03	non-toxic^
Penthiopyrad	Apple	Fungicide						0.1						0.03	non-toxic
Phosmet	Apple	Insecticide		41.1				73.45						1.4	high
Piperonyl butoxide	Crops/Residential	Synergist		0.22			1.63								non-toxic^
Prometon	Weeds	Herbicide						0.06						0.01	non-toxic
Propazine	Weeds	Herbicide		0.44				0.93						0.03	unknown
Pyraclostrobin	Apple	Fungicide			trace								1.2		non-toxic^
Pyrimethanil	Apple	Fungicide		0.86										0.07	non-toxic
Simazine	Weeds	Herbicide			trace								8		non-toxic
Spinetoram	Apple/Peach	Insecticide					15.04	276.08						0.07	high
Tebuconazole	Crops/Residential	Fungicide						1.2						0.28	non-toxic
Thiamethoxam	Peach/Cherry	Insecticide	trace	11.6		0.61	8.32	9.18					4	0.07	high^
Trifloxystrobin	Crops/Residential	Fungicide		0.62			15.56	3.77						0.03	non-toxic

Table 3.5. Pesticides detected on pollen samples collected from honey bee corbicula foraging in and around apple orchards during and after apple bloom. Pesticides are listed in alphabetical order, and we include what is known about their use in the environment, the type of pesticide, the detection in parts per billion (ppb) by season and year, and the toxicity to bees. An asterisk next to the season indicates that the sample was analyzed by United States Department of Agriculture, National Science Laboratory, and the caret next to the toxicity indicates the pesticide has known synergistic effects on bees.

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CHAPTER 4: FINAL DISCUSSION

Assessing project implications

Prior studies have examined the relationship of honey bees' benefits during bloom toward apple orchards yield or have quantified pesticides exposure during bloom; however, few studies include a comprehensive analysis of how orchard landscapes affect honey bees across entire foraging seasons. Our study determined that apple orchards served as a significant food resource during bloom, but even more so after the bloom. Thirty-three different plant taxa were identified through palynology, a majority (21) of which could be attributed to plants found within apple orchards.

Contrary to our expectations, apple blooms did not drive foraging behaviors as reflected by the decoded waggle dances. Although we found honey bees forage in apple orchards during bloom, they foraged more in the same orchards after the apple bloom had ended. We used forests as a comparison because they provide early spring blooms that could potentially create a more attractive alternative to apple blossoms, and forests immediately surround the orchards in which we placed our bees and comprised a major part of the foraging area (c. 35%). We found that honey bees forage more in forests than apple orchards during and sometimes after bloom, but honey bees increase their foraging notably in apple orchards after bloom.

One of the most resounding takeaways from this research is that bees continue to use the orchards even more after bloom. This finding provides an interesting contrast to the typical management of commercial beekeeping. Honey bees are typically brought into apple orchards during bloom then promptly taken out after the bloom period has ended. This management style became increasingly frequent with the conversion of landscapes to large scale monocultures. As migratory and commercial beekeeping persists to meet the pollination demands of modern agriculture, research such as this, show that these environments are potentially able to nutritionally support honey bees year-round. The implications of converting landscapes to monocultures profoundly impacted pollinators, while allowing ground cover and surrounding natural lands improved pollinator diversity and populations (1-3).

As described in previous studies, summertime has the potential to be a period of foraging dearth (4, 5); our results corroborate this, with communicated foraging distance highest in late summer. According to our pollen samples, corn was one of the major available pollen sources during late summer, a crop described as of limited nutritional value to honey bees, and not necessarily what pollinators would frequent given the choice of a higher quality resource (6). It may be that in late summer, alternative options are limited. Previous work suggests that diversity of available floral resources throughout the foraging season are essential to honey bee health and functions such as brood development, consistent honey yield, and reducing the risk of mortality due viral pathogens and pests (7, 8). Therefore, these data could mean that summer might represent a time when there is a dearth of food and therefore could contribute to overall poor health outcomes. Although we did not specifically test this issue, anecdotally we did experience nine hive deaths during our research, especially during late summer (four observation hives replaced during summer in 2019).

