ASSESSING HONEY BEE HIVE STOCKING DENSITY AND COLONY STRENGTH FOR NORTHERN HIGHBUSH BLUEBERRY POLLINATION

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ABSTRACT

Pollination of commercial northern highbush blueberry fields is often achieved through rented colonies of the managed honey bee, *Apis mellifera.* Recommendations for blueberry pollination emphasize hive stocking density, or number of hives per acre, for growers to meet their pollination needs. However, the strength of these colonies may also contribute to pollination success. Measuring *Apis mellifera* colony strength is expected to provide growers, who depend on strong colonies for sufficient crop pollination, a more accurate estimate of the number of foragers available for pollination services. In this thesis, I report a study to investigate the influence of honey bee colony number, size, and activity on honey bee density in the field and pollination success in commercial highbush blueberry systems of the cultivar 'Bluecrop'. During 2021 and 2022, honey bee density in blueberry fields was not influenced by stocking density, but there was a positive relationship between farm-level colony strength and honey bee density in the field. Honey bee density in the field was a significant predictor of the seed number per berry and the estimated partial yield. I also compared the standard cluster count method for colony size estimation to two non-invasive methods: counting the number of foragers returning to hives and measuring the thermal signature of the same colonies using a hand-held infrared camera. Returning forager counts at colony entrances were positively correlated with colony cluster counts in both years. There was no relationship between infrared sampling and the other methods in 2021, whereas a positive correlation was found in 2022. My results indicate that colony strength, which can be effectively measured using non-invasive methods, is an important indicator of farm-level honey bee density, which can be used to predict pollination success in blueberry farms.

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Assessing honey bee hive stocking density and colony strength for northern highbush blueberry pollination

Introduction

Pollination is the process of pollen grain transfer from the anther of a flower to the stigma of the same species, resulting in fertilization of the ovaries leading to production of seeds. Complete fertilization leads to full development of grain, nut, vegetable, and fruit crops. Many staple food crops including rice, wheat, corn, and other cereal grains, are pollinated after the dispersal of pollen by gravity or wind. However, most of the most prominent food crops grown directly for human consumption and which provide essential micronutrients (Eilers et al. 2011) are reliant on animals for movement of pollen to achieve pollination. This includes a variety of vegetables, fruits, and nuts, with 87 of 124 main sources of human nutrition reliant upon or improved by animal pollinators (Klein et al. 2007). The demand for pollination continues to increase as the fraction of agriculture dedicated to production of high-value pollinator-dependent crops grows (Aizen & Harder 2009). In the U.S, production of crops that are directly dependent on pollinators was valued at over $15 billion annually (Calderone 2012). This farm gate valuation likely under-represents the full value given the processing of many of these crops into value-added products and their importance for supporting rural economies.

Bees as pollinators

Several vertebrate species, including small mammals and birds, can act as animal pollinators (Ratto et al. 2018); but insects, especially bees, are recognized as some of the most important

pollinators of pollination-dependent crops (Free 1970). The relationship between bees and flowers is mutualistic: as a reward for facilitating floral fertilization, bees receive pollen and nectar from the flowers they visit. The co-evolution of bees and flowers has led some bee species to become highly specialized pollinators of certain plants (Johnson & Steiner 2000).

Wild bees are often the most efficient pollinators of many crop species (Garibaldi et al. 2013, Eeraerts et al. 2019). However, due to various factors including land-use intensification and pesticide exposure, wild bees are often at low abundance in agricultural landscapes dominated by crop production (Isaacs & Kirk 2010) and therefore cannot meet the demand for crop pollination in these commercial settings. To ensure adequate pollination, growers can employ several different managed bee species during crop bloom. Most commonly, managed honey bees are rented from beekeepers to maintain pollination at levels needed for economic productivity (Hung et al. 2018, Hristov et al. 2020). Honey bees are estimated to contribute $11.7 billion to U.S. agriculture through the increase in yield and quality of the crop species where they are used for pollination (Calderone 2012).

Honey bees and pollination

History and native range

The western honey bee, *Apis mellifera,* is a eusocial hymenopteran insect in the family Apidae. *Apis mellifera* is native to Europe, the Middle East, and Africa, but European subspecies have now been naturalized on all continents excluding Antarctica. Documentation from over 10,000 years ago provides evidence of human exploitation of this species for honey production (Crane 1984). In the 17th century, *A. mellifera* was brought by European settlers to North America to provide wax and honey (Crane 1984). In the 19th century, domestication of honey bees was

revolutionized by the discovery of “bee space” by Lorenzo Langstroth in 1851, leading to the modern method of beekeeping that is still in use today.

Biology of honey bees

The western honey bee is cavity-nesting, forming colonies in hollow trees, natural crevices, or hives provided by humans. The members of the honey bee colony can be split up into three castes: the fertile female queen who is mainly responsible for reproduction and egg-laying, the male drones who fertilize queens, and the infertile female workers. The worker caste is of most interest to humans, as it is responsible for pollen and nectar foraging, and subsequent honey production and pollination services. The life cycle of the honey bee colony involves foraging and colony growth in the summer and fall, survival on food stores in the winter, and colony reproduction in the spring.

Role as pollinators

Honey bees were initially domesticated for their wax and honey-producing abilities but have since proven their added value to humans through their role as crop pollinators. Honey bees are generalist foragers, meaning they collect pollen and nectar from a wide variety of flowering plants, including many crops used for human consumption. However, they exhibit floral constancy, a behavior in which individual foraging bees collect pollen and nectar from one floral species during a single foraging trip. As a honey bee forages for nectar and pollen, it quickly moves from flower to flower, accidentally transferring pollen from its body onto the receiving flower, facilitating pollen transfer and subsequent fertilization. A single honey bee colony provides tens of thousands of foragers available for pollination services. Thus, growers of

pollination-dependent crops rely on managed honey bee colonies to help meet their pollination demands (Mayer & Delaplane 2000; Garibaldi et al. 2017).

Colony stocking density

When deciding on how to pollinate their crop, growers select a stocking rate, or number of honey bee colonies per acre, to ensure sufficient pollination (Mayer & Delaplane 2000, Rollin & Garibaldi 2019). Optimal stocking densities vary depending on the crop and location, but generally, higher stocking densities are associated with greater crop yields. Through the meta-analysis of 22 studies across 16 crops, Rollin & Garibaldi (2019) found a positive linear relationship between fruit set and honey bee colony density.

However, because there is a cost to renting colonies, there may be limits to the benefits of increasing colony stocking density, since the return on investment should decline after a certain density is reached, and the most profitable density may not be the same as the one causing highest yields. There is also a potential for disease transmission among colonies. For example, in a study focused on apiary configuration, Dynes et al. (2019) found that colonies placed in a high-density, visually similar configuration spaced 1 m apart in a single line had higher parasite loads and lower survival rates than colonies in a low-density, visually complex pattern arranged as a circle with colonies spaced 10 m apart. Therefore, appropriate, crop-specific stocking density recommendations are needed to reduce the potential negative consequences of overstocking and maximize the benefits of honey bee pollination for crop yields.

Colony strength

Honey bee colony strength refers to the overall population size of a colony. A strong colony has many workers, particularly of foraging age, and enough brood to sustain colony growth and productivity (Nasr et al. 1990, Delaplane et al. 2013). There is considerable variability in forager populations between colonies of different sizes (Farrar 1937, Beekman et al. 2004), ranging from 10,000 to 60,000 bees per colony. In addition, foragers from larger colonies have been observed making longer foraging trips and collecting larger nectar loads than those of smaller colonies (Eckert et al. 1994). Consequently, large colonies may provide more pollination than small colonies at similar stocking rates (Geslin et al. 2017, Grant et al. 2021). In the second chapter of this thesis, I explore how a combination of colony stocking density and strength, deemed "stocking strength," contributes to honey bee abundance at the field-level in blueberry farms and the provision of pollination services.

Highbush blueberry pollination

History and value

Highbush blueberry, *Vaccinium corymbosum* L., is a species of blueberry native to North America. Cultivation of highbush blueberry for commercial crop production began in the early 1900's (Eck & Childers 1966), and throughout the 20th century advances in breeding and cultivation have led to improved yields (Moore 1965, Edger et al. 2022). Today, the cultivation of highbush blueberries has expanded across the globe, with the United States, Canada, Chile, and Peru leading in commercial production (Protzman 2021). In the U.S, Michigan is one of the top producers of northern highbush blueberries, producing over 100 million pounds each year in

good production years, but only 60-70 million pounds in years with production challenges, including from poor pollination (USDA NASS, 2022).

The highbush blueberry industry is a major contributor to the U.S. economy, generating an estimated $4.7 billion annually nationwide, and $530 million in Michigan alone (U.S. Highbush Blueberry Council, 2020). Less straightforward to quantify but impossible to overstate is the nutritional value of blueberries. Blueberries are rich in antioxidants, which provide anti-inflammatory benefits and promote good health in humans (Kalt et al. 2020). As a result, highbush blueberries are a popular food choice globally, and their demand continues to increase (Protzman 2021).

Flowering and pollination

Northern highbush blueberry bloom takes place from early May through mid-June in Michigan. Within each cultivar, flowers will expand, open, and reach petal fall over the course of 2-3 weeks. After opening, individual blueberry flowers are viable and receptive to pollen for up to four days, depending on temperature (Kirk & Isaacs 2012; Ch abert [unpublished]). During this time, highbush blueberry flowers require deposition of 50-125 compatible pollen tetrads to reach full fruit set and berry weight (Dogterom et al. 2000).

Certain cultivars of highbush blueberry exhibit parthenocarpy, or the production of fruit without fertilization. In many crops, the purpose of breeding for this trait is to reduce pollinator dependence (Ehlenfeldt 2007, Allsopp et al. 2008), but in highbush blueberry, pollinator visitation during bloom is required for high levels of fruit set and for the production of berries of marketable size (McGregor 1976, MacKenzie 1997). To run a profitable business, it is crucial for

highbush blueberry growers to invest in pollination to ensure high yields, considering the high input costs of production (Safley 2006).

Common pollinators

Over 100 species of wild bees have been found in Michigan highbush blueberry farms during bloom (Tuell et al. 2009). In small, non-commercial highbush blueberry fields with diverse landscapes, wild bees are the dominant pollinators (Isaacs and Kirk 2010). Some species, such as *Andrena carolina*, are specialized pollinators of blueberry (LaBerge 1980). The common eastern bumble bee, *Bombus impatiens,* is a native pollinator in North America, and thanks to domestication and commercial rearing (Velthuis & Van Doorn 2006), is also commonly used as a managed pollinator of highbush blueberry in Michigan. Additionally, *Osmia* bees have some potential for management as blueberry pollinators, but their domestication is still in its infancy (Stubbs et al. 1997, Kraemer et al. 2014).

Some bee species, including honey bees, may have difficulty accessing the anthers and collecting pollen and nectar from the narrow, bell-shaped corolla of the blueberry flower (De Luca 2013, Russell et al. 2017, Cooley & Vallejo-Marín 2021). However, bumble bees and several other wild bees are excellent pollinators of highbush blueberry due to a special foraging technique known as “buzz pollination” (Buchman 1983). In this behavior, a bee holds on to the corolla of a flower (Cardinal 2018) and emits vibrations of a certain frequency (King 1993), causing the expulsion of pollen from the flower onto the bee. The presence or absence of this co-evolved interaction makes different pollinators highly variable in their pollination efficiency of blueberry flowers (Javorek et al. 2002).

The importance of honey bees for commercial highbush blueberry pollination is well documented (DeVetter et al. 2022), but their efficiency as pollinators of the crop is debated. Research in highbush blueberry cv. 'Bluecrop' has been extensive because this is a widely planted cultivar. Studies of this cultivar in British Columbia showed that nectar foraging honey bees are more common floral visitors than pollen foragers (Dogertom & Winston 1999), indicating that nectar availability is an important component to attract honey bees to a plant. A recent study in Poland in the same cultivar found that nectar mass peaks between the sixth and ninth day of flower opening (Bozek 2021), which does not coincide with the maturity of anthers during the first four days of flower opening. This potential suspension of floral attractiveness to honey bees during the critical pollination period, as well as the honey bee's inability to buzz pollinate, may make them less efficient pollinators of highbush blueberry per bee than other bee species.

Despite a lack of overlap in their phenology and poorly matched morphological traits, several studies have shown that honey bees can successfully pollinate highbush blueberry. For example, a study conducted by Benjamin & Winfree (2014) on 'Duke' and 'Bluecrop' in New Jersey found that honey bees deposit pollen on a per-visit basis as effectively as native bees, regardless of whether they are collecting nectar or pollen. This finding was confirmed by Hoffman et al. (2018) who discovered that even unintentional contact between the head or legs of a honey bee and a blueberry flower's stigma can transfer a significant proportion of the total pollen tetrads needed for fertilization. These findings, in addition to the manageability and transportability of honey bees, have made them the primary pollinator of highbush blueberry.

Honey bee colony strength assessment

Growers and commercial beekeepers often engage in pollination agreements or contracts to guide the delivery of honey bee colonies to farms during crop bloom (Goodrich 2017). In these contracts, beekeepers agree to rent out their hives to be placed on the land of the grower during crop bloom. In turn, growers pay the beekeepers a fee for their pollination services. These contracts specify terms of agreement, such as the number of hives to be provided, the timing of placement, and a minimum standard for colony size based on a certain number of frames covered with bees. In the almond crop system, a third-party broker will often be hired to open randomly selected hives and verify their strength to the grower.

A measurement of colony size provides growers with assurance that the product they have paid for is at the expected hive strength (Goodrich and Goodhue 2020) and an estimation of foragers available for pollination services (Harbo 1986). In addition, there is potential for beekeepers to maximize rental revenues through providing strong colonies. For example, in California almond production, beekeepers are paid a premium for colonies of a certain strength (Goodrich 2019). Thus, there has been increasing emphasis placed on the provision of strong honey bee colonies during crop bloom.

Estimating colony cluster size

The cluster count is a method of colony strength assessment first described by Nasr et al. (1990). It involves opening the colony, then each box is viewed from above and below, and, without removing frames, the observer identifies the tops and bottoms of frames which are completely or partially covered in bees, estimated to the nearest half frame. This method was developed to reduce disturbance to the colony and the amount of time needed to perform evaluations. Previous

assessments were far more intensive, requiring the removal of individual frames with the colony open for 10 – 20 minutes at a time (Nasr et al. 1990). Although the cluster count method can be done with a high level of accuracy between experienced and naïve observers (Chabert et al. 2020), there is still potential for human estimation error and bias. This method is only effective if done at temperatures below 15º C, or else clusters will disperse (Nasr et al. 1990). In addition, hives should not opened without the explicit permission or assistance of their beekeeper, making this method impossible for growers who want to evaluate colony strength on their own.

Since its conception, the cluster count method has remained one of the most common methods of colony strength assessment for commercial crop pollination (Delaplane & Mayer 2000). It has been more than 30 years since its development, and technological advancements in other areas of agriculture, such as the use of remote sensing technology (Poblete-Echeverría & Fuentes 2020), indicate the potential for development and validation of novel apicultural practices, including innovative methods of colony strength assessment. Several alternative methods to the cluster count have been proposed for rapid, non-invasive assessment of colonies contracted for pollination services including the returning forager count method and infra-red image analysis.

The returning forager count method

In a 2011 extension document, Sagili and Burgett suggested that growers count the number of foragers returning to the hive to gauge colony strength. This method involves standing a few feet away from the hive, focusing on the entrance, and counting the number of bees that enter the hive over a one-minute period. Based off preliminary data, they suggested that a count of 100 incoming bees per minute is a sufficiently sized colony for pollination. However, this reference

number was for a given temperature range, which is not sufficiently high enough for the general blueberry bloom period and thus poses a limitation on its use. This method was recommended as a way for growers to estimate pollinator activity on their farms without the needed apicultural experience to evaluate rented hives through opening them.

Ten years later, this method was validated by Grant et al. (2021), who found that counts of returning foragers were strongly correlated with adult bee population in the hive. They also observed that counts of returning foragers were related to variation in yields of highbush blueberry. Results of their study were the first to indicate that growers may be able to predict yield outcomes by measuring colony strength at the hive entrance.

Infrared image analysis

The use of infrared (IR) cameras for honey bee colony research is decades old. For example, infrared imaging has been used to investigate brood nest incubation by worker bees (Bujok et al. 2002), to measure thermogenesis and insulation by bees in the winter (Stabentheiner et al. 2003), and to model thermoregulation mechanisms and heat distribution throughout the colony (Eskov et al. 2009). Since these earlier studies, the use of infrared imaging has also been suggested as a rapid, non-invasive method of colony size estimation.

Shaw et al. (2011) reported the first application of infrared imaging to assess honey bee colony population size without opening the hive, demonstrating a strong positive linear relationship between thermal radiance data and cluster count. This technique uses an IR camera to capture a thermal heat signature from the hive, which can be used to estimate the bee population inside the hive. Fernandez et al. (2018) developed another non-invasive approach to

quantify honey bee colony population using thermal imaging, constructing 4D models which integrated metric information from 2D images and thermographic data from infrared images.

The ability to measure colony strength rapidly and accurately can help to maximize pollination efficiency by allowing the identification of sub-standard hives for remedial management. Additionally, this can support the provision of only strong hives for pollination services. In the third chapter of this thesis, I will compare the cluster count method for colony size assessment to the two non-invasive methods previously described, to determine their usefulness for commercial crop production settings.

Thesis objectives

In the context of the preceding information on crop pollination, the importance of pollination for blueberry yield, and honey bee colony assessment, the goals of the research presented in this thesis were 1) investigate the influence of honey bee colony density and strength on pollination services, and 2) assess alternative methods of performing honey bee colony strength assessments.

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CHAPTER 2:

The importance of honey bee hive stocking density and colony strength for pollination of northern highbush blueberry

Introduction

Insects are responsible for pollination of approximately 82% of all flowering plants (Ollerton et al. 2011, Rodger et al. 2021) and over 70% of global food crops (Klein et al. 2007, Gallai et al. 2009). Wild insects are often the most efficient pollinators of many crop species (Garibaldi et al. 2013, Eeraerts et al. 2019) but they are typically at low abundance in intensively managed agricultural landscapes (Isaacs & Kirk 2010) and are therefore inadequate for large commercial crop production settings. To achieve pollination at levels needed for economic production of pollinator-dependent crops, honey bees (*Apis mellifera*) are the primary managed pollinator brought to farms during crop bloom (Hung et al. 2018, Hristov et al. 2020). By contracting with beekeepers, growers can control the placement and density of colonies on their farm thereby, affecting the density of bees available for pollination during crop bloom.

Highbush blueberry requires insect-mediated pollination for high levels of fruit set and to produce berries of marketable size (McGregor 1976, MacKenzie 1997). The importance of honey bees for commercial highbush blueberry (*Vaccinium corymbosum* L.) production is well documented (DeVetter et al. 2022). For example, Isaacs & Kirk (2010) found that honey bees are responsible for approximately 88.2% of the yield increases from pollination in Michigan northern highbush blueberry farms, and Gibbs et al. (2016) found that honey bee abundance on bushes was the primary factor affecting fruit set, berry weight, and seed set of northern highbush blueberry in Michigan and British Columbia.

Bloom occurs over 2-3 weeks for each cultivar of northern highbush blueberry, so in production systems containing several cultivars with staggered bloom timings, the total bloom period is 4-6 weeks. However, this bloom period is subject to variation due to weather (Tuell & Isaacs 2010). After opening, a blueberry flower is viable and receptive to pollen for up to four days depending on the temperature (Kirk & Isaacs 2012; Chabert [unpublished]). Pollen deposition on stigmatic surfaces leads to pollination and fertilization, with 50-125 pollen tetrads required to reach maximum fruit set and berry size in 'Bluecrop' (Dogterom et al. 2000). Thus, to maximize potential yield, it is critical that sufficient pollinators are present during bloom while flowers are open and receptive to pollen (Kirk 2013). Wild bees provide some of this service to blueberry production (Reilly et al. 2020), but to ensure full pollination of their crop, most commercial highbush blueberry growers stock their fields with honey bee colonies.

The stocking rate of honey bee colonies (number of colonies per unit area) is selected by growers to meet their specific pollination demands (Mayer & Delaplane 2000, Rollin & Garibaldi 2019), and this may vary by cultivar and crop maturity. It is typical for growers to stock their fields at 1-4 colonies per acre. Several studies have documented the relationships between highbush blueberry pollination components and honey bee colony stocking density. In Washington fields of ‘Duke’ blueberry, Arrington & DeVetter (2018) found higher honey bee visitation, berry weight, and seed number per berry when honey bee colony densities were increased from 10 to 20 per hectare, but fruit set was unaffected. However, in a Florida study in southern highbush blueberry fields across multiple cultivars, Mallinger et al. (2021) found that honey bee floral visitation was associated with higher yield estimates but was not predicted by stocking density. Thus, increased colony density was not directly associated increased yield estimates.

Recently there has been more attention paid to the size of honey bee colonies and the role of this parameter in blueberry crop pollination. In Oregon, Grant et al. (2021) found that highbush blueberry yields increased with average honey bee colony strength. Due to considerable variability in forager populations between colonies of different sizes (Farrar 1937, Beekman et al. 2004), large colonies are expected to provide more pollination at similar stocking rates (Geslin et al. 2017, Grant et al. 2021). Thus, standards for colony strength during pollination typically include a statement on minimum size, usually expressed as frames of bees per hive (Goodrich & Goodhue 2020). Given the importance of both stocking density and colony strength for blueberry pollination in the previous studies, one might predict that both parameters contribute to the number of honey bees available for pollination services, as well as the pollination and yield outcomes. To test this hypothesis, measurements of honey bee visitation, pollination, and yield are needed to test whether stocking density, colony strength, or a combination of both, which I will call "stocking strength," are most predictive of pollination outcomes. These measurements were used to: 1) determine if stocking density, colony strength, or stocking strength contribute to honey bee density and floral visitation during blueberry bloom, and 2) determine which aspects of honey bee abundance and activity best predict blueberry pollination.

Methods

Study system

This study was conducted at six highbush blueberry farms across West Michigan, in fields of cv. 'Bluecrop' that were at least 5 acres. In each year, a pair of fields was selected at each of six farms, with the two fields at each farm managed in a similar way. Two fields used in

2021 were replaced by similar fields managed by the same grower in 2022. Sampling locations within each pair of selected fields were separated by ≥ 2 km, except for in two instances where the distance was ≥ 1.25 km. All fields were at least six years old.

Within each pair of fields, one farm was stocked with honey bee colonies at the density typically used by the grower, and the other was stocked with an additional 2 – 3.5 colonies per acre to increase honey bee abundance at that field to create a high stocking density (Figure 2.1). To control for potential effects of colony spatial configuration, all colonies were placed in clumps at each field. The same beekeeper was used to supply colonies to each field within a pair. A total of five beekeepers were used in the study, as the same beekeeper supplied colonies to two of the pairs. Treatments were randomly assigned to fields within a pair to control for variables such as farm size and surrounding landscape. In 2022, the number of colonies at each field was carefully recorded within a 1 km radius of each focal field. This was done by driving around the host farm and all fields within 1 km, marking the location of colonies on a map, along with the number of colonies, and the number of boxes on each hive. This was done to compare the grower-reported stocking density to the actual number of colonies that was ultimately placed in the field.

Figure 2.1. Map of blueberry pollination study site locations across West Michigan in 2022. Northern fields are zoomed in for clarity. Pins are colored by farm pair, with pointed pins indicating fields stocked with honey bee colonies at the typical densities (1.5-4 hives per acre). Starred pins indicate fields stocked with honey bee colonies at increased densities (3.5 – 6 hives per acre).

Colony strength assessments

Ten colonies were randomly selected from each farm and tagged with a nursery tab labeled with a unique number and stapled onto the hive to ensure repeated sampling of the same colonies. For each of these colonies, cluster counts were assessed as described by Nasr et al. (1990) and returning forager counts were conducted on the same colonies as described by Sagili & Burgett (2011) and validated by Grant et al. (2021).

To perform cluster counts of the honey bee colonies at each site, each of the 10 sampled colonies was opened and observed visually without removing frames from the box (Figure 2.2A). The observer identified full seams of bees between frames in both the top and bottom of each box, to the nearest half frame. A full seam of bees was defined as the space between frames which is completely covered with bees when viewed from above or below (Figure 2.2B). The number of frames from above and below were averaged for each box, and the values from each box were summed to obtain the total number of frames for each hive. Counts from medium and shallow sized boxes were multiplied by ¾ and ½, respectively, to account for the differences in frame size from a standard deep hive box. Cluster counts were performed in both years, once at the beginning of bloom and once at the end of bloom, except for one farm in 2022 when colonies were picked up before cluster counts could be performed. In each year, the number of frames at the beginning and end of bloom were averaged to obtain an average cluster count per colony. The resulting cluster counts from each of the 10 randomly selected colonies at each field were averaged and multiplied by the number of colonies per acre to calculate the stocking strength as the average cluster count per acre for each field in each year.

To determine the number of returning foragers at the 10 selected honey bee colonies in each field, bee activity at each hive entrance was recorded for one minute with a smartphone camera. Returning forager videos were recorded between 9 A.M. and 4 P.M., when the sun was shining, at temperatures above 15°C but not exceeding 32°C, when there was no precipitation, and windspeeds were below 15 km/h. To ensure as little obstruction of forager flight path as possible, the observer stood to the side of the colony and zoomed in to the hive entrance to make recordings in 2021. In 2022, a tripod was used to further stabilize the video and standardize placement of the camera. All recordings were taken in landscape mode with the colony ID and

all possible entrances, including auger holes, visible in the frame (Figure 2.3). If necessary, grass in front of the hive entrance was tamped or cut down to improve visibility for recordings. Once all video files were uploaded to a computer drive, they were replayed on a large computer screen and slowed down to count the number of honey bees that entered the hive for one minute. Two observers independently conducted this assessment for each recording, and the two values were averaged to obtain a returning forager count for each hive. Returning forager counts from each of the 10 randomly selected colonies at a site were averaged and multiplied by the number of colonies per acre to determine the stocking strength as the average returning forager count per acre for each field in each study year.

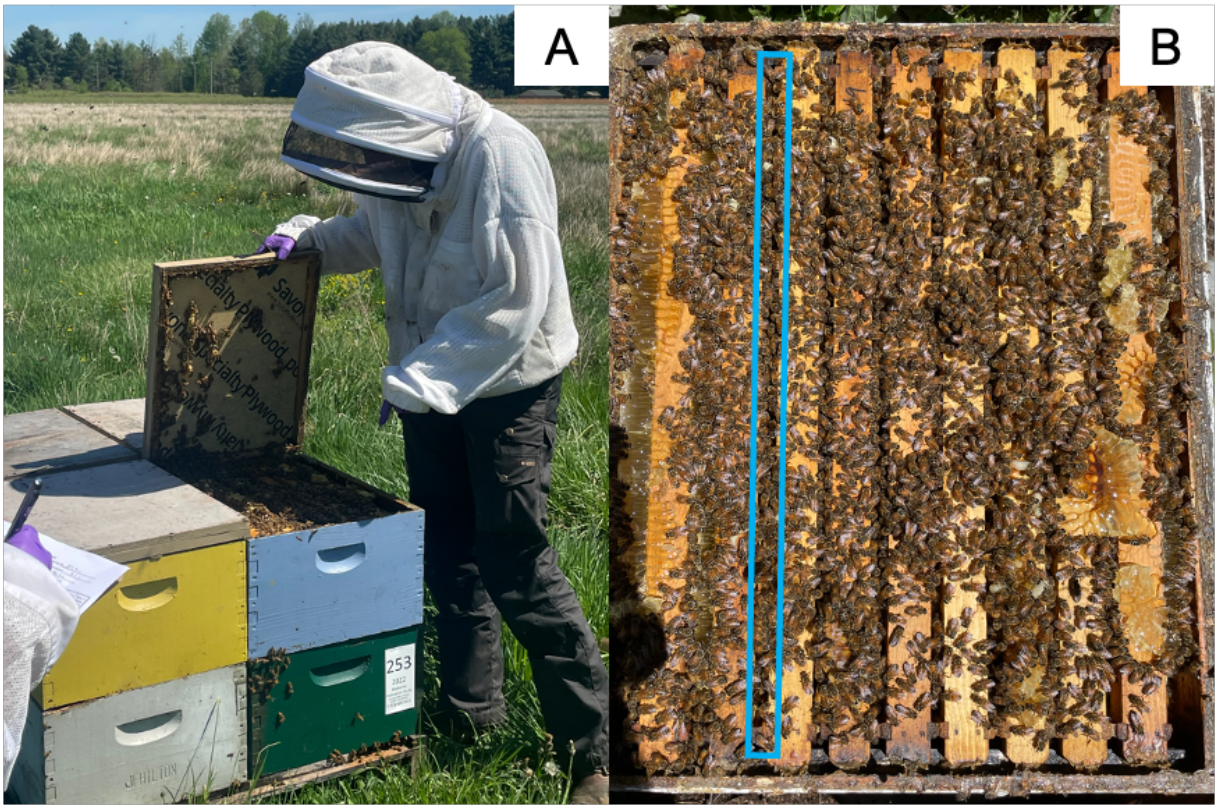

Figure 2.2. (A) An observer performing a manual cluster count of honey bees from above without removing frames from the hive. (B) Point of view of observer performing cluster count. Blue rectangle indicates one full seam of bees between frames.

Figure 2.3. Ideal filming angle for returning honey bee forager video assessments. There is no grass obstructing the hive entrance, and the entire entrance and colony ID tag are in frame.

Honey bee density and floral visitation assessments

To estimate honey bee density and floral visitation in the blueberry fields, a scan sampling method as described by Vaissiére et al. (2011) was used and adapted for this crop system. Sampling was done along four 100 m transects per farm, with transects at least 9 m into the field and running perpendicular to the field edge. Transects were separated by at least five meters (Figure 2.4). Observers walked down a transect for 10 minutes and recorded the number of honey bees visiting open blueberry flowers on the facing half of each bush in the transect. This sampling occurred within the same hour that returning forager counts were taken at the nearby honey bee colonies. Honey bee colonies were located near to the edge of the field where the assessment transect started. The four honey bee counts from individual transect samples were

averaged together to determine honey bee density as the average number of honey bees per 10-minute sample at each field in each year.

To estimate the number of open flowers observed during honey bee scan sampling, five bushes per transect were randomly selected to count the number of open flowers visible on the half of the bush facing the observer. For each year and field, the average value was multiplied by the number of bushes per transect to determine flowers per transect for subsequent calculation of floral visitation as the number of honey bees per 100 open flowers.

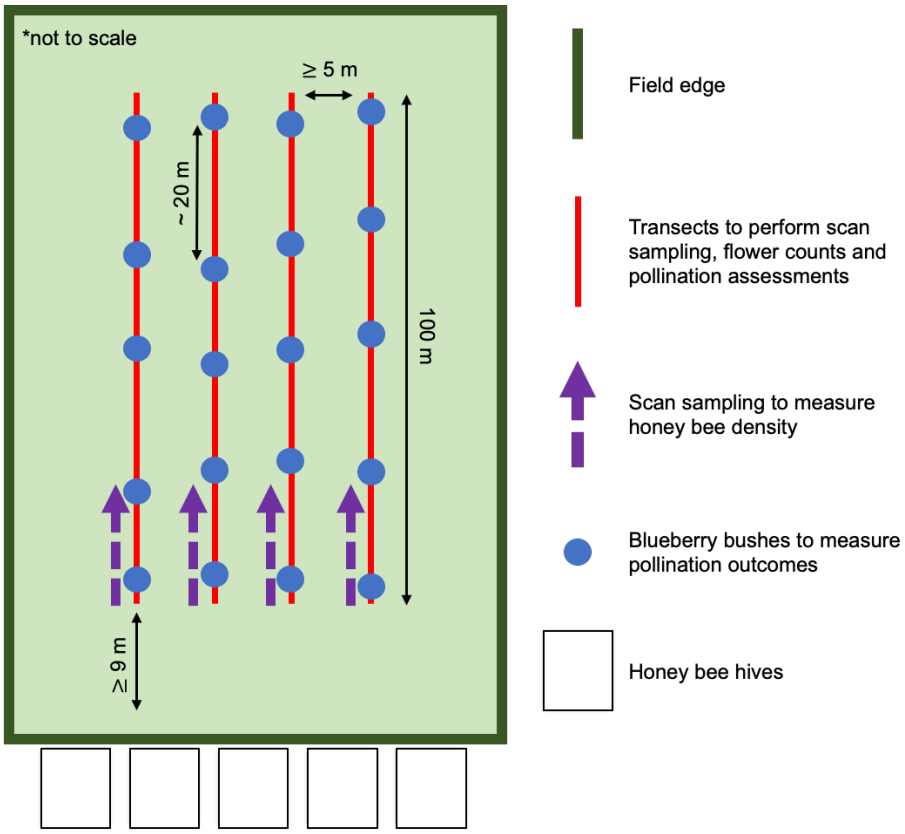

Figure 2.4. Experimental layout for assessing honey bee density and floral visitation (scan sampling), flower density, and pollination in highbush blueberry fields. Transects (red lines) ran along the rows of blueberry bushes, starting at least nine meters from the field edge (dark green box) and spaced at least five meters apart from one another. In each transect, five blueberry bushes (blue circles) were selected along the transect for assessing pollination. Sampled honey bee colonies were located near to the edge of the field where the assessment transect started.

Pollination assessments

To measure the levels of pollination in each blueberry field, in the early spring of both years, five bushes in each transect (n = 20 per farm) were selected to determine fruit set, berry weight, and seed set. The selected healthy plants were at least 9 meters from the edge of the field and spaced through the transect (Figure 2.4). Selected bushes were spaced out and were the healthiest bushes available. On each selected bush, two similar branches were selected with healthy one-year old growth with abundant flower buds at mid-canopy height. These branches were assigned one of two treatments: exposed to the insect community in the field (open) or excluded from pollinators (bagged). Prior to flowering, branches were flagged with different colored tape for identification, and all flowers distal to the flagging tape were counted to determine the number of flowers per shoot. After counting flowers, marked branches in the bagged treatment were covered with a gallon-sized fine-mesh bag secured with a twist tie at the base of the shoot. Immediately after fruit set, mesh bags were also placed over all branches assigned to the open treatment to protect the developing fruits from harvesters and predators and to ensure similar conditions for both treatments during development.

Berry collections began when the open treatment clusters were 25-50% ripe. Due to asynchronous ripening within and among the selected clusters, ripe (blue) berries were harvested 2-3 times at each field in each year. After each berry collection, mesh bags were replaced to avoid accidental fruit loss or predation. Ripe berries were placed in labeled resealable bags and transported in insulated coolers back to the laboratory, where they were stored at approximately 4ºC.

Within 48 hours of harvesting, ripe berries were counted and weighed to the nearest 0.1g. Percent fruit set was calculated for each branch by dividing the number of ripe berries by the

number of open flowers counted during sampling. After determining berry number and weight, berries were frozen until seed number could be measured. One berry per branch per treatment was randomly selected for seed number determination. Each berry was placed in a clear reclosable plastic bag, defrosted, and squeezed until all fruit contents were released into the bag so seeds could be counted. Plump, fertilized seeds counted towards total seed number per berry, and small, flat, and unfertilized seeds were excluded from seed count assessments.

Because some cultivars of highbush blueberry exhibit parthenocarpy (Eck, 1988), it can be useful to determine the contribution of insects to pollination outcomes, also known as pollination service. Following the approach of Garratt et al. (2021), the contributions of pollination service in this study were calculated by subtracting the values for bagged branches from the values for open branches for values of percent fruit set, berry weight, and seed set for each shoot. The data from individual branches were used to calculate average percent fruit set, average berry weight, and average seed set for each field (bagged, open, and pollination service).

Partial yield estimations

To estimate partial yields for each field, the number of open flowers per acre was calculated by multiplying the number of open flowers per bush during scan sampling by the number of bushes per acre. Bush density was calculated using row and bush spacing. The resulting number of open flowers per acre was multiplied by the percent fruit set and average weight per berry (in grams) on open treatment branches. These values were then converted to pounds of fruit per acre for each field, using Eq. 1:

$$EPY = \frac{Flowers}{Acre} \times FS_o \times W_o$$

Where EPY is the estimated partial yield, FS_o is the fruit set from open branches, and W_o is the berry weight from open branches. These values were considered partial yields because they are based on the open flowers during one sample during bloom which is therefore is an underestimate of bloom density.

Statistical analysis

All data were analyzed in R-Studio statistical software (v4.2.2; R Core Team 2022). To determine if stocking density, colony strength, or stocking strength contribute to honey bee density and floral visitation during blueberry bloom, linear mixed effects models (function *lmer,* package *lmerTest*, Kuznetsova et al. 2017) were developed to model the relationships between the honey bee socking density, the cluster count per acre, the returning forager count per acre, and honey bee density in the blueberry fields. To determine which aspects of honey bee abundance and activity best predict blueberry pollination, linear mixed effects models were developed to model the relationships between honey bee density in the field, floral visitation, and the pollination metrics. These models included the explanatory variables and year as fixed effects and farm as a random effect. These models were also analyzed without the inclusion of the year effect to determine direct relationships between the parameters. A coefficient of determination (marginal R^2) was then produced to estimate the proportion of variance explained by the model (function *r.squaredGLMM,* package *MuMIn,* Bartoń 2022), with ANOVA used to estimate the statistical significance of the coefficient of determination.

To compare measured and reported stocking densities in 2022, independent sample t-tests were conducted. Levene's test was used to determine homogeneity of variances, and if the assumption of similar variance was not met, Welch's non-parametric t-test was used to compare

values between treatments. To compare differences between years, paired samples t-tests were conducted. The Shapiro-Wilk test was used to determine whether the differences between years followed a normal distribution, and if the assumption of normality was not met, the Wilcoxon non-parametric t-test was used to compare values between years. To model the relationships between reported and measured stocking density and honey bee density and floral visitation in 2022, linear regression was used.

To observe differences in weather between years, temperature data from the Michigan State University Enviroweather weather station was downloaded and compiled. The daily maximum temperature from May 5th – May 31st was plotted for each year for comparison.

Results

Stocking densities in blueberry fields

In 2021, reported stocking densities ranged from 1.6 – 5.3 colonies per acre across all fields. In 2022, the reported and measured stocking densities ranged from 1.6 – 6 and 1.5 – 5.3 colonies per acre, respectively. In 2022, there was no significant difference in average colony number per farm, cluster count per acre, or returning forager count per acre between reported and measured stocking densities (Table S2.1). Further, the predictive power of the 2022 regression analysis of honey bee density and floral visitation as a function of honey bee stocking density was not improved by using the measured compared to the reported stocking density values (Table S2.2), so subsequent analyses were conducted using only the reported colony stocking densities.

Honey bee colony strength

Colony sampling in 2021 revealed a wide range of sizes, with cluster counts ranging from 2.5 to 21.0 frames of bees per hive. The number of returning foragers was also highly variable with from 1 – 192 bees observed entering the hive per minute. In 2022, cluster counts had a similar range, from 0 to 19.8 frames per hive. The number of returning foragers per minute also was similar to 2021, ranging from 0.5 to 250.5 bees entering the hive per minute. Pooling data across all farms, there was no significant difference in average cluster count (Figure 2.5A, Table S2.3) or average returning forager count (Figure 2.5B, Table S2.3) between years.

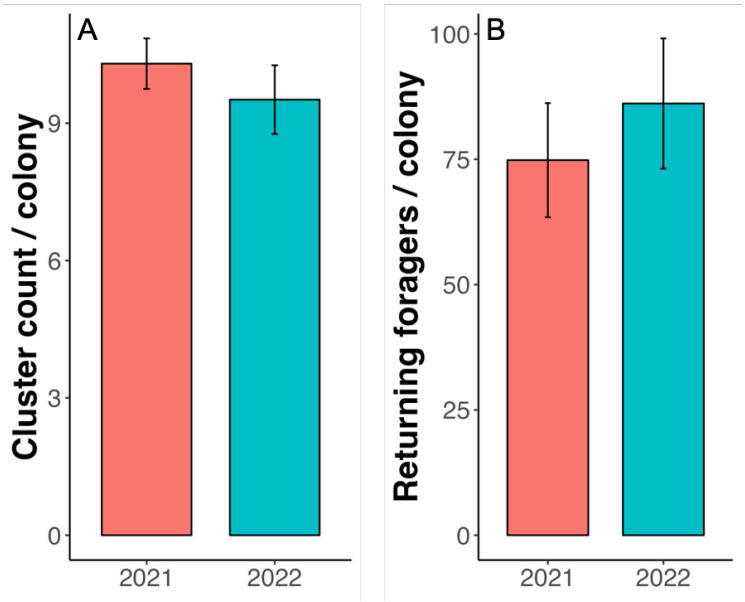

Figure 2.5. Comparison of the average ± S.E. A) cluster count per colony and B) number of returning foragers per colony of rented honey bee colonies used for blueberry pollination in Michigan during 2021 (pink) and 2022 (teal). For full statistical results, see Table S2.3.

Influence of stocking density and colony strength on floral visitation

In 2021, honey bee density ranged from 7.5 – 139 bees per 10 minute transect sample, flower density ranged from 6,426 – 28,318 open flowers per transect sample, and honey bee floral visitation ranged from 1.2 to 3.5 bees per 100 flowers. In 2022, honey bee density ranged

from 0 – 73.6 bees per 10 minute transect sample, flower density ranged from 16,500 – 83,727 open flowers per transect, and honey bee floral visitation ranged from 0.03 – 0.83 bees per 100 flowers. Pooling data across all farms, the average honey bee density in transects per 10-minute sample was at least three times higher in 2021 than in 2022 ($p<0.001$; Figure 2.6A, Table S2.3). The opposite pattern was found for the number of open flowers per transect during sampling, with this being significantly lower in 2021 than 2022 ($p<0.001$; Figure 2.6B, Table S2.3). Finally, the number of honey bees per 100 flowers was about five times higher in 2021 than 2022 ($p<0.001$; Figure 2.6C, Table S2.3).

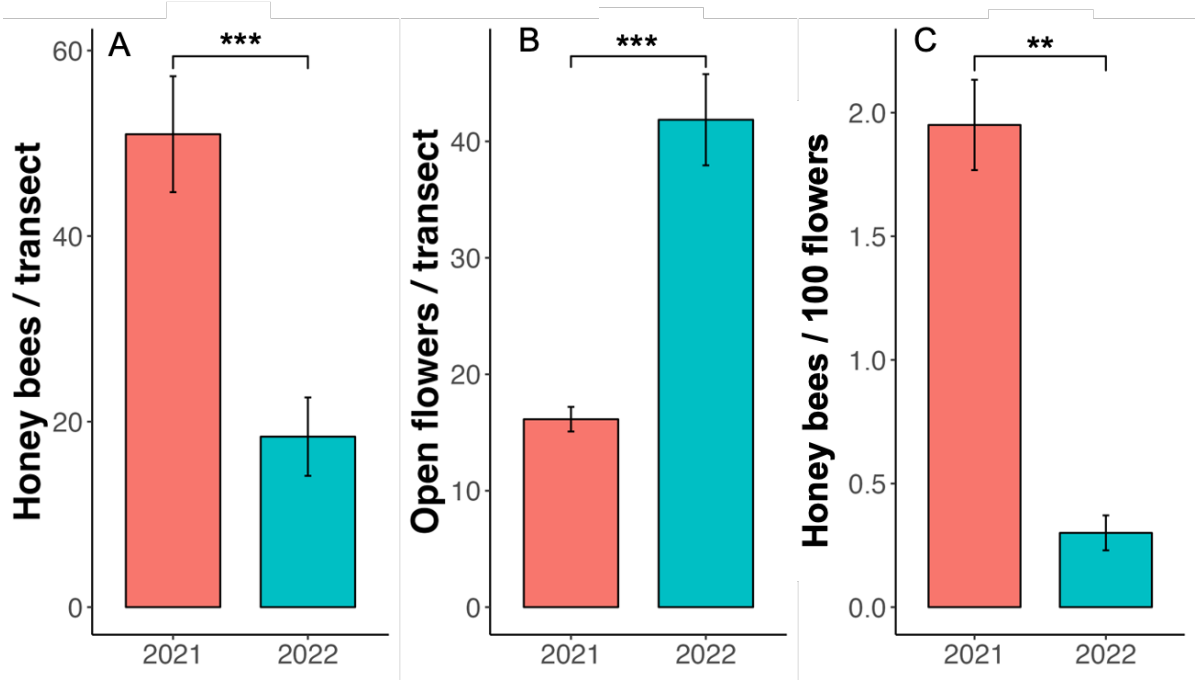

Figure 2.6. Comparison of the average ± S.E. A) number of honey bees per transect, B) number of open flowers per transect (per 1,000 flowers), and C) number of honey bees per 100 flowers between 2021 (pink) and 2022 (teal) across 12 fields of 'Bluecrop' blueberries during bloom in Michigan. Symbols represent results of independent t-tests (** = $p < 0.01$, *** = $p < 0.001$). For full statistical results, see Table S2.3.

When year was included as a factor in the linear model, there were no significant relationships between the honey bee density in transects and the stocking density (Figure 2.7A,

Table S2.4) or the average cluster count per colony (Figure 2.7B, Table S2.4). However, honey bee density in transects was positively related to the average returning forager count per colony (R^2=0.56, p=0.01; Figure 2.7C, Table S2.4), the cluster count per acre (R^2=0.56, p=0.03; Figure 2.7D, Table S2.4), and the returning forager count per acre (R^2=0.61, p<0.01; Figure 2.7E, Table S2.4). In addition, there were no significant relationships between the number of honey bees per 100 flowers and any of the measures of stocking density, colony strength, or stocking strength (not shown).

When the year factor was not included in the linear models, there were no significant relationships between honey bee density in transects or the number of honey bees per 100 flowers and any of the measures of stocking density, colony strength, or stocking strength (not shown).

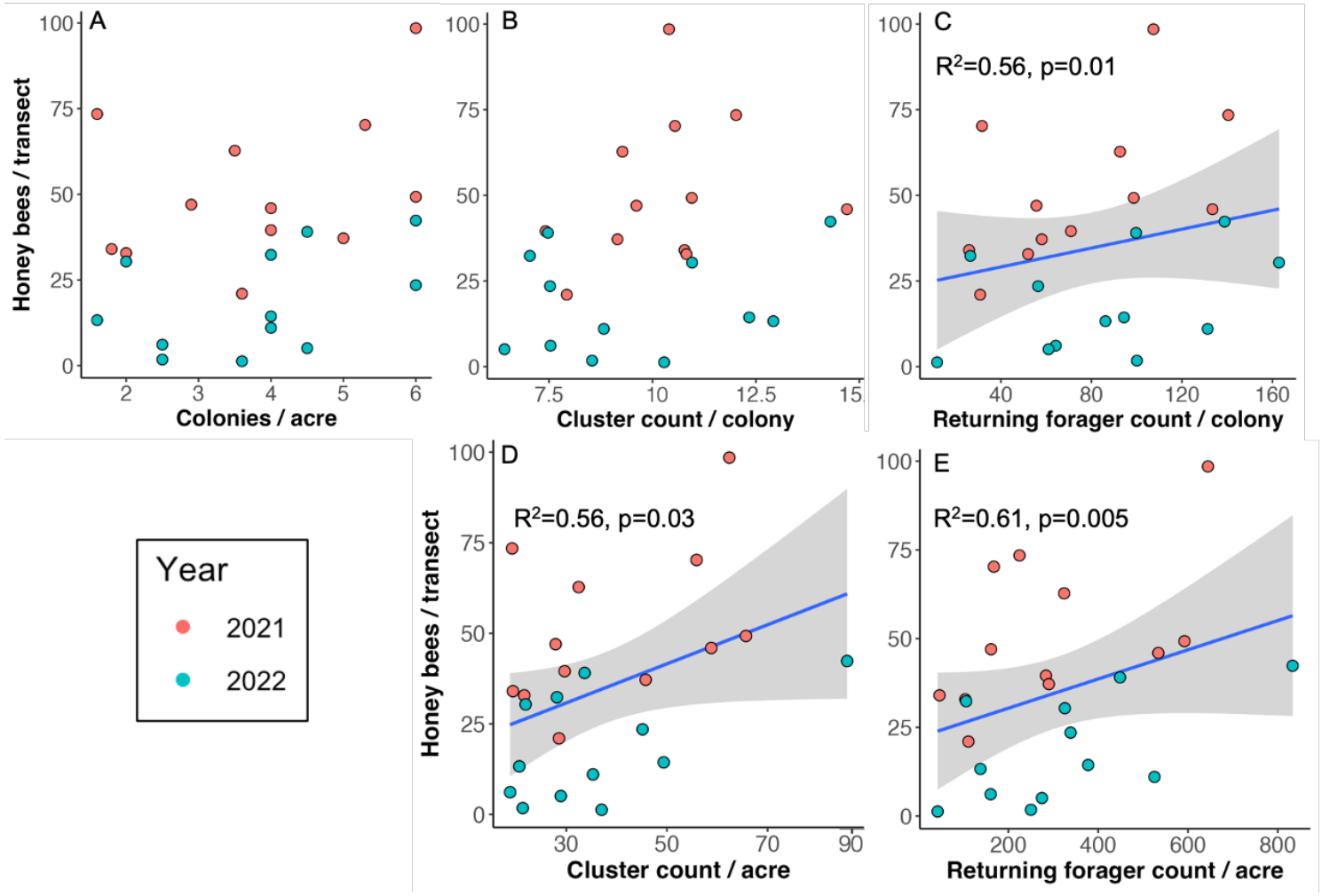

Figure 2.7. Relationships between the number of honey bees observed visiting blueberry flowers in 10 minutes and the A) stocking density (colonies per acre), B) cluster count per colony, C) returning foragers per colony (1 minute sample), D) cluster count per acre, and E) returning forager count per acre (1 minute sample) across 12 fields of 'Bluecrop' blueberries during bloom over 2021 (pink0 and 2022 (teal). Blue lines indicate significant results of linear regression. Grey shaded areas show 95% confidence intervals. For full statistical results, see Table S2.23.

Influence of honey bee abundance on fruit set

In both years, the fruit set of open branches ranged from 0 – 100%. Pooling data across all farms, the average fruit set of open branches was not significantly different between years (Figure 2.8A, Table S2.5). In both years, the fruit set of bagged branches ranged from 0 – 100%. Pooling data across all farms, average fruit set of bagged branches was significantly lower in 2021 than 2022 ($p=0.001$; Figure 2.8B, Table S2.5). The fruit set from pollination (open treatment – bagged treatment) ranged from 11.23 – 95.17% in 2021, and -18.64 – 54.89% in 2022. Pooling data across all farms, average fruit set from pollination was much higher in 2021 than 2022 ($p<0.001$; Figure 2.8C, Table S2.5).

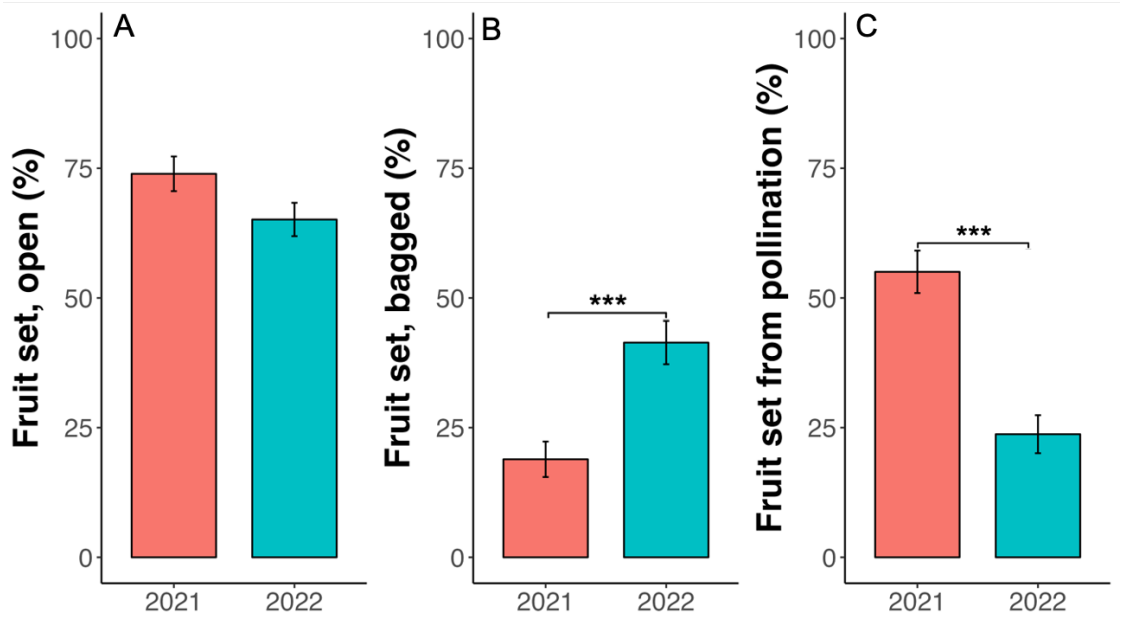

Figure 2.8. Comparison of the average ± S.E. percent fruit set of A) branches in an open treatment exposed to pollinators, B) branches in a bagged treatment excluded from pollinators, and C) pollination service (open – bagged treatment) between 2021 (pink) and 2022 (teal) across 12 fields of 'Bluecrop' blueberries in Michigan. Symbols represent results of independent t-tests (*** = $p<0.001$). For full statistical results, see Table S2.5.

When the effect of year was included in the linear models, I found no significant relationships between any of the measures of honey bee abundance and the fruit set of open branches (not shown). Values for the bagged branches were much lower than those for open branches, and there were also no significant relationships between the fruit set of bagged branches and the stocking density, average cluster count per colony, cluster count per acre, returning forager count per acre, or the number of honey bees per 100 flowers (not shown). The fruit set in bagged branches decreased with the average returning forager count per colony (R^2=0.54, p=0.03) and the honey bee density in transects (R^2=0.52, p=0.04). There were no significant relationships between the fruit set from pollination (open – bagged treatments) and any of the measures of stocking density, colony strength, or stocking strength, or the number of honey bees per 100 flowers (not shown). The fruit set from pollination was strongly positively related to the honey bee density in transects (R^2=0.71, p=0.005).

When year was not included in the linear models, there were no significant relationships between the fruit set of open branches and any of the measures of stocking density, colony strength, or stocking strength, or the honey bee density in transects (Figure 2.12A). Fruit set of open branches was weakly associated with the number of honey bees per 100 flowers (R^2=0.10, p=0.05; Figure 2.13A, Table S2.6). There were no significant relationships detected between any of the measures of honey bee stocking density, colony strength, or stocking strength and the fruit set of bagged branches. The fruit set of bagged branches decreased with the increase of honey bee density in transects (R^2=0.49, p<0.001; Table S2.6) and the number of honey bees per 100 flowers (R^2=0.42, p=0.002; Table S2.6). There were no significant relationships between the fruit set from pollination and any of the measures of stocking density, colony strength, or stocking strength. Finally, the fruit set from pollination increased significantly with the honey bee density

in transects (R^2=0.61, p<0.001; Table S2.6) and the number of honey bees per 100 flowers (R^2=0.61, p<0.001; Table S2.6).

Influence of honey bee abundance on berry weight

The average ripe berry weight from open branches ranged from 0.49 – 2.82 grams per berry in 2021, and 0.42 – 2.31 grams per berry in 2022. Pooling data across all farms, average berry weight from open treatment branches was significantly higher in 2021 than in 2022 (p=0.01; Figure 2.9A, Table S2.5). The average ripe berry weight (per branch with fruit set > 0%) from bagged branches ranged from 0.20 – 2.20 grams per berry in 2021, and 0.33 – 2.36 grams per berry in 2022. Pooling data across all farms, average berry weight from bagged branches was significantly lower in 2021 than 2022 (p<0.001; Figure 2.9B, Table S2.5). The average ripe berry weight from pollination ranged from 0.20 to 1.93 grams per berry in 2021, and -0.12 to 0.99 grams per berry in 2022. Pooling data across all farms, average berry weight from pollination was significantly higher in 2021 than 2022 (p<0.001; Figure 2.9C, Table S2.5).

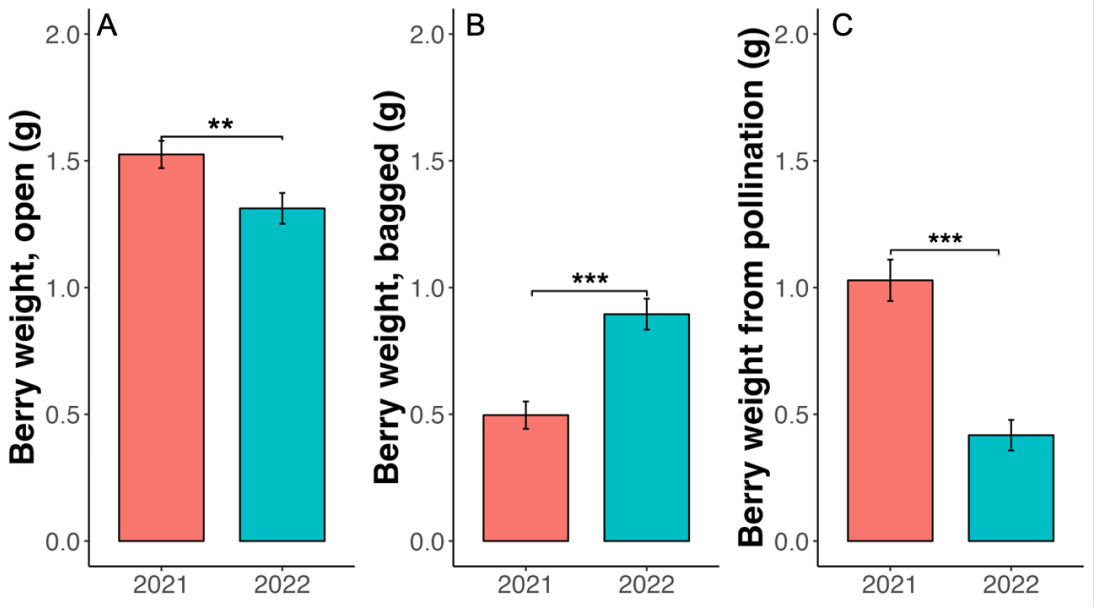

Figure 2.9. Comparison of the average ± S.E. berry weight of A) branches in an open treatment exposed to pollinators, B) branches in a bagged treatment excluded from pollinators, and C) pollination service (open – bagged treatment) between 2021 (pink) and 2022 (teal) across 12 fields of 'Bluecrop' blueberries in Michigan. Symbols represent results of independent t-tests (*** = p <0.001, ** = p<0.01). For full statistical results, see Table S2.5.

When year was included as a factor in the linear models, I found no significant relationships between the measures of honey bee abundance and the berry weight of open branches (not shown). This was also the case for bagged branches (not shown), except for the negative relationship with average returning forager count per colony (R^2=0.67, p=0.05) and bagged branches. There were no significant relationships between any of the measures of honey bee abundance and the berry weight from pollination (not shown).

When year was not included in the linear models, the berry weight of open branches increased with the number of honey bees per 100 flowers (R^2=0.16, p=0.05; Figure 2.13B, Table S2.6), but not any of the other metrics of bee abundance (Figure 2.12B). For bagged branches, berry weight decreased with the honey bee density in transects (R^2=0.31, p=0.004; Table S2.6) and the number of honey bees per 100 flowers (R^2=0.38, p=0.001; Table S2.6), but not the other

measures of honey bee abundance. The berry weight from pollination was increased with the honey bee density in transects (R^2=0.33, p=0.003; Table S2.6) and the number of honey bees per 100 flowers (R^2=0.41, p<0.001; Table S2.6), but not the stocking density, colony strength, or stocking strength.

Influence of honey bee abundance on seed set

The seed set from open branches ranged from 1 to 51 seeds per berry in 2021, and 0 to 36 seeds per berry in 2022. Pooling data across all farms, average seed set from open branches was significantly higher in 2021 than 2022 (p<0.001; Figure 2.10A, Table S2.5). As expected, the number of fertilized seeds per berry in the open branches was positively related to berry weight in open branches (R^2=0.43, p=0.009). In contrast, the seed set from bagged branches was similar between years (Figure 2.10B, Table S2.5), ranging from 0 to 40 seeds per berry in 2021, and 0 to 35 seeds per berry in 2022. There was no relationship between the number of seeds per berry and the weight of berries from bagged branches. The seed set from pollination ranged from 14.20 to 29.65 seeds per berry in 2021, and -1.15 to 12.70 seeds per berry in 2022, and was significantly higher in 2021 than 2022 (p<0.001; Figure 2.10C, Table S2.5).

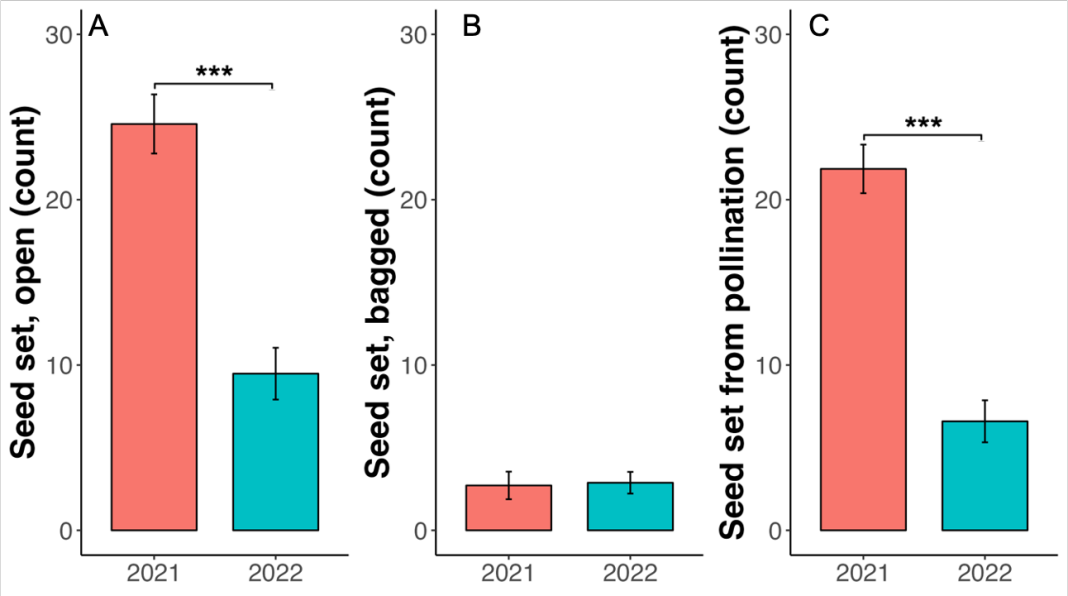

Figure 2.10. Comparison of the average ± S.E. seed number per berry of A) branches in an open treatment exposed to pollinators, B) branches in a bagged treatment excluded from pollinators, and C) pollination service (open – bagged treatment) between 2021(pink) and 2022 (teal) across 12 fields of 'Bluecrop' blueberries in Michigan. Symbols represent results of independent t-tests (*** = p <0.001,). For full statistical results, see Table S2.5.

When year was included in the linear models, there were no significant relationships between seed set in open branches and most of the measures of honey bee abundance (not shown), except for the average cluster count per colony (R^2=0.70, p=0.03), which was positive. Seed set in bagged branches was not affected by honey bee abundance (not shown), whereas seed set from pollination was positively corelated with average cluster count per colony (R^2=0.79, p=0.01), but not the other honey bee abundance metrics (not shown).

When year was not included in the linear models, seed set in open branches increased with honey bee density in transects (R^2=0.49, p<0.001; Figure 2.12C, Table S2.6) and the number of honey bees per 100 flowers (R^2=0.54, p<0.001; Figure 2.13C, Table S2.6).There were no significant relationships between seed set in bagged branches and any measure of honey bee abundance. Seed set from pollination increased with the average cluster count per colony

(R^2=0.16, p=0.05; Table S2.6), the honey bee density in transects (R^2=0.53, p<0.001; Table S2.6), and the number of honey bees per 100 flowers (R^2=0.62, p<0.001; Table S2.6).

Yield estimates

The yield estimate for the sampled 'Bluecrop' blueberry fields ranged from 2,418 – 4,238 pounds per acre (2710 – 4750 kg/ha) in 2021, and 1,223 – 3,997 pounds per acre (1371 – 4480 kg/ha) in 2022. The average yield estimate was significantly higher in 2021 than 2022 (Figure 2.11, Table S2.7; p=0.02).

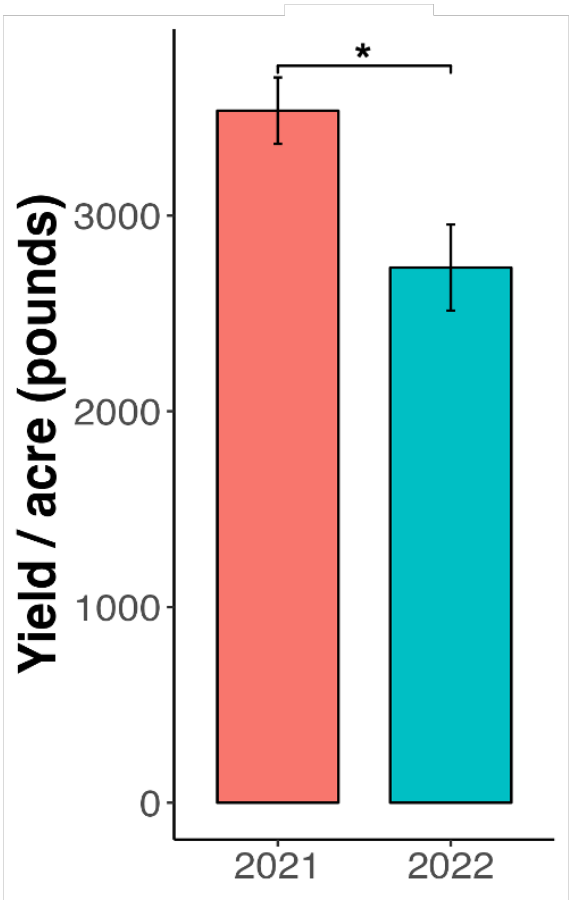

Figure 2.11. Comparison of the average ± S.E. estimated partial yield in pounds of fruit per acre between 2021 (pink) and 2022 (teal) across 12 fields of 'Bluecrop' blueberries in Michigan. Symbol represent results of a t-test (* = p <0.05). For full statistical results, see Table S2.7.

When year was included in the linear model, there was a significant relationship between estimated partial yield and the returning forager count per acre (R^2=0.23, p=0.04). None of the other bee density metrics were statistically significant (not shown). When year was not included in the linear model, estimated partial yield increased with honey bee density in transects (R^2=0.25, p=0.01; Figure 2.12D, Table S2.8) and the number of honey bees per 100 flowers (R^2=0.27, p=0.01; Figure 2.13D, Table S2.8), but not the other bee density metrics.

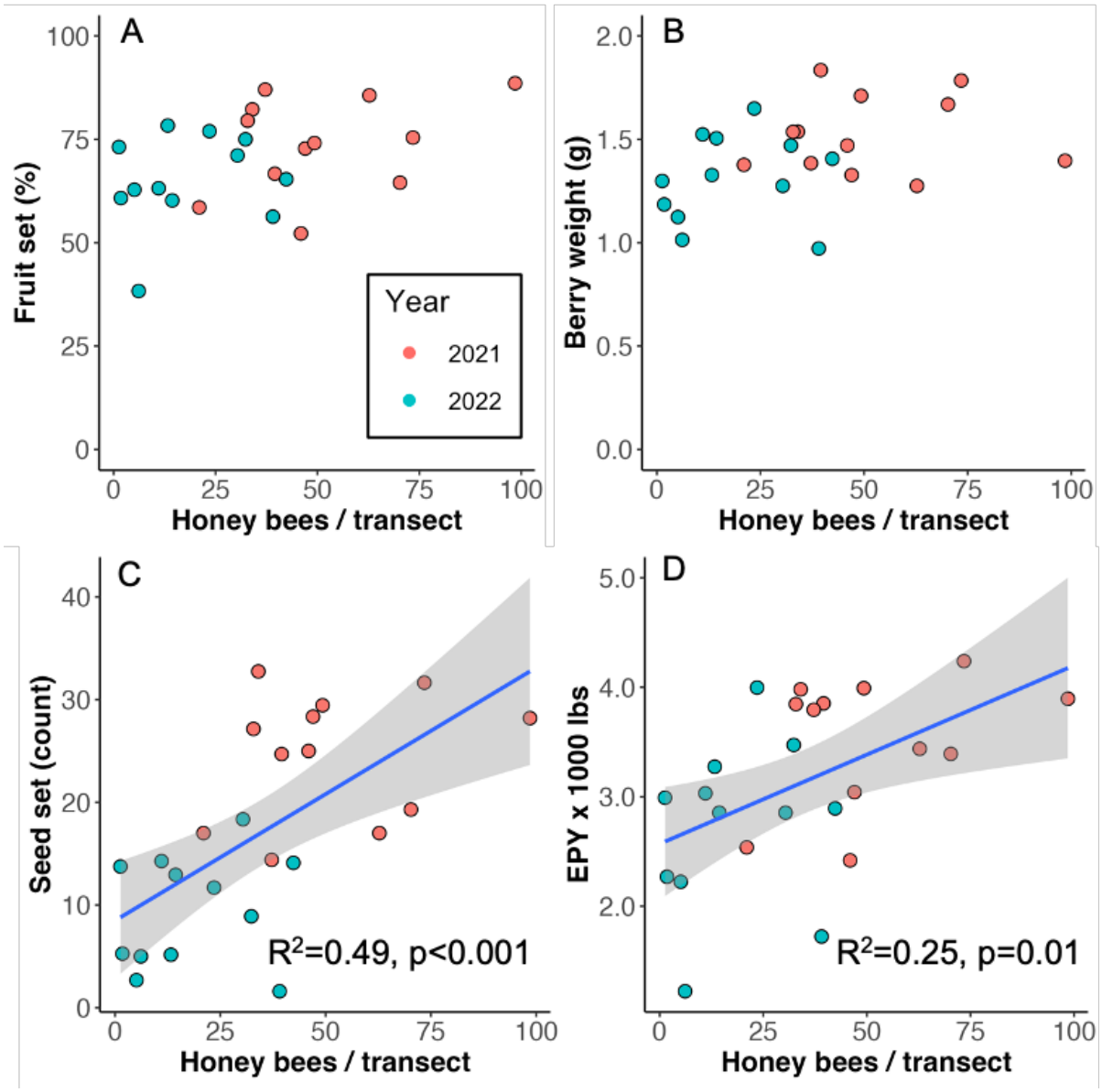

Figure 2.12. Relationship between honey bee density during bloom (honey bees per 100 m transect, 10-minute sample) and A) percent fruit set, B) berry weight, C) seed number per berry, and D) estimated partial yield (1000 pounds per acre) across 12 fields of 'Bluecrop' blueberries in Michigan sampled in 2021 (pink) and 2022 (teal). Blue lines indicate significant results of linear regression without the effect of year included in the model. Grey shaded areas show 95% confidence intervals. For full statistical results of significant relationships, see Table S2.6 (C); Table S2.8 (D)

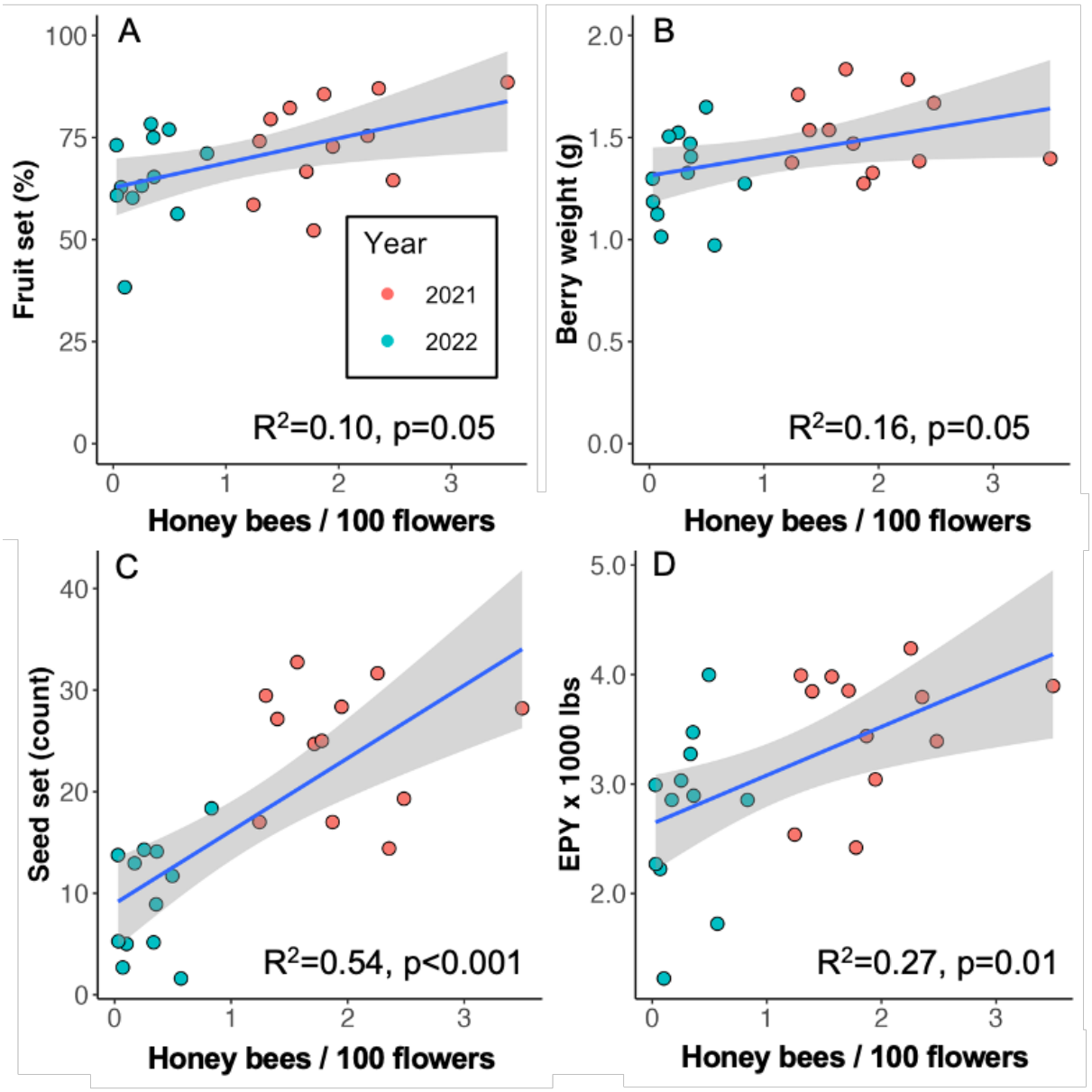

Figure 2.13. Relationship between honey bee floral visitation during bloom (honey bees per 100 flowers) and A) percent fruit set, B) berry weight, C) seed number per berry, and D) estimated partial yield (1000 pounds per acre) across 12 fields of 'Bluecrop' blueberries in Michigan sampled in 2021 (pink) and 2022 (teal). Blue lines indicate significant results of linear regression without the effect of year included in the model. Grey shaded areas show 95% confidence intervals. For full statistical results of significant relationships, see Table S2.6 (A, B, C); Table S2.8 (D)

Discussion

This two-year study on highbush blueberry pollination revealed that honey bee colony stocking strength, measured as the combination of honey bee colony stocking density and colony size, was a significant predictor of honey bee density in fields during blueberry bloom. In addition, honey bee colony activity, measured as the number of honey bee foragers returning to the hive in a one-minute sample, was also predictive of honey bee density in fields. In turn, this study revealed that honey bee density in the field was a significant predictor of seed number per berry and estimated partial yield. My results show that when honey bee colony size is variable across farms, the stocking density alone is insufficient for predicting pollination. Rather, the results highlight the importance of considering both colony density and strength for predicting the foraging force available for crop pollination in nearby fields. As the contrast between the two sampling years indicates, these interactions are also highly affected by the weather during bloom. This provided a wide range of bee foraging activity and flower density in the farms across this study, illustrating the importance of these aspects for pollination of the crop and the realized yield.

When considered alone, none of the measurements of stocking density, colony strength, or stocking strength were significant predictors of the number of honey bees per flower, and I suspect this is because of the wide range in flower densities between sites, ranging from 11,258 - 53,402 open flowers per transect sample averaged across years. From these results, I conclude that floral density is an overlooked but important factor to consider when making colony stocking decisions. I found that the number of honey bees per 100 flowers was a highly significant predictor of all pollination components, which indicates that sufficient honey bee

floral visitation at the field-level is the target that growers should be aiming for to maintain a large enough foraging force of pollinators to transfer pollen among flowers.

The very different weather conditions during the two years of this study were also influential to our results. In 2021, temperatures gradually warmed through most of May, allowing for an even progression of blooming. However, in 2022, after a brief cold start in early May, temperatures rapidly increased from 60 to 90 ºF (15.5 – 32.2 ºC) in only six days (Figure S2.1). The rapid accumulation of growing degree days and associated development of bloom emergence led to double the density of flowers requiring pollination in a much shorter period (Figure 2.6B, Table S2.3). Consequently, despite the similar average colony stocking and strength parameters across years, the number of honey bees per 100 flowers was over six times higher in 2021 than in 2022 (Figure 2.6C, Table S2.3). The much lower bee visits per flower in 2022 help explain the much lower average berry weight (Figure 2.8A, Table S2.5), seed set (Figure 2.9A, Table S2.5), and yields (Figure 2.10, Table S2.7) across all farms that year. By conducting data analysis without the effect of year in the linear model, I was able to investigate these impacts of field-level honey bee abundance at a greater scale.

The density of foraging bees at a particular field can be magnified by landscape factors, as is now well documented for wild bees (Connelly et al. 2015, Evans et al. 2018, Martins et al. 2018). A similar pattern has been recently reported for honey bees in Washington blueberry farms where honey bee density was best predicted by the number of colonies in a 1000 m radius, rather than the field -level colony density (Eeraerts et al. 2022). The results from this study suggest that a similar pattern may be present in Michigan blueberry farms, with honey bee density predicted by the combined density and strength of colonies at the field level and in the surrounding landscape, leading to higher levels of pollination service than field-level colonies

alone could provide. This may also explain why stocking strength is predictive of honey bee density in the field, whereas it is not a predictor of floral visitation, pollination components, or estimated partial yields.

The relationship between honey bee floral visitation and blueberry fruit set and berry weight from pollination service was generally significant and positive (Figure S2.2), but these results require some explanation. The positive trends were a result of the difference between pollination in open branches, which increased with honey bee floral visitation, and the pollination in bagged branches, which declined with honey bee floral visitation. The experiments included bagged branches to provide an internal control for the variation in horticultural practices among fields, and I expected the pollination in bagged branches to be similar across the gradient of honey bee abundance. Theoretically, the resulting levels of pollination in bagged branches could have then been attributed to parthenocarpy, infiltration of small, non-honey bee pollinators, or human error in bagging. However, the decrease in fruit set and berry sizes in bagged branches observed with increasing honey bee floral visitation in the sampled fields was too significant to be ignored. Biologically, this inflation of pollination service estimates could be due to resource reallocation (Stephenson 1981). Through pollen supplementation experiments, Zimmerman & Pyke (1988) found that flowers with pollen added were prioritized at the expense of unmanipulated flowers, resulting in inflated estimates of pollen limitation. Since then, several review papers have suggested improvement of the methodology used in pollen limitation experiments to prevent spurious results (Knight et al. 2006, Wesselingh 2007). In the case of this study, the patterns of fruit set in bagged branches may have been caused by greater resource allocation to well-pollinated branches on the rest of the unbagged flower clusters. This is supported by the positive trend of seed set in open branches and honey bee floral visitation in

this study, theoretically increasing the fitness of the plant to pass on its genes in the form of more viable seeds. More research is needed to determine the influence of pollinator visitation on blueberry resource partitioning, using bagged treatment experiments with varying proportions of the bush excluded from pollinators, to determine if this hypothesis is supported. These results also highlight that calculations of pollination service from comparing flowers where pollinators are excluded or not should be interpreted with caution, particularly if a small portion of the plant's flowers are being used for pollinator exclusion.

Given the results of this study, highbush blueberry growers would benefit from more guidance on the implications of flower density for making decisions about colony stocking rates. For example, in a year like 2022, a higher density of pollinators may have buffered against the deleterious effects of rapid bloom on yield component outcomes. Although measurements of stocking density and colony strength at the farm-level did not directly predict bees per flower, yield components, or estimated partial yields in this study, growers can still benefit from evaluating a subset of their rented colonies for strength. Growers or consultants who want to gauge field-level honey bee density through colony strength assessments should perform returning forager counts, rather than cluster counts, for better estimates. This also avoids the need to open colonies, which provides greater flexibility. For even better estimates of field-level honey bee density, growers should assess stocking strength by multiplying an estimate of average colony strength by the number of hives per acre. To estimate pollination outcomes and yields, growers or crop scouts may perform honey bee scan sampling during ideal weather conditions by walking down a 100 m row for 10 minutes and counting the number of bees on blueberry flowers. From my results, a target of greater than 50 bees per 10-minute sample would provide some confidence that moderate to high levels of pollination are being delivered. This result is

specific to ‘Bluecrop,’ and may not hold for other cultivars which may have varying pollination requirements. For the optimization of highbush blueberry pollination, there remains a need to uncover the interacting effects of pollinator abundance, horticultural practices, floral density, surrounding landscape, climate, and other factors on subsequent pollination services.

APPENDIX

Table S2.1. Comparison of reported and measured stocking densities of honey bee colonies in blueberry farms in 2022. Table presents three measures of honey bee abundance across 12 fields of 'Bluecrop' blueberries in Michigan.

Variable	Reported mean (SD)	Measured mean (SD)	Mean difference (95% CI)	t (df)	*P*[a]
Colonies / acre	3.77 (1.42)	3.17 (1.32)	0.6 (-0.56, 1.76)	1.07 (1, 22)	0.30
Cluster count / acre	35.51 (18.60)	28.43 (9.44)	7.08 (-5.40, 19.57)	1.18 (1, 22)	0.25
Returning foragers / acre[b]	318.45 (216)	249.27 (124)	69.17 (-80.02, 218.37)	0.96 (1, 22)	0.35

[a] Independent *t*-test. [b] Sampling time = one minute.

Table S2.2. Model comparisons between reported and measured stocking densities of honey bee colonies. Table reports results of statistical relationships between honey bee floral visitation (bees per transect) and three measures of honey bee abundance across 12 fields of 'Bluecrop' blueberries during bloom in 2022. AICc weight: proportion of the total amount of predictive power provided by the model being assessed.

Model	Reported			Measured		
	AICc weight	df	*P*[a]	AICc weight	df	*P*[a]
Colonies / acre	0.78	10	0.15	0.22	10	0.94
Cluster count / acre	0.83	10	0.10	0.17	10	0.72
Returning foragers / acre[b]	0.73	10	**0.05**	0.27	10	0.15

[a] Linear regression. [b] Sampling time = one minute.

Table S2.3. Comparison of average honey bee and floral parameters during crop bloom between 2021 and 2022 across 12 fields of 'Bluecrop' blueberries in Michigan. *P*-values in bold are significant at α = 0.05.

Variable	2021 Mean (SD)	2022 Mean (SD)	Mean difference (95% CI)	*t* (df)	*P*[a]
Cluster count / colony	10.3 (1.91)	9.51 (2.6)	0.79 (-1.1, 2.68)	0.92 (1, 11)	0.38
Returning foragers / colony[b]	74.82 (39.4)	86.13 (44.96)	-11.31 (-46.61, 24)	-0.7 (1,11)	0.5
Honey bees / transect[c]	50.98 (21.64)	18.38 (14.64)	32.6 (20.58, 44.62)	5.97 (1, 11)	**<0.001**
Open flowers / transect (10^4)[d]	1.61 (0.37)	4.19 (1.36)	-2.57 (-3.47, -1.67)	-6.28 (1, 11)	**<0.001**
Honey bees / 100 flowers	1.95 (0.63)	0.3 (0.24)	1.65 (1.30, 1.99)	10.53 (1, 11)	**<0.001**

[a] Paired samples *t*-test. [b] Sampling time = one minute. [c] Sampling time = 10 minutes. [d] Per 1000 flowers.

Table S2.4. Results of linear regression analysis (with fixed effect of year) between honey bee density (bees per transect) and five measures of honey bee abundance during crop bloom across 12 fields of 'Bluecrop' blueberries in Michigan over 2021 and 2022. *P*-values in bold are significant at α = 0.05.

Variable	Slope (95% CI)	$R^2_{marginal}$	*F* (df)	*P*
Colonies / acre	4.72 (-0.59, 9.98)	0.52	2.99 (1, 12.26)	0.11
Cluster count /colony	2.54 (-0.91, 6.05)	0.49	2.1 (1, 20.83)	0.16
Returning foragers /colony[a]	0.21 (0.06, 0.35)	0.56	7.39 (1, 17.77)	**0.01**
Cluster count / acre	0.51 (0.09, 0.96)	0.56	5.53 (1, 16.57)	**0.03**
Returning foragers / acre[a]	0.05 (0.02, 0.08)	0.61	9.6 (1, 20.86)	**0.005**

[a] Sampling time = one minute.

Table S2.5. Comparison of average fruit set, berry weight, and seed set in bagged, open, and pollination service (open – bagged) treatments between 2021 and 2022 across 12 fields of 'Bluecrop' blueberries in Michigan. *P*-values in bold are significant at α = 0.05.

Yield metric	Treatment	2021 Mean (SD)	2022 Mean (SD)	Mean difference (95% CI)	*t* (df)	*P*[a]
Fruit set (%)	**Open**	73.93 (11.58)	65.12 (11.12)	8.81 (-1.53, 19.14)	1.88 (1, 11)	0.09
	Bagged	18.9 (11.85)	41.39 (14.46)	-22.49 (-33.9, -11.09)	-4.34 (1, 11)	**0.001**
	Pollination service	55.03 (14.16)	23.73 (12.69)	31.30 (17.24, 45.37)	4.9 (1,11)	**<0.001**
Berry weight (g)	**Open**	1.53 (0.19)	1.31 (0.21)	0.21 (0.06, 0.37)	3.02 (1, 11)	**0.01**
	Bagged	0.5 (0.19)	0.9 (0.13)	-0.4 (-0.56, -0.24)	-5.53 (1, 11)	**<0.001**
	Pollination service	1.03 (0.28)	0.42 (0.21)	0.61 (0.44, 0.78)	8.05 (1, 11)	**<0.001**
Seed set (count)	**Open**	24.58 (6.19)	9.48 (5.42)	15.10 (9.67, 20.53)	6.12 (1, 11)	**<0.001**
	Bagged	2.72 (2.88)	2.88 (2.3)	-0.16 (-1.65, 1.32)	-0.24 (1, 11)	0.81
	Pollination service	21.86 (5.1)	6.6 (4.4)	15.27 (10.21, 20.32)	6.65 (1, 11)	**<0.001**

[a] Paired samples *t*-test.

Table S2.6. Significant results of linear regression analysis (without fixed effect of year) on measures of honey bee abundance and the fruit set, berry weight and seed set of 'Bluecrop' blueberries. Table displays results from an open treatment group accessible to pollