Row crop environments provide an all-you-can-eat buffet and pesticide exposure to foraging honey bees

Mary Silliman

Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science

in

Entomology

M. J. Couvillon, Committee Chair
S. Taylor, Co-advisor
R. Schürch

20 May 2021
Blacksburg, VA

Keywords: waggle dance, Apis mellifera, foraging ecology, row crops
Row crop environments provide an all-you-can-eat buffet and pesticide exposure to foraging honey bees

Mary Silliman

Scientific Abstract

The western honey bee, *Apis mellifera*, provide invaluable economic and ecological services while simultaneously facing stressors that may compromise their health. For example, agricultural landscapes, such as a row crop system, are necessary for our food production, but they may cause poor nutrition in bees from a lack of available nectar and pollen. Row crops are largely wind or self-pollinated, and while previous studies have focused on the impact of bees to row crops, fewer studies have examined the reciprocal relationship of the row crops on honey bees. Here we investigated the foraging dynamics of honey bees in a row crop environment. We decoded, mapped, and analyzed 3460 waggle dances, which communicate the location of where bees collected food, for two full foraging seasons (April – October, 2018-2019), and concurrently collected pollen from returning foragers. We found that bees foraged mostly locally (< 2 km) throughout the season. The shortest communicated median distances (0.48 and 0.32 km), indicating abundant food availability, occurred in July in both years, which was when our row crops were in full bloom. We determined, by plotting and analyzing the communicated locations, that most mid-summer foraging was in row crops, with at least 40% of honey bee recruitment dances indicating either cotton or soybean fields. Bees also largely foraged for nectar when visiting row crop fields, only returning to the hive with *Glycine* spp. pollen, and foraging on nearby trees and weeds for pollen. Foragers were exposed to thirty-five different pesticides throughout the foraging season, based on pesticide residues in collected pollen. Overall, row crop fields are contributing a surprising majority of mid-summer forage to honey bee hives and suggests that
similar agricultural landscapes may also provide abundant, mid-summer forage opportunities for honey bees, however, at the risk of pesticide exposure.
Row crop environments provide an all-you-can-eat buffet and pesticide exposure to foraging honey bees

Mary Silliman

General Audience Abstract

Declines in the number of honey bee hives have been observed in the United States and western Europe throughout the last century, driven by environmental stressors such as poor nutrition caused by anthropogenic landscape change and pesticide exposure. Agricultural landscapes, for example, contain monocultures and often necessitate pesticide use, which may be detrimental to bee health. Because of these effects, it is necessary to understand how honey bees forage in these systems and what potential health risks they face. We investigated honey bees foraging dynamics in a row crop environment, observing honey bee waggle dance recruitment behavior and gathering forager-collected pollen to better understand when, where, and what honey bees forage on throughout the season (April – October). We found that bees largely foraged near the hive throughout the season, indicating that sufficient resources were available, particularly in July when crops were in full bloom. During full bloom bees considerably foraged in cotton and soybean fields. We found that bees collected minimal row crop pollen, apart from soybean pollen, largely foraging on trees and flowering weeds for pollen. Through pollen foraging bees were exposed to thirty-five pesticides, ranging in toxicity and mode of action. Overall, honey bees foraging in a row crop system foraged substantially in row crop fields during the mid-summer. Row crops systems may be able to provide abundant forage during the mid-summer, but could come at the risk of exposure to pesticides.
TABLE OF CONTENTS

CHAPTER 1: LITERATURE REVIEW .............................................................................1
General honey bee life history and importance .........................................................1
Honey bee waggle dance behavior ............................................................................2
Honey bee stressors ...................................................................................................3
Solutions to address stressors ..................................................................................5
Row crops ..................................................................................................................6
REFERENCES ..............................................................................................................8

CHAPTER 2: ROW CROP FIELDS PROVIDE A MID-SUMMER, ALL-YOU-CAN-EAT BUFFET FOR FORAGING HONEY BEES ..............................................................17
Introduction ..............................................................................................................17
Methods ....................................................................................................................20
Study organism and experimental set-up ...................................................................20
Study location, crops, and bloom times ...................................................................21
Data collection – waggle dance filming ...................................................................22
Data collection – waggle dance decoding .................................................................23
Data management and validation ..........................................................................24
Data analysis – distance ..........................................................................................25
Data analysis – percent foraging in fields of interest ..............................................26
Results .....................................................................................................................27
Honey bees in row crops forage mostly locally with some long-range events .......27
Foraging distance varies by month and year ............................................................28
Median foraging distance varies during crops’ full bloom .....................................28
Most mid-summer honey bee foraging is in row crop fields ...............................28
Discussion ...............................................................................................................29
Tables and Figures ..................................................................................................33
REFERENCES ..........................................................................................................43
CHAPTER 3: WEEDS AND WILDFLOWERS IN A ROW CROP ENVIRONMENT PROVIDE POLLEN AND PESTICIDE EXPOSURE TO FORAGING HONEY BEES

Introduction ........................................................................................................................................ 54

Methods ............................................................................................................................................... 55

- Study organism and experimental set-up ........................................................................ 55
- Study location, crops, and bloom times ............................................................................ 55
- Data collection – waggle dance filming ............................................................................. 55
- Data collection – waggle dance decoding ........................................................................... 56
- Data collection – pollen collection and sorting ................................................................. 56
- Data analysis – percent foraging in fields of interest by food type .................................... 57
- Pollen preparation .................................................................................................................... 57
- Pollen processing and counting of palynomorphs ............................................................... 57
- Pesticide residue analysis ........................................................................................................ 59
- Data management and validation .......................................................................................... 59

Results .............................................................................................................................................. 59

- Visitation to cotton and soybean fields is nectar driven .................................................. 59
- Honey bees only collected soybean pollen ....................................................................... 60
- Honey bees were exposed to pesticides through the entire foraging season .................. 60

Discussion .......................................................................................................................................... 60

Tables and Figures .......................................................................................................................... 63

REFERENCES .................................................................................................................................. 67

CHAPTER 4: FINAL DISCUSSION ......................................................................................................... 73

- Assessing project implications ............................................................................................... 73
- Tables and Figures ...................................................................................................................... 75

REFERENCES ..................................................................................................................................... 76
List of Tables and Figures

Chapter 2

Figure 2.1. Approximate bloom times of row crop cultivars in Suffolk, Virginia. ..............33

Figure 2.2. Communicated distance travelled by foragers (km) throughout the foraging season in 2018 and 2019, Suffolk, Virginia. ........................................................................................................34

Figure 2.3. Median foraging distance travelled (km) by month (n = 3460) in 2018 and 2019, Suffolk, Virginia. ........................................................................................................35

Figure 2.4. Median foraging distance travelled (km) by bloom time per crop in 2018 (n = 2067) and 2019 (n = 1393). ........................................................................................................36

Figure 2.5. Mean percent (95% CI) foraging, centered around the hives, in peanut, soybean, corn, and cotton fields during bloom intervals (pre-bloom, during bloom, post-bloom) in 2018 and 2019. ........................................................................................................37

Table 2.1. Percent land cover type, rounded to the nearest degree, within an area that represents the majority of dances (i.e., a radius of the median 95th% foraging distance from our decoded dances each year). ........................................................................................................41

Table 2.2. Mean percent (95% CI) foraging in fields of interest by bloom in 2018 and 2019, as determined by waggle dance decoding, mapping, and analysis, within the 95% median foraging distance by year........................................................................................................42
Chapter 3

Figure 3.1. Mean percent (95% CI) foraging, centered around the hives, in peanut, soybean, corn, and cotton fields during bloom intervals (pre-bloom, during bloom, post-bloom) and by forage type (peanut and nectar, peanut, nectar) in 2018 and 2019. .........................................................63

Figure 3.2. Contaminants detected (in ppb) in samples from forager-collected pollen throughout the foraging season of 2018 and 2019 (April – October). .................................................................65

Table 3.1. Mean percent (95% CI) foraging in fields of interest by bloom and forage type (pollen or nectar) in 2018 and 2019, as determined by waggle dance decoding, mapping, and analysis, within the 95% median foraging distance by year.................................................................66

Chapter 4

Figure 4.1. The flow of research from previous work, to the overview of this thesis, to potential research questions arising from this thesis.................................................................75
CHAPTER 1: LITERATURE REVIEW

General honey bee life history and importance

The western honey bee, *Apis mellifera*, within Insecta, is one of 1-30 million different species estimated to exist within the class (Caron and Connor 2013). Within Insecta is the order Hymenoptera, which is comprised of sawflies, ants, wasps, and bees. Honey bees are further placed in Apidae, a family of long-tonged bees, some of which are eusocial. *A. mellifera* also falls within the clade Corpiculata, which are the bees possessing pollen baskets (Martins et al. 2014).

The Italian honey bee, *Apis mellifera linguistica*, is a subspecies of the western honey bee. *Apis mellifera* is indigenous to Africa, Asia, and Europe and ranges from southern Africa to southern Scandinavia (Winston 1991); however, domestication and shipping have contributed to the species spreading to all continents except Antarctica (Hung et al. 2018). Honey bees are eusocial insects, coexisting in hives composed of three distinct castes: workers, drones, and a queen. All castes undergo six molts before reaching adulthood, a process that takes between 16-24 days depending on caste (Science and Education Administration 1980, Caron and Connor 2013). Within the all-female worker caste, individuals exhibit division of labor by age, including acting as nurse bees, facilitating in resource storage, serving as guard bees (in select cases), and actively foraging outside the hive, and they generally live for about six weeks in the summer and for many months while overwintering. Honey bees possess a distinctive proboscis, 5.3-7.2mm when extended, which allows foragers to probe flowers for nectar. Proboscis length can vary by race (Ruttner et al. 1978) and is correlated with flower visitation based on corolla depth (Inouye 1980). To collect pollen from floral resources and propolis and transport it back to the hive, honey bees aggregate these materials in their corbiculae. Largely through their pollination services, *A.*
*mellifera* contribute $14.6 billion (inflation adjustment to 2021: $22) annually to the US economy (Morse and Calderone 2000).

**Honey bee waggle dance behavior**

The waggle dance is a method of forager recruitment observed in honey bees, in which a dancing bee repetitively oscillates her abdomen, moving linearly (waggle run phase), and then stopping to turn either to the left or right as she moves back to her start position (return phase), where she typically then begins another waggle run, after which she stops and turns to the opposite direction (i.e., figure-eight pattern). The waggle run encodes the information regarding the distance and direction to a resource in the landscape relative to the hive position (von Frisch 1967). Honey bees are central place foragers, residing at a nest site, venturing outside the nest to collect resources or mate, and subsequently returning to the nest (Charnov 1976). During the dance, a dancer will either turn to the right or to the left, typically alternating directions after each iteration. In order to orient directionally, bees use the sun’s azimuth. While honey bees most commonly dance for nectar or pollen resources, they may also dance for water, resin, or to communicate the location of new nest sites. Though dancers communicate a specific location to their nest mates, dance accuracy and precision can be variable due to comb cell use, dance duration, physiological constraints, and other factors that are still unknown (Couvillon et al. 2012, Tautz 1996, Tanner and Visscher 2010, Preece and Beekman 2014). The waggle dance is a filtered signal, meaning that it is typically indicative of a higher quality resource and dancing has been shown to be representative of the relative profitability (energetic efficiency) of a source at the present time (Seeley 1994). Therefore, foraging distance is a proxy of resource availability since bees are economic foragers and will not needlessly fly long distances. Because vector information is given (i.e., duration and angle of dance), communicated behavior in the hive can be translated [by researchers] to the actual forage
(i.e., nectar and pollen) sources in the landscape. By training individually marked bees on a feeder at a set location and decoding their dances, past studies have been successful at translating forager communicated duration (dance run) to the equivalent distance traveled in the landscape (Schürch et al. 2013, Schürch et al. 2016, Schürch et al. 2019). Since Karl von Frisch’s groundbreaking work understanding and interpreting the waggle dance, decoding methods have progressed from live measurements and calculations to digital analysis methods post factum (Couvillon et al. 2012, Seeley 1994, Beekman and Ratnieks 2000) and even recent advancements in automated decoding (Wario et al. 2015). By decoding and interpreting waggle dance behavior, researchers hope to better understand and to assess the ability of a landscape to feed pollinators (Couvillon et al. 2015), suggesting that honey bees could serve as bioindicators to evaluate landscape health and suitability for bees (Couvillon and Ratnieks 2015, Couvillon et al. 2014a, Couvillon et al. 2014b, Quigley et al. 2019).

**Honey bee stressors**

There has been a 50% reduction in the number of managed *A. mellifera* hives in the United States in the last century (Neumann and Carreck 2010, Aizen and Harder 2009b, Levy 2011, Ellis et al. 2010), a trend which has also been observed in western Europe. Many different stressors, including pests, pathogens, pesticides, and poor nutrition, contribute to these declines, either singularly or in combination (Dolezal et al. 2019), and [in some instances] arise or are exacerbated by man-made processes (Kluser et al. 2010, Goulson et al. 2015). *Varroa destructor*, a prolific bee pest, has largely been spread, along with their vectored diseases, via migratory beekeeping. (Rosenkranz et al. 2010, Alger et al. 2018, Ramsey et al. 2019). Mites exhibit two major life cycle phases and feed upon the fat bodies (Rosenkranz et al. 2010, Ramsey et al. 2019), transmitting harmful diseases such as deformed wing virus (DWV) and Israeli acute paralysis virus (IAPV)).
Mite damage can be observed on an individual scale (e.g., reduced flight performance, reduced cognition in terms of learning and ability to navigate) and on a colony scale (e.g., fewer swarms, reduced overwintering survival). Colony-level damage is more dependent upon overall colony health and survival. *Apis mellifera* are also susceptible to various additional pathogens, including *Nosema spp.* and American Foulbrood (AFB). Exposure to *Nosema apis* and *Nosema ceranae* may result in digestive disorders and reduced honey production (Chen et al. 2008). AFB only affects larval stages, resulting in increased mortality when larvae are exposed to *Paenibacillus larvae*, a spore-forming bacterium (Genersch 2010, DeGraaf et al. 2006). Agrochemicals, though necessity to protect global food supply, can have deleterious effects on insect pollinators (Henry et al. 2012, Gill et al. 2012, Whitehorn et al. 2012, Alkassab and Kirchner 2017). Brandt et al. (2016) observed overall reductions in bee immunity when honey bees were exposed to sublethal (field-realistic) concentrations of various neonicotinoids (e.g., thiamethoxam, thiacloprid, imidacloprid, and clothianidin) while El Hassani et al. (2005) observed impaired retention and learning in honey bees exposed to fipronil. Other studies have acknowledged that it can difficult to assess true field exposure because impact may vary between life stages, environmental conditions, the frequency and duration of exposure, and overall impact (i.e individual vs. colony-level) (Fairbrother et al. 2014, Goulson et al. 2015, Carreck and Ratnieks 2014). Poor nutrition generally results from loss in abundance, diversity, and quality of floral resources and can lead to a decreased ability to cope with other health stressors or starvation. (Goulson et al. 2015, Moret and Schmid-Hempel 2000). Nutritional requirements vary on a colony and life-stage (adult, larvae) scale (Brodschneider and Crailsheim 2010). Nectar provides vital carbohydrates, essential for overwintering honey storage and metabolism in adults. On a larval level, nectar quantity affects the number of larvae that are reared. Pollen provides essential proteins, aiding in adult longevity and overwintering survival. On
a larval level, pollen supports brood development, behavior, and physiology while diminishing the
likelihood of the cannibalization of brood when protein stocks are low (Di Pasquale et al. 2013).
Poor nutrition is considered the linchpin of other stressors due to the necessity for bees to
constantly forage despite potential consequences. Stress from pathogens may up-regulate immune
response, causing increased food consumption, followed by potential pesticide exposure (Goulson
et al. 2015).

Solutions to address stressors

Lack of available forage in the landscape for feeding pollinators has recently captured the
attention of people at multiple levels, from federal through commercial to private citizens. For
example, under the Obama administration, a Presidential Memorandum was enacted to improve
pollinator health through data-driven colony loss prevention, habitat restoration projects, and
public education (Presidential Memorandum 2014). In 2017, the General Mills company Cheerios
launched a “Bring Back the Bees” campaign, encouraging concerned citizens to plant flowers, in
addition to providing interested individuals with an (albeit controversial) seed mix (Fears 2007).
Lastly, awareness of and concern for pollinator health has grown among everyday citizens, as
evidenced by the increased interest in personal pollinator gardens (Garbuzov and Ratnieks 2014).
While all these mentioned efforts are meritorious, aid is most effective when it can be targeted,
which requires a deep understanding of the interaction of a pollinator within a particular landscape.
While our knowledge of pollinator foraging dynamics within urban and suburban environments is
improving (Bates et al. 2011, Wojcik and McBride 2012, Kaluza et al. 2016, Garbuzov et al. 2015),
the interaction of flower-visiting insects with agricultural lands, particularly those outside
orchards, is less understood.
Row crops

While all environmental stressors impact overall bee health, the compounding effect may be most detrimental (Goulson et al. 2015). Many of these stressors (e.g., pesticides, poor nutrition) may be prevalent within row crop production systems, and less research has been done to uncover how honey bees forage in these environments. In 2012, approximately 137 million hectares were used for harvested cropland (Bigelow and Borchers 2017). In terms of pollinators, there are several factors that might, at first glance, appear to prelude honey bees from foraging in these systems. Row crop systems are largely comprised of monocultures, including corn, cotton, soybeans, and peanuts being notable in eastern North America. This system allows for mass production, but may limit nutritional diversity; however, it is unclear as to whether this has a significant effect on fitness (Goulson et al. 2015). Cotton flowers, although containing extrafloral nectaries that may be attractive to pollinators, may also preclude visitation because of low sugar quality and large pollen structure (Vaissière and Vinson 1994, Konzmann et al. 2019). Pesticide exposure affects floral visitation as certain pesticides increase or preclude foraging (Kessler et al. 2015). Pesticide application in the US has declined overall while herbicide use has grown (Fernandez-Cornejo et al. 2014). While herbicides largely indirectly target insects, usage in agricultural systems diminish the floral resources available for pollinators (Goulson et al. 2015). Though broadcast applications of insecticides have decreased, seed treatment use is higher than ever (Douglas and Tooker 2015) and bees can be exposed to dust that contains insecticide residues during planting (Krupke et al. 2012). When insecticides are sprayed on flowering crops, honey bees and other pollinators are at risk of exposure.

Since row crops are largely wind- or self-pollinated, they do not necessitate visitation from a pollinator and consequently, there has been less emphasis on understanding the impact of
pollinators on row crops (Science and Education Administration 1980, Malerbo-Souza 2011). Though there is much debate regarding the suitability of these habitats, honey bees and native bees in particular, are able to extract nectar and pollen and, in some experiments, visitation from pollinators has resulted in increased yields (McGregor et al. 1955, Klein et al. 2007, Klatt et al. 2014, Konzmann et al. 2019). In soybeans, honey bee hives added to the landscape increased seed number in high yield conditions (Blettler et al. 2018). Cage studies, which exclude flower-visiting insects from blooms, showed that honey bees can improve yield in some cotton cultivars, including total boll and seed mass (Rhodes 2002). However, while most of the few previous studies have focused on the impact of the honey bees to the row crop, less is known about the reciprocal impact of the row crop to honey bees. Our research seeks to fill these knowledge gaps.
REFERENCES


Douglas, Margaret R., and John F. Tooker. 2015. "Large-scale deployment of seed treatments has driven rapid increase in use of neonicotinoid insecticides and preemptive pest management in US field crops." *Environmental science and technology* 49 (8): 5088-5097.


Garbuzov, Mihail, Margaret J. Couvillon, Roger Schürch, and Francis L.W. Ratnieks. 2015. "Honey bee dance decoding and pollen-load analysis show limited foraging on spring-


Kluser, Stéphane, Peter Neumann, Marie-Pierre Chauzat, and Jeffery S. Pettis. 2010. *Global Honey Bee Colony Disorder and Other Threats to Insect Pollinators*. UNEP.


CHAPTER 2*: ROW CROP FIELDS PROVIDE A MID-SUMMER, ALL-YOU-CAN-EAT BUFFET FOR FORAGING HONEY BEES

* Submitted 04-19-2021 to the journal of Agriculture, Ecosystems & Environment

Introduction

Nearly 90% of flowering plants have evolved to utilize animal, particularly insect, pollination (Willmer 2011). About 35% of major food crops are insect pollinated (Klein et al. 2007). Largely because of this reliance in pollinator-dependent crops, flower visiting insects are estimated to provide c. $200-250 billion worldwide in critical benefits to agricultural ecosystems (Gallai et al. 2009). Currently the demand for food crop pollination is outpacing the availability of pollinators (Garibaldi et al. 2011, Aizen and Harder 2009a), as approximately 41% of insect species are declining globally (Sánchez-Bayo and Wyckhuys 2019, Hallmann et al. 2017, Kosior et al. 2007). Of these insect species, major classes of pollinators, such as Hymenoptera and Lepidoptera, are some of the most affected (National Research Council 2007).

The decrease in abundance and diversity of insect pollinators is associated with four stressors: pests, pathogens, pesticides, and poor nutrition (Kluser et al. 2010, Goulson et al. 2015, Dolezalet al. 2019). These stressors arise or are exacerbated by man-made processes. Agrochemicals, including insecticides, are essential for protecting our food supply and sublethal exposure of insect pollinators to these compounds has been shown to cause impairment to their longevity, navigation, learning and memory, queen reproductive health, overall colony survival, and behavior (Henry et al. 2012, Gill et al. 2012, Whitehorn et al. 2012, Alkassab and Kirchner 2017). Migratory beekeeping, where honey bees (Apis mellifera) are repeatedly moved in and out of different orchards during bloom, has become the norm in some countries, including the USA,
as a way for a limited supply of honey bees to be deployed through multiple landscapes and different crop bloom times. The practice has consequently contributed to the spread of pests, such as the mite *Varroa destructor*, and their vectored diseases like Deformed Wing Virus (Rosenkranz et al. 2010, Alger et al. 2018, Ramsey et al. 2019). Lastly, increased urbanization and the development of previously unmanaged rural land is important for the growing world population; however, these anthropomorphic landscape changes are decreasing the abundance and quality of available forage for pollinators in some instances (National Research Council 2007, Otto et al. 2016, Carvell et al. 2006, Simanonok et al. 2020), which in turn has detrimental effects on pollinator health, both directly through starvation and indirectly through their decreased ability to cope with other stressors (Goulson et al. 2015, Moret and Schmid-Hempel 2000).

Lack of available forage in the landscape for pollinators has recently captured the attention of people at multiple levels, from federal through commercial to private citizens. For example, under the Obama administration, a Presidential Memorandum was enacted to improve pollinator health through data-driven colony loss prevention, habitat restoration projects, and public education (Presidential Memorandum 2014). In 2017, the General Mills company Cheerios launched a “Bring Back the Bees” campaign, encouraging concerned citizens to plant flowers, in addition to providing interested individuals with an (albeit controversial) seed mix (Fears 2017). Lastly, awareness of and concern for pollinator health has grown among everyday citizens, as evidenced by the increased interest in personal pollinator gardens (Garbuzov and Ratnieks 2014). While all these efforts are meritorious, aid is most effective when it can be targeted, which requires a deep understanding of the interaction of a pollinator within a particular landscape. While our knowledge of pollinator foraging dynamics within urban and suburban environments is improving
(Bates et al. 2011, Wojcik and McBride 2012, Kaluza et al. 2016), the interaction of flower-visiting insects with agricultural lands, particularly those outside orchards, is less understood.

Harvested cropland, land that includes row and sown crops, tree fruits and nuts, and vegetables, is a dominant feature in the rural landscape, covering an estimated 137 million hectares in the USA (Bigelow and Borchers 2017). Row crop systems are largely comprised of monocultures, including corn, cotton, peanuts, and soybeans. Interestingly, although row crops are usually wind- or self-pollinated, many of these crops produce pollen and even nectar and attract pollinators (Science and Education Administration 1980, Gill and O’Neal 2015). Additionally, previous studies have demonstrated that insect pollinators can increase yield in these crops (Klein et al. 2007, Klatt et al. 2014, Konzmann et al. 2019), even if they are not essential. In soybeans, the addition of honey bee hives to the landscape increased seed number in high yield conditions (Blettler et al. 2018). Cage studies, which exclude flower-visiting insects from blooms, showed that honey bees can improve yield in some cotton cultivars, including total boll and seed mass (Rhodes 2002). However, despite these yield improvements and perhaps because of row crop non-dependency on pollinators, pollinators in row crop environments remain understudied. It is unclear if these habitats can provide adequate nutrition for pollinators: while previous studies have focused on the impact of insect pollinators to row crops, few studies have investigated the reciprocal relationship of the row crop habitat on the insect pollinators.

The honey bee is a valuable, managed insect pollinator, contributing c. $14 - 20 billion annually to the US economy (Morse and Calderone 2000). Similar to the declines in wild pollinators, the number of honey bee hives is decreasing in the United States, Britain, and many western European countries (Aizen and Harder 2009a, Aizen and Harder 2009b): in the United States alone, there has been a 50% reduction in the number of hives in the last century (Neumann
Honey bees use a recruitment behavior called the waggle dance to communicate the location of a good source of food, which is usually nectar or pollen (von Frisch 1967, Couvillon 2012). Nestmate recruits then use the waggle dance to determine the location of a profitable food source, which they can then forage on themselves. The communication therefore allows the hive to exploit the resource in an efficient and timely manner. The dance is also visible to the eye and can be observed and decoded by scientists to determine how, where, and when the forager is collecting food within a landscape (Schürch et al. 2013, Couvillon et al. 2014a).

Here we monitored honey bee foraging in southeastern Virginian agricultural land, which includes numerous corn, cotton, soybean, and peanut fields. We allowed freely flying honey bee foragers to collect nectar and pollen in a row crop environment for two complete foraging years and analyzed the honey bee waggle dances. Our objectives were: (1) to determine the foraging distance, as this serves as a proxy for food availability, across the seasons within a row crop environment and (2) to calculate the percentage of foraging by honey bees in peanuts, soybeans, corn and cotton. Our unique study design allowed us to observe not only where honey bees prefer to forage (i.e., by decoding the waggle dance), but also to calculate how much of that foraging occurs in fields of interest.

Methods

Study organism and experimental set-up

We studied three predominantly Apis mellifera ligustica honey bee colonies (labeled “A-C”) that included a queen and approximately 5000 workers plus brood housed in plexiglass-walled observation hives containing three American Standard Deep frames. We suspended plumb lines...
made of fishing lines at the top of the observation hive and secured a washer or bolt at the bottom of each line. The plumb lines were spaced out in 5cm intervals and appeared as white lines on the camera, which provides a vertical reference. The observation hives were placed inside a shed (approx. 14 ft x 8 ft), and the colonies were connected to the outside via a 3cm x 30cm plastic tube, which afforded the foragers unimpeded access to the landscape. We wired the shed for electricity to provide diffuse lighting and, when needed, air conditioning or heating. When supplemental feeding with sugar solution was needed, we provided it after the filming was done for the day using a canning jar with small holes in the lid inverted over a mesh opening in the observation hive. We managed the colonies using standard beekeeping techniques to prevent swarming and to keep approximately the same population levels in each hive.

*Study location, crops, and bloom times*

We located the colonies at the Virginia Tech Tidewater Agricultural Research and Extension Center (TAREC) (36°41'06.4"N 76°46'01.6"W) in Suffolk, Virginia. The 465-acre facility researches southeastern Virginia row crops (e.g., corn, cotton, peanuts, soybeans, sorghum) and swine. The TAREC comprised the immediate area around the honey bee hives, but within a wider foraging range (approx. 8.2 km radius) there was a mixed-use landscape of cropland, developed land, forest, grassland/pasture, and wetlands (USDA-NASS 2018, 2019), with cropland and forest as the dominant land types (Table 2.1). Our crops of interest were peanuts, soybeans, corn, and cotton, which accounts for circa 5 – 19% of the landscape (Table 2.1).

Row crops vary in their bloom times (Figure 2.1). Early season cover crops, including wheat, rye, canola, crimson clover, and vetch, bloom from early April to mid-May. Major row crops typically begin to bloom around mid-June to early July. For the purpose of this study, we were interested in examining honey bee foraging on peanuts, soybeans, corn, and cotton because
they all bloom around the same time, beginning between mid-June to early July with the bloom season lasting until mid- to late-August. Additionally, it is often anecdotally assumed that bees prefer soy to cotton, but, to our knowledge, it has never formally been investigated. In our calculations of percent foraging of specific fields of interest (see below), we restricted the date ranges to the time intervals when c. 75% of the crop is in bloom (Figure 2.1).

Data collection - waggle dance filming

Foragers may perform waggle dances to recruit nestmates to high quality resources, generally 100 meters to many kilometers from the hive (von Frisch 1967). Importantly, foragers only dance for the best resources at any given time (Seeley 1994, Couvillon 2012), and this allows us to determine the location of where the bees are most preferring to forage, not the location of all the available forage including low quality forage. We filmed each colony for about an hour per day (~ 9:30am – 12:30pm, but usually 10-11am), 4-5 days per week using Canon Vixia HF R82 cameras. Videos were recorded to SanDisk Extreme SD cards and then later uploaded to a Google Team Drive (GTD) for dance decoding (see below). Filming was conducted on days with a minimum temperature of 14°C and no rain or adverse weather. Plumb lines (fishing wire suspended from the top of the observation hive with a push pin with a washer or bolt at the bottom) spaced 5cm apart were used as guides to adjust cameras so that approximately 20-25cm of the dance floor was visible. Prior to filming, we recorded the daily meta-data (e.g., date, time, hive, blooming crops, outside temperature). We filmed during the entire foraging season for two years (23 April – 31 October, 2018, and 10 April – 18 October, 2019). Overall, we generated one-hour long video per day from 3 observation hives, usually 4 days per week with 4 weeks per month, for 6.5 months per year and for 2 years to make approximately 624 hours of video data.
Data collection – waggle dance decoding

Dance decoding was based on Couvillon et al. 2012. Briefly, we converted video files filmed at 30 frames per second to AVI using Ubuntu (v. 2004.2021.222.0) and imported them into ImageJ (version 1.52i). At the start of each video, we measured our vertical reference plumb line to determine the angle offset (i.e., if the cameras and/or observation hive are not completely vertical, then measuring the angle offset corrects for that, usually only by a few degrees or fractions of a degree). Then we played the video from the beginning until we found the first dancer, which usually occurred within the first few seconds of the video. A returning forager performing a waggle dance will repetitively oscillate her abdomen while moving linearly (waggle run phase) across the comb, will stop, turn either to the left or right, and then walk back (return phase) to the starting position. She may then do another waggle run, repeating this pattern of waggle run and return phase 1-100+ times (von Frisch 1967, Seeley et al. 2000). Dance decoding involves extracting two pieces of information per waggle run to obtain a vector: the duration of the run and the direction of the run relative to vertical (Couvillon et al. 2012). We assessed the duration by noting the start and end frame of each waggle run and calculating the difference, which we then convert from number of frames to seconds of duration by dividing by the 30 fps. We assessed the direction of each waggle run by using the straight-line tool in ImageJ to measure the angle from vertical that the dancer makes with her body as she performs a waggle run. This measurement is automatically corrected for the angle offset (see above) in Excel. Lastly, since honey bees orient relative to the sun but dance relative to vertical, we noted the time of day and date during filming and corrected later for the solar azimuth to obtain the bearing.

For each dance, we decoded four mid-dance, consecutive waggle runs per dancer, with an equal number of left and right-handed turns (von Frisch 1967, Seeley et al. 2000). We averaged
the four waggle run durations and azimuth-corrected angles to obtain a single duration and direction for each dancer. This number and selection of waggle runs was chosen because the calculated mean direction and mean duration was not significantly different from the means obtained if all the waggle runs per dance are decoded (Couvillon et al. 2012). We converted duration to distance using the Virginia calibration reported by (Schürch et al. 2019). Taken together, these averaged components (direction + distance) give the approximate location of the advertised resource.

Once the first dance within that video frame had been decoded, we then decoded the other dances that were occurring simultaneously on the dance floor. In videos with few dancers present, we watched the entire video and decoded every dance. However, in videos with higher activity (20+ per hour), we skipped ahead 6 minutes (or 10,789 frames) after decoding all the simultaneous dances that occurred at the start of the video. By skipping ahead, we avoided potential pseudo-replication, where we sample from the same dancer twice [i.e., a single dancer can dance 1-100 times (Seeley et al. 2000) depending on the resource quality, which can last many minutes and can involve the dancer moving in and out of the screen]. Our objective was to achieve at least 20 dances per hour of video. These dances should provide a good representation of colony foraging during the respective hour of filming (Couvillon et al. 2012, Sponsler et al. 2017). In all, we decoded 3456 dances, 2063 from 2018 and 1393 from 2019.

Data management and validation

For waggle dance decoding, we recorded all raw video data on SD cards, which were subsequently uploaded to a Google Team Drive (GTD) as a permanent, online, cloud-based repository. Each dance decoder selected a video file from the GTD, downloaded it, and worked with the file locally. We immediately entered decoded dance data into Excel, and each decoder
would upload his or her Excel file daily to the GTD. We received results as Excel files, which we also uploaded to the GTD. Lastly, we created a GitHub repository to manage R files.

We validated dance data before analysis. Data were scanned and rows with missing values, due to human error, were removed. Next, we examined our data for erroneous values by calculating, for each dance, the standard deviation (SD) per dance for each dance component (i.e., the SD between the four runs for waggle run duration and for waggle run angle). We then identified the highest ten values (i.e., the dances with the most intra-dance variation) within the data set for each respective dance component (i.e., duration, angle). We located these 20 dances and examined them on the data entry sheet to determine if the SD was due to an obvious entry error. If the error source was unclear, we located the dances on the original videos to verify whether high SD was based on error inherent in the dance (i.e., a “noisy” dancer with high intra-dance variation), which is normal (Schürch and Couvillon 2013, Couvillon et al. 2014a, Schürch et al. 2019) and acceptable, or due to human error (i.e., dance decoding error), in which case we re-decoded the dance. We noted any changes to initial values in our dataset. The process of examining the 10 dances with the highest SD values in either duration or angle was repeated until all 10 dances’ SDs were solely from error inherent in the dance (i.e., were just noisy dances). Overall, this required us to validate six rounds of highest SD value dances for both duration and angle and involved our correcting (re-decoding) 30 dances out of a dataset of 3456 dances.

Data analysis – distance

Honey bees have evolved to be economic in their foraging decisions and, considering the energetic impact of a flight, a bee will only fly and recruit her nestmates to a resource as necessary, especially given the distance from the hive (Schmid-Hempel et al. 1985, Seeley 1986, von Frisch 1967, Couvillon et al. 2014b). Therefore, communicated foraging distance can serve as a proxy
for the availability of nectar and pollen (i.e., when bees recruit for a resource relatively further away, it is likely because food cannot be found closer to the hive). We interpreted the communicated foraging distance as a function of time to determine seasonal fluctuations in forage availability. We began by plotting the raw data as distance across time, which included a LOESS regression. We then aggregated time by month, which allowed us to compare median foraging distance per month to previous studies (Couvillon et al. 2014a). Lastly, because our study was in an environment with semi-predictable bloom times highly relevant to our study, we aggregated median foraging distance across the growing season (e.g., pre, during, and post bloom) by crop. We expected to see communicated foraging distance to decrease as we go from pre- to during bloom and increase as we go from during bloom to post-bloom if the crop is impacting food availability. We used RStudio (v. 1.4.1106), R (v. 4.0.3) (R Core Team 2020) to perform the analysis.

Data analysis - percent foraging in fields of interest

We were primarily interested in honey bee foraging in soybean, cotton, corn, and peanut fields (Figure 2.1, Table 2.1) because of our regional land cover, the timing of the crops, and the gaps in the previous research. To calculate percent foraging in our fields of interest by crop, we downloaded the 2018 and 2019 National Cropland Data layers and imported layers into ArcGIS Pro (v. 2.5.1). We created a 95% median foraging distance buffer, 1.911 km and 1.883 km for 2018 and 2019 respectively, using the buffer function in ArcGIS and used the extract by mask function to extract the cells of a raster that correspond to the areas defined by the mask, the 95% median foraging ranges by year. We then we converted the raster file to a shapefile and exported the layer package (including .cpg, .dbf, .prj, .sbn, .sbx, .shp, .shp.xml, and .shx files) into our R project. We selected for the field of interest by grid code number in the dataset.
Honey bees are imprecise and inaccurate in their dance communications and there is an inherent error in both components of the dance (von Frisch 1967, Schürch and Couvillon 2013, Okada et al. 2014). Therefore, predicting and mapping waggle dances as exact foraging locations, as was done in the past (Beekman and Ratnieks 2000, Steffan-Dewenter and Kuhn 2003), overrepresents our certainty. Instead, we developed a methodology where individual dances are decoded, simulated, and then mapped as a probability cloud (Schürch et al. 2013, Couvillon et al. 2014a, Couvillon et al. 2014b), which considers the error in the dance. To calculate the percent foraging in fields of interest, we predicted each observed dance’s advertised location 1000 times, accounting for uncertainty (Schürch et al. 2019). For each dance we then picked one prediction and calculated the percentage of dances in fields of interest. This procedure was repeated 10,000 times, each time with different combinations of predictions from each dance, and resulting in a distribution of 10,000 percentages. From the distribution of percentages, we then took the median percentage as our point estimate and the 2.5th and 97.5th percentile for our confidence interval. Using this procedure, we calculated percent foraging for pre-, during, and post- bloom per crop to understand how foraging in our fields of interest changes throughout the season and with crop bloom. All data and code will be made available through Virginia Tech’s data repository after conclusion of a larger, on-going project in the lab.

Results

*Honey bees in row crops forage mostly locally with some long-range events*

The median distance foragers recruited to in the landscape during the study (April – October, 2018 and 2019) was 0.703 km (n = 3460) with a range from 0.058 to 8.214 km (Figure 2.2). The median distance was 0.700 km (range 0.051 to 7.131 km) (n = 2067) and 0.706 km (range
0.050 to 8.214 km) (n = 1393) in 2018 and 2019, respectively. The maximum distance in 2019 (8.214 km) was larger than in 2018 by 1 km.

Foraging distance varies by month and year

Foraging distance varied by month in both years (2018: \(\chi^2 = 105.06, df = 6, p < 0.0001, n = 2067\); 2019: \(\chi^2 = 274.98, df = 6, p < 0.0001, n = 1393\)). In 2018, the highest median foraging distances were in April (0.852 km), May (0.831 km), and August (0.791 km), suggesting these are months when forage is less available. In 2019, the highest median foraging distances were in April (0.850 km), June (1.335 km), and August (0.811 km). June 2019 was the highest median foraging distance for both years, which we suspect was driven by drought (see discussion). The lowest median foraging distances were observed in July in both years, which suggests that forage is most available at this time. July is the month when all target row crops are blooming (Figure 2.1).

Median foraging distance varies during crops’ full bloom

The median foraging distance travelled during pre, during, and post bloom followed our prediction for corn (2018, 2019), peanut (2019), and soy (2019) (Figure 2.4). Cotton (2019) did demonstrate a decrease in distance during bloom, but post-bloom decreased even further, presumably because additional forage became available closer to the hive (see discussion).

Most mid-summer honey bee foraging is in row crop fields

Percent visitation of foragers to fields of interest [per crop] increased from pre-bloom to bloom, as we would expect if bees were foraging in the crops, in all years and all crops except corn in 2019. This increase was most evident in soybean and corn in 2018 and in cotton in 2019, especially if one considers the representation of these crops (%) in the wider landscape (Table 2.2). For example, cotton accounts for 14% of the foraging range landscape in 2019, and yet honey bees
are foraging upon and recruiting to cotton 30% of the time. As these row crops bloom over roughly the same period of time, taken together, the row crops account for a majority of foraging during summer full bloom (c. 45-57% in total, Table 2.2, % foraging during bloom), representing an overwhelmingly large effort by honey bee hives in exploiting row crops for forage.

**Discussion**

Here we have shown that honey bees in an agricultural landscape forage extensively in nearby row crops, especially during the mid-summer full bloom period (Figure 2.5). Across the entire study, foraging for nectar and pollen was generally local, less than 2 km away from the hive throughout the foraging year (April - October) (Figure 2.2). We found some variation per month, with communicated foraging distance increasing (i.e., forage availability decreasing) in April, May, June, and August (Figure 2.3). The month where distance was shortest, indicating that food was most abundant, was observed in July, the only month when all target crops (peanuts, soybeans, corn, cotton) were in full bloom. Our study suggests that row crops provide abundant forage for honey bees during the mid-summer, a period that has previously presented as a time of forage dearth in other landscapes (Couvillon et al. 2014a, Couvillon et al. 2014c).

Other studies have investigated honey bee foraging dynamics in row crops, with the majority focusing on the pollination services bees provide to crops (McGregor et al. 1955, Pires et al. 2014, Blettler et al. 2018, Erickson 1975). Row crops are wind or self-pollinated and do not depend upon insects for successful pollination; however, some still have showy flowers or extrafloral nectaries that attract pollinators and provide forage. Our study demonstrates that, when placed in a row crop environment, honey bees will forage extensively in row crops, primarily during the periods of full and, sometimes, post-bloom (Table 2). Percent visitation during full
bloom was especially high in cotton and soybean fields—honey bee hives used cotton (30%) and soybeans (16%) as major forage sources. Interestingly, there was an additional increase from bloom to post-bloom in 2018. This is likely due to the indeterminate nature of cotton plant blooming (i.e., blooms are often available at some level if the climate is favorable and plants have not reached physiological cut-out although the percentage of blooming plants is less than 75%) (Quisenberry and Roark 1976, Ritchie et al. 2007). Alternatively, additional forage may have become available within the fields, such as blooming weeds, because herbicide applications are terminated at row closure.

Previous investigations have used our waggle dance decoding and mapping methods (Balfour and Ratnieks 2017, Bänsch et al. 2020, Garbuzov et al. 2015, Carr-Markell et al. 2020, Sponsler et al. 2017), demonstrating its wide suitability for determining how honey bees use particular landscapes, fields, or flora for food. Overall, how honey bees use a landscape is a complex interaction of colony requirements, season, distance to field/crop of interest, and availability of alternative forage. In the springtime, when abundant alternative forage will be present, honey bees visit oilseed rape fields 0-26%, depending on whether the hives were in an urban or rural location respectively (Garbuzov et al. 2015). In our study location, at least 40% of all foraging in mid-summer was in row crops, especially cotton and soybean. Both cotton and soybeans produce nectar and pollen, and cotton secretes nectar through extrafloral nectaries; however, these are not viewed as high quality food sources (Science and Education Administration 1980).

Our bees largely foraged locally (median foraging distance = 0.70 km), with some long-range recruitment indicating as far as ~8 km. While honey bees can fly several kilometers from the hive for resources, they will not typically do so unless resources near the hive are extremely
scarce (Seeley 1994, Couvillon et al. 2014a) or if a highly rewarding alternative suddenly becomes available (Beekman and Ratnieks 2000). One recent study that investigated distance over time, as indicated by the waggle dance, reported that the maximum foraging distance for bees in Southern England was ~5 km (Couvillon et al. 2014b). Therefore, an 8 km dance should be viewed as an interesting and unusual event. In particular, these long-distance foraging mostly occurred in the months of May and June in 2018 and 2019, which may be due to the abnormally dry conditions in the first quarter of 2018 and in June of 2019.

The distance bees travelled for resources also varied by month and year, a trend observed in other studies and largely driven by season, major blooms, and dominant landscape features (Beekman and Ratnieks 2000, Couvillon et al. 2014a). For our study, the shortest foraging distance, when forage is most available, occurred in July, likely due to surrounding row crops blooming concurrently. Foraging distance was relatively consistent throughout the rest of the foraging season (Figure 2.3). June 2019 represents one exception to this general rule, where our honey bees recruited to a median foraging distance of 1.3 km, representing a 126.7% increase from the previous year’s June. Although we cannot determine the exact reasons, anecdotally we think that drought, and the related loss of two hives, contributed to these distances. Rainfall events during that month were largely seen during the first week, whereas most of the rest of the month (c. 60%) was without rainfall. Drought could represent an additional stressor that impacts available forage or forage quality through a reduction in nectar or pollen available, subsequently impacting overall colony health (Minckley et al. 2013, Le Conte and Navajas 2008). Perhaps because of the challenging conditions, we had two hives die in early July 2019, and although they were quickly replaced with additional observation hives, there was a small gap in our data that overlapped with the bloom of corn (Figure 2.1), which already possesses a relatively shorter full bloom period.
These colony deaths and the few days it took to replace them might also explain why the bees seemed not to use corn as much compared to the previous year (Figure 2.5).

Overall, our honey bees foraged in rows crops of peanut, soybeans, corn, and cotton much more than initially predicted, especially given that the select row crops are not pollinator-dependent, are generally poor nectar sources, were not overly represented in the landscape (Table 1), and can present a cumbersome challenge in pollen transport in the case of cotton (Vansell 1944, Vaissière and Vinson 1994, Jones and McCurry 2012). Although our study is limited to one study site, and its location on a mixed crop research farm concentrated multiple crops within the immediate vicinity, it nevertheless presents the tracking of honey bee foraging through waggle dance decoding that spanned two full foraging years. As such, it represents the most complete effort to date to determine how honey bees eat in a row crop environment and suggest that, in a similar agricultural landscape, hives can be sustained by such crops during the summer. However, several unknown remain: just because honey bees can feed themselves a majority of the time on row crops does not mean that it is the ideal option for their health and well-being. Future studies should determine how honey bees might exploit row crops in landscapes with greater or lesser heterogeneity, especially given that periods of forage dearth have been observed in less heterogeneous landscapes (Dolezal et al. 2019) and, importantly, further research should also investigate whether a diet of row crop forage might also represent an over-exposure to agricultural chemicals. Overall, row crop fields are contributing a surprising majority of mid-summer forage to honey bee hives and suggests that similar agricultural landscapes will provide abundant, mid-summer foraging opportunities for honey bees that may also have implications for pest management.
Tables and Figures

**Figure 2.1.** Approximate bloom times of row crop cultivars in Suffolk, Virginia. Bloom intervals are divided based on the percentage of bloom (i.e., > 75% bloom refers to greater than 75% of fields of interest in bloom). We investigated percent honey bee foraging in fields of interest when > 75% of the crop of a field was actively flowering.
Figure 2.2. Communicated distance travelled by foragers (km) throughout the foraging season in 2018 and 2019, Suffolk, Virginia. Raw data is fitted using LOESS regressions (span = 0.1). Each point is a single decoded dance.
**Figure 2.3.** Median foraging distance travelled (km) by month (n = 3460) in 2018 and 2019, Suffolk, Virginia. Months with different letters are different (p < 0.05) in post hoc comparison. Post hoc results are capitalized for year one and lowercase for year two and results show comparisons between the months in each respective year.
Figure 2.4. Median foraging distance travelled (km) by bloom time per crop in 2018 (n = 2067) and 2019 (n = 1393). Distance travelled during pre-bloom, bloom, and post-bloom are shown based on bloom intervals for each crop (e.g. peanut, soybean, corn, cotton). Bloom signifies full bloom, when > 75% of fields are in bloom. Post hoc results are between the bloom intervals for each crop by year. 2018 post hoc results are capitalized and 2019 results are lowercase. Colors correspond to the crop bloom times from Figure 2.1.
Figure 2.5. Mean percent (95% CI) foraging, centered around the hives, in peanut, soybean, corn, and cotton fields during bloom intervals (pre-bloom, during bloom, post-bloom) in 2018 and 2019. During bloom is the period when > 75% of the fields of interest are in bloom (full bloom). The fields of interest (peanut, soybean, corn, cotton) within the median 95% foraging range (a 1.911 km radius from the hives in 2018, 1.883 km in 2019) are included as black polygons. Decoded dances are overlaid on the maps, and areas in red signify highest probability of forage. Concentric circles are in 0.5 km intervals with the outermost ring signifying the respective median 95% foraging range distance. Dashed lines represent the percent land cover (95% foraging range) in 2018 and 2019.
Table 2.1. Percent land cover type, rounded to the nearest degree, within an area that represents the majority of dances (i.e., a radius of the median 95th% foraging distance from our decoded dances each year). Land-type percentages are from the 2018 and 2019 National Cropland Data layers and therefore are subject to slight yearly variations.

<table>
<thead>
<tr>
<th></th>
<th>2018</th>
<th>2019</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1.911 km radius</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cropland</td>
<td>49.5</td>
<td>47.4</td>
</tr>
<tr>
<td>Peanuts</td>
<td>7.0</td>
<td>5.4</td>
</tr>
<tr>
<td>Soybeans</td>
<td>19.5</td>
<td>11.4</td>
</tr>
<tr>
<td>Corn</td>
<td>12.6</td>
<td>15.9</td>
</tr>
<tr>
<td>Cotton</td>
<td>10.3</td>
<td>14.6</td>
</tr>
<tr>
<td>Other</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>1.883 km radius</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Developed land</td>
<td>3.5</td>
<td>4.3</td>
</tr>
<tr>
<td>Forest</td>
<td>17.5</td>
<td>23.8</td>
</tr>
<tr>
<td>Grassland/pasture</td>
<td>0.1</td>
<td>0.0</td>
</tr>
<tr>
<td>Wetlands</td>
<td>17.3</td>
<td>19.3</td>
</tr>
</tbody>
</table>
Table 2.2. Mean percent (95% CI) foraging in fields of interest by bloom in 2018 and 2019, as determined by waggle dance decoding, mapping, and analysis, within the 95% median foraging distance by year. During bloom is the period when > 75% of the fields of interest are in bloom. Pre- and post-bloom periods comprise percent foraging in fields of interest before and after the period of full bloom. Percent land cover displays the proportional representation of that land type within the wider landscape encompassing 95% of the median foraging distance (circle with radius 1.911 km in 2018, 1.883 km in 2019). All values rounded to nearest percent.

<table>
<thead>
<tr>
<th></th>
<th>Percent land cover (95% foraging distance radius)</th>
<th>Pre-bloom (95% CI)</th>
<th>During (95% CI)</th>
<th>Post-bloom (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanuts</td>
<td>2018</td>
<td>7</td>
<td>4 (3 to 5)</td>
<td>4 (3 to 5)</td>
</tr>
<tr>
<td></td>
<td>2019</td>
<td>5</td>
<td>6 (5 to 7)</td>
<td>8 (6 to 10)</td>
</tr>
<tr>
<td>Soybeans</td>
<td>2018</td>
<td>19</td>
<td>13 (11 to 14)</td>
<td>16 (14 to 19)</td>
</tr>
<tr>
<td></td>
<td>2019</td>
<td>11</td>
<td>8 (6 to 9)</td>
<td>10 (7 to 12)</td>
</tr>
<tr>
<td>Corn</td>
<td>2018</td>
<td>12</td>
<td>10 (9 to 12)</td>
<td>11 (9 to 13)</td>
</tr>
<tr>
<td></td>
<td>2019</td>
<td>16</td>
<td>11 (9 to 12)</td>
<td>9 (7 to 11)</td>
</tr>
<tr>
<td>Cotton</td>
<td>2018</td>
<td>10</td>
<td>15 (13 to 16)</td>
<td>14 (12 to 15)</td>
</tr>
<tr>
<td></td>
<td>2019</td>
<td>14</td>
<td>16 (14 to 18)</td>
<td>30 (27 to 33)</td>
</tr>
</tbody>
</table>
REFERENCES


Klein, Alexandra-Maria, Bernard E. Vaissière, James H. Cane, Ingolf Steffan-Dewenter, Saul A. Cunningham, Claire Kremen, and Teja Tscharntke. 2007. "Importance of pollinators in

Kluser, Stéphane, Peter Neumann, Marie-Pierre Chauzat, and Jeffery S. Pettis. 2010. *Global Honey Bee Colony Disorder and Other Threats to Insect Pollinators.* UNEP.


CHAPTER 3: WEEDS AND WILDFLOWERS IN A ROW CROP ENVIRONMENT PROVIDE POLLEN AND PESTICIDE EXPOSURE TO FORAGING HONEY BEES

Introduction

The United States is comprised of 137 million hectares of cultivated cropland. While there have been declines overall in agricultural land cover within the last century, particularly in the Northeast and Southeast, the Corn Belt contained 25% of US cropland in 2012. The focus of crop production has also shifted to corn and soybean in the past several decades (Bigelow and Borchers 2012). Pollinators provide an estimated $200-250 billion worldwide in ecosystem services (Gallai et al. 2009), with honey bees contributing c. $14-20 billion annually to the US economy (Morse and Calderone 2000). Despite the importance of stable crop production globally and the current surge in demand, the availability of pollinators is declining (Garibaldi et al. 2011, Aizen and Harder 2009). About 41% of insect species are declining globally (Sánchez-Bayo and Wyckhuys 2019, Hallmann et al. 2017, Kosior et al. 2007), with Hymenoptera and Lepidoptera among the most affected pollinators (National Research Council 2007).

Agriculture comprises a significant portion of the US landscape and it is important to understand the interaction of these ecosystems and pollinator populations. Previously it was shown that bees forage in self-pollinating crops and utilize both nectar and pollen in soybean and cotton fields (Blettler et al. 2018, St. Clair et al. 2020, Cunningham 2014, Silliman et al. in review). What remains to be understood are the risks associated with foraging in these systems, in terms of pesticide exposure, and what specific resources are being used (i.e., whether bees are utilizing crops or flowing weeds and nectar or pollen) when honey bees forage in row crop fields.
We monitored honey bee pollen foraging in southeastern Virginia agricultural land, which includes numerous corn, cotton, soybean, and peanut fields for two foraging seasons (Apr-Oct). Honey bee waggle dances from foraging bees returning with and without pollen were analyzed to determine foraging locations and likely resource type (i.e., nectar or pollen). Forager-collected pollen was collected and analyzed for plant species and pesticide residues. Our objectives were: (1) to determine whether honey bee foraging to row crops was driven by food type (nectar or pollen), (2) to identify what flora honey bees forage on in a row crop environment based on forager-collected pollen, and (3) to determine what pesticide residues are brought into the hive relative to bloom time. Our study allowed us to observe what plants honey bees forage on in an agricultural system and what pesticides they are exposed to through foraging for pollen.

Methods

Study organism and experimental set-up

Methods were based on Silliman et al. in review (see chapter 2).

Study location, crops, and bloom times

Methods were based on Silliman et al. in review (see chapter 2).

Data collection - waggle dance filming

Methods were based on Silliman et al. in review (see chapter 2).
Data collection – waggle dance decoding

Methods were based on Silliman et al. in review (see chapter 2). Of the waggle dances decoded, 1265 and 767 were from nectar foragers and 802 and 626 were from pollen foragers in 2018 and 2019 respectively.

Data collection – pollen collection and sorting

We collected forager-gathered pollen twice per week concurrently with the hour of filming. The collection was done by placing a pollen trap, made of pvc pipe and 5-mesh hardware cloth, over the circular entrance to the observation hive. The mesh was large enough to allow the returning foragers to crawl through, but 40-60% of the pollen carried in the bees’ corbiculae was dislodged and would collect at the bottom of the pollen trap or into plastic containers that we placed below the entrance. A returning forager may then go inside the observation hive and perform a waggle dance. It was sometimes possible to see some remaining pollen in the corbiculae, which allowed us to distinguish the dances of pollen versus nectar dancers. However, for the rest of the non-pollen collecting time, filled pollen baskets were easily visible. At the end of the filming for that day, we placed the pollen pellets in tubes labelled with the date and from what hive. We stored the tubes at -20°C and transported them back to the main campus of Virginia Tech for later analysis.

Our goal was to collect two tubes per week for four weeks per month for the entire foraging season for the two years (ideally, 104 tubes would have been collected). There were some days when the foraging bees did not bring back any or very little pollen because the colony was not actively rearing brood. In total, we obtained 72 tubes. There were some instances where it was necessary to pool the pollen collected from the three hives (A, B, C) on a particular date to ensure
a large enough sample for analysis (at least 1 gram) since adjustments had to be made to extraction reagents in the event that the sample weight was too light.

*Data analysis – percent foraging in fields of interest by food type*

Methods were based on Silliman et al. in review (see chapter 2). We then took a subset of the dataset, looking specifically at whether dances were indicating pollen forage or nectar forage (see above). Once the dataset was parsed, we calculated percent foraging for pre-, during, and post-bloom by individual crop.

*Pollen preparation*

We divided the pollen samples (n = 72 tubes) roughly into thirds, ensuring that each third had samples from evenly spaced time periods throughout the foraging season (April – October). Usually, each tube represents the pollen collected on one day from multiple hives. For the purposes of this study, we were interested in investigating what flora bees foraged on in the surrounding landscape by identifying the pollen returned to the hive from foragers and what pesticide residues were present in forager-collected pollen.

*Pollen processing and counting of palynomorphs*

Pollen was identified using microscopy (n=20). Of the twenty samples, seventy-nine individual pollen pellets, divided by date and color, were sent. The processing technique used was developed by Dr. Vaughn M. Bryant at Texas A&M University for use with the study of bee-collected pollen pellets. The processing was conducted by Global Geolab Limited. First, the vials of pollen pellets were thoroughly mixed. Next, 2 grams of pellets was weighed out and placed in a sterile 15 mL screw top centrifuge tube. Glacial acetic acid (GAA) was added to the test tube to
dehydrate the pollen, and then the sample was thoroughly mixed until all pellets were dissolved. Next, the samples were heated in a heating block at 80°C for 5 minutes, stirred regularly and then vortexed again to ensure that all pellets were dissolved properly and were fully mixed. Immediately after being vortexed, a sterile pipette was inserted into the middle of the mixture and about 4-5 mL of liquid was extracted. The extracted liquid was then placed into a new, sterile 15 mL test tube, each tube was filled with glacial acetic acid, and centrifuged at 3,500 rpm for 3 minutes, after which the GAA was poured off. Eight to nine mL of acetolysis was added and then the mixture was heated at 80°C for about 8 minutes and stirred regularly. The acetolysis chemical treatment (a mixture of sulfuric acid and acetic anhydride) is designed to remove lipids, waxes, and cytoplasm to allow easier identification of the pollen grains. The samples were then removed, centrifuged, and the acetolysis was decanted. The samples were then washed three times in distilled H₂O. This batch of samples was not stained with Safrarin-O. The sample was then rinsed in ETOH, centrifuged, placed into 2 mL vials, and centrifuged again. The ETOH was poured off, 10-12 drops of glycerine were added, and the samples were vortexed to mix the pollen with the glycerine.

The tabulation of samples was done by Dr. Sophie Warny, a palynologist in the CENEX laboratory at LSU. The vial content was stirred thoroughly for one minute each, a small drop of the well mixed sample was collected and placed on a slide, then diluted with glycerine. The drop was mounted on a 75x25 mm microscope slide and covered with an 18 x18 mm #1-thickness glass coverslip, all new and pre-washed to avoid contamination. The cover slips were sealed with clear nail polish to prevent leakage. Once dried, the samples were examined at 600x and 1000x magnification with an Olympus BX41 to identify the pollen types present. To establish statistically valid, relative abundance for each taxon, a minimum of 300 pollen grains was counted.
for each of the samples using traverses that prevented any duplication of counts (same pollen being counted twice). For the rare samples that were poor, the entirety of the residues was tabulated. In making quantitative counts, each pollen type was identified to the family, genus, or in some cases species level when possible.

**Pesticide residue analysis**

In order to analyze pesticide residues in our pollen samples, a subset of pollen samples were sent to the McArt lab at Cornell University (n=19) or to the United States Department of Agriculture, National Science Laboratories, Gastonia, NC (n=11). Using known standards, the McArt lab and USDA labs used liquid chromatography-mass spectrometry and gas chromatography-mass spectrometry to detect residues, 266 and 193 compounds respectively. Both methods were used since some compounds can only be detected with a specific methodology.

**Data management and validation**

Methods were based on Silliman et al. in review (see chapter 2).

**Results**

**Visitation to cotton and soybean fields is nectar driven**

Visitation to cotton and soybean fields during full bloom (> 75% of fields of interest in bloom) was largely driven by nectar foraging (Figure 3.1). For example, in 2018, overall visitation (pollen and nectar foraging) to soybean fields was 16% (14 to 19, 95% CI) during full bloom, with 15% (12 to 18, 95% CI) of pollen dances indicating visitation to soybean fields and 16% (12 to 20, 95% CI) of nectar dances indicating visitation to soybean fields. Overall visitation to cotton fields in 2019 was 30% (27 to 33, 95% CI) during full bloom, with 23% (20 to 28, 95% CI) of
pollen dances indicating visitation to cotton fields and 38% (32 to 43, 95% CI) of nectar dances indicating visitation to cotton fields (Table 3.1). There was a dramatic increase in visitation to cotton fields during full bloom in 2019.

**Honey bees collected soybean pollen**

*Glycine* spp. (soybean) was the only row crop identified in forager-collected palynology samples. Soybean pollen was detected in samples between late July to mid August during the period of full bloom. Within the remaining pollen, other sources of pollen collected included *Lagerstroemia indica* (crepe myrtle) and *Portulaca* spp. (purslane) from mid July to early September and mid July to early October, respectively.

**Honey bees were exposed to pesticide residues throughout the foraging season**

Thirty-five different pesticides were detected in pollen samples collected from returning honey bee foragers over the foraging season, with all samples containing at least one pesticide (Figure 3.2). In total there were eight herbicides, nine insecticides, seventeen fungicides, and one synergist. By parts per billion, alachlor and metolachlor were the most abundant herbicides. Chlorantraniliprole and acephate were the most abundant insecticides, while chlorothalonil and fludioxonil were the most abundant fungicides. Piperonyl butoxide was the only synergist detected.

**Discussion**

Here we have shown that honey bees in an agricultural landscape mostly forage for row crops to obtain nectar and are exposed to a variety of pesticides when pollen is collected in this environment. The most striking examples of this were seen in soybean in 2018 and in cotton in 2019, where nectar foraging visitation in the fields increased 25 and 153% in the move from pre
to full bloom. Through waggle dance filming and decoding, we were able to indicate whether bees were pollen or nectar foragers based on whether a pollen pellet or residue was visible on a dancer’s corbiculae. However, there may have been instances when residue was missed, either due to decoder error or poor video quality. This may have contributed to some dances for corn fields being labelled as nectar forage, which is problematic since corn does not produce nectar.

Soybean pollen was the only row crop pollen present in our samples demonstrating that, as a self-pollinating crop, soybean potentially provides an important pollen resource. The lack of other row crop pollen may be due to difficulties in packing and transporting certain pollen types because of their structure (Vaissière and Vinson 1994, Konzmann et al. 2019). In previous work gathering forager-collected pollen in an agricultural landscape from late April to early May, mass-flowering woody plant species were the most prevalent (Richardson et al. 2015). While we collected pollen later in the remaining season, we also found multiple pollens from trees, including *Lagerstroemia indica* (crepe myrtle).

Pesticide residues were detected in pollen samples through the foraging season. Previous analyses of forager-collected pollen have yielded similar results, with 62% of pollen samples, collected from foragers at hives managed under organic production protocols, contained at least one screened pesticide (Tosi et al. 2018). In contrast, 100% of our pollen samples, brought back to the hive by foraging honey bees, had at least one pesticide. Clorpyrifos, an organophosphate, was present in samples and is one of the most prevalent compounds detected in honey bee hives throughout the US (Mullin et al. 2010). Chlorothalonil, a broad-spectrum fungicide, had the highest parts per billion count out of all detected pesticides in any pollen sample. Although a fungicide, chronic exposure, in combination with other pesticides, has been shown to increase
mortality in bee larvae (Zhu et al. 2014). Fungicides may also increase the risk of *Nosema* prevalence among honey bees (Pettis et al. 2013).

Overall, our honey bees foraged for nectar when visiting row crops and visited other flowering plants for pollen forage. Through pollen forage we found that bees were exposed to thirty-five different pesticides; however, it is unclear what specific plants the bees foraged upon to be exposed to pesticides. Future studies should investigate whether the availability of forage in row crops is beneficial despite the potential exposure to pesticides.
Tables and Figures

**Figure 3.1.** Mean percent (95% CI) foraging, centered around the hives, in peanut, soybean, corn, and cotton fields during bloom intervals (pre-bloom, during bloom, post-bloom) and by forage type (peanut and nectar, peanut, nectar) in 2018 and 2019. During bloom is the period when > 75% of the fields of interest are in bloom (full bloom). Dashed lines represent the percent land cover (95% foraging range) in 2018 and 2019.
**Figure 3.2.** Contaminants detected (in ppb) in samples from forager-collected pollen throughout the foraging season of 2018 and 2019 (April – October). Pesticides are grouped by pesticide type. Samples with an asterisk indicate samples analyzed by the USDA.
Table 3.1. Mean percent (95% CI) foraging in fields of interest by bloom and forage type (pollen or nectar) in 2018 and 2019, as determined by waggle dance decoding, mapping, and analysis, within the 95% median foraging distance by year. During bloom is the period when > 75% of the fields of interest are in bloom. Pre- and post-bloom periods comprise percent foraging in fields of interest before and after the period of full bloom. All values rounded to nearest percent.

<table>
<thead>
<tr>
<th>Field</th>
<th>2018</th>
<th>2019</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-bloom</td>
<td>During</td>
</tr>
<tr>
<td></td>
<td>(95% CI)</td>
<td>(95% CI)</td>
</tr>
<tr>
<td>Peanuts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2018</td>
<td>Pollen</td>
<td>3 (2 to 4)</td>
</tr>
<tr>
<td></td>
<td>Nectar</td>
<td>4 (3 to 5)</td>
</tr>
<tr>
<td>2019</td>
<td>Pollen</td>
<td>5 (4 to 7)</td>
</tr>
<tr>
<td></td>
<td>Nectar</td>
<td>7 (5 to 9)</td>
</tr>
<tr>
<td>Soybeans</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2018</td>
<td>Pollen</td>
<td>16 (13 to 19)</td>
</tr>
<tr>
<td></td>
<td>Nectar</td>
<td>12 (10 to 13)</td>
</tr>
<tr>
<td>2019</td>
<td>Pollen</td>
<td>7 (5 to 10)</td>
</tr>
<tr>
<td></td>
<td>Nectar</td>
<td>7 (5 to 9)</td>
</tr>
<tr>
<td>Corn</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2018</td>
<td>Pollen</td>
<td>12 (9 to 15)</td>
</tr>
<tr>
<td></td>
<td>Nectar</td>
<td>9 (8 to 11)</td>
</tr>
<tr>
<td>2019</td>
<td>Pollen</td>
<td>10 (7 to 12)</td>
</tr>
<tr>
<td></td>
<td>Nectar</td>
<td>11 (9 to 13)</td>
</tr>
<tr>
<td>Cotton</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2018</td>
<td>Pollen</td>
<td>14 (11 to 16)</td>
</tr>
<tr>
<td></td>
<td>Nectar</td>
<td>16 (14 to 17)</td>
</tr>
<tr>
<td>2019</td>
<td>Pollen</td>
<td>17 (14 to 20)</td>
</tr>
<tr>
<td></td>
<td>Nectar</td>
<td>15 (13 to 17)</td>
</tr>
</tbody>
</table>
REFERENCES


Richardson, Rodney T., Chia-Hua Lin, Douglas B. Sponsler, Juan O. Quijja, Karen Goodell, and Reed M. Johnson. 2015. "Application of ITS2 metabarcoding to determine the provenance of pollen collected by honey bees in an agroecosystem." Applications in Plant Sciences 3 (1): 1400066.


Seeley, Thomas D., Alexander S. Mikheyev, and Gary J. Pagano. 2000. "Dancing bees tune both
duration and rate of waggle-run production in relation to nectar-source profitability."
Journal of Comparative Physiology A 186: 813-819.

Silliman, Mary R., Sean Malone, Roger Schürch, Sally Taylor, Margaret J. Couvillon, and Roger
Schürch. in review. "Row crop fields provide a mid-summer, all-you-can-eat buffet for
foraging honey bees." Agriculture, Ecosystems and Environment.

Sponsler, Douglas B., Emma G. Matcham, Chia-Hua Lin, Jessie L. Lanterman, and Reed M.

St. Clair, Ashley L., Adam G. Dolezal, Matthew E. O'Neal, and Amy L. Toth. 2020. "Pan Traps

Steffan-Dewenter, Ingolf, and Arno Kuhn. 2003. "Honeybee foraging in differentially structured

Tosi, Simone, Cecilia Costa, Umberto Vesco, Giancarlo Quaglia, and Giovanni Guido. 2018. "A
3-year survey of Italian honey bee-collected pollen reveals widespread contamination by

Published crop-specific data layer [Online]. Available at


CHAPTER 4: FINAL DISCUSSION

Assessing project implications

Prior studies have examined how honey bees benefit row crop yields and or measured estimated pesticides exposure resulting from foraging; however, few studies include a comprehensive analysis of how crops benefit – or harm – honey bees. Our study determined that row crops served as a significant food resource during the mid-summer, with bees most prevalent in cotton and soybean. Though minimal row crop pollen was collected, apart from soybean, honey bees do visit row crops fields for nectar, especially cotton. The highest cotton nectar foraging was found in 2019 during a prolonged period of low to no rainfall. Perhaps one reason why cotton might continue to provide an important nectar source for foraging honey bees during dry conditions is that cotton, compared to other row crops, is more drought tolerant.

In some previous studies, summertime represented a period of foraging dearth (Couvillon et al. 2014, Garbuzov et al. 2015), which was in contrast to our results, suggesting that a row crops could provide an important food reservoir for bees when other sources are less available. However, what is not known is the health impact of such heavy food use of just one crop. Previous work suggests that diverse floral resources are essential for brood development, consistent honey yield, and reducing the risk of mortality via viral pathogens (deGroot et al. 2021, Brodschneider and Crailsheim 2010, Dolezal et al. 2019).

Bees were also exposed to a wide variety of compounds through pollen foraging. Based on these results, more research is needed to assess whether the combination of waggle dance decoding and analysis and pesticide residue analysis from forager-collected pollen can be used to better understand how and from what plants bees are likely to be exposed to pesticides and whether,
ultimately, a row crop environment serves as a suitable habitat for honey bees. Lastly, all pollen samples had at least one pesticide detected. Future studies should conduct similar research in environments with less landscape heterogeneity and varying levels of pesticide use to better understand if row crop environments are suitable habitats for honey bees.
Tables and Figures

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