Bees were also exposed to a wide variety of contaminating compounds (41) through pollen foraging. Based on these results, more research is needed to determine if the combination of waggle dance decoding analysis and pesticide residue analysis from forager-collected pollen can be used to better understand how and from what bees are likely to be exposed to pesticides. A study conducted in Italy did look at the floral origin of pesticide residues found in pollen loads collected by managed honeybees during and after apple bloom. They similarly found that the amount of insecticide residues present in pollen samples increased after apple bloom (9). Lastly, and to no surprise, given the prolific usage in orchard landscapes, all pollen samples had at least three pesticides detected, with the most at 22 (from post bloom) and on average 11.16 pesticides were detected per sample.

How this thesis fits into the larger field

This thesis adds to an existing albeit limited body of knowledge of bees in fruit orchards (Figure 4.1, teal). Specifically, we previously knew that although honey bees are important apple pollinators, there is some evidence to suggest that they might not be the most efficient at the task, although their worker numbers might help cover this deficiency. Additionally, we knew previously that a diverse community of post bloom plants support bee communities, although the details of what resources specifically remained unknown, especially for honey bees. My research (Figure 4.1, orange) furthered the field by answering many of the outstanding questions left open from the previous work. Moving forward (Figure 4.1, yellow), future studies should look at synergy of multiple interacting chemicals picked up by non-target organisms. It also would be important to further explore the interactions of different stressors affecting pollinators at the landscape level to inform the risk assessment process and conservation policy decisions, such as determining if it is more beneficial to remove a potentially important source of forage if it eliminates the risk of toxic pesticide exposures. Lastly, we hope that this research might help to inform better pollinator decision-making for orchardists and land managers.

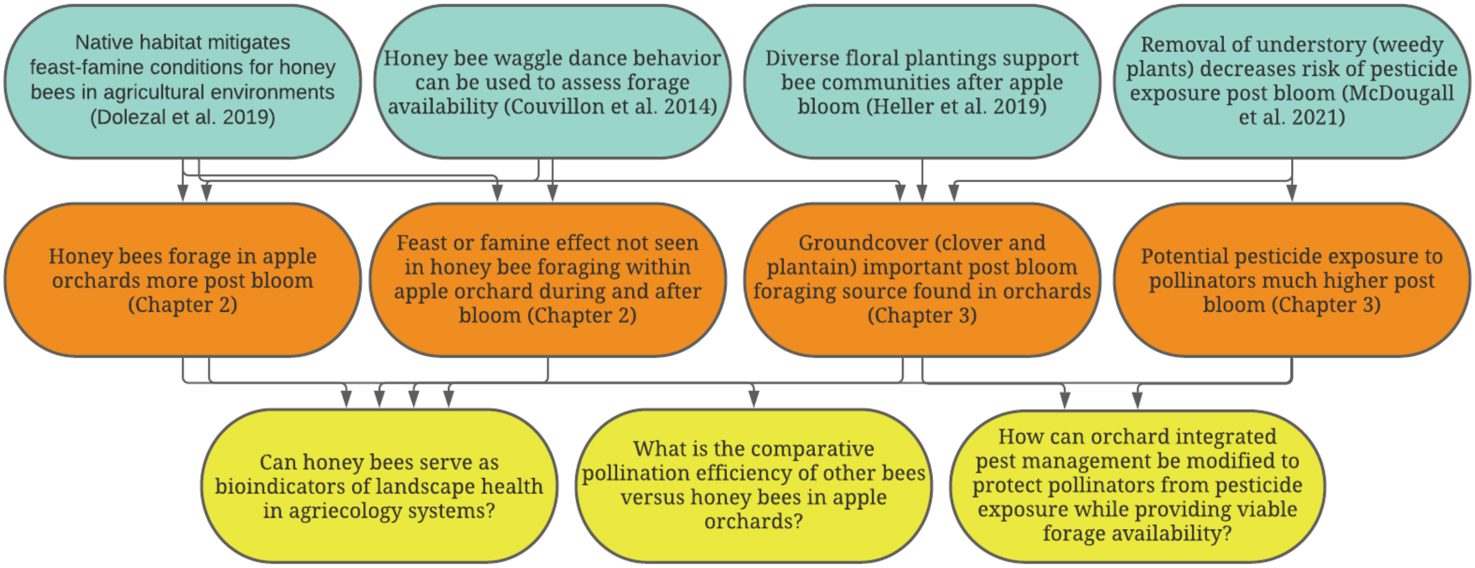

Figure 4.1. The flow of research from previous work (teal), to the overview of this thesis (orange), to potential research questions arising from this research (yellow).

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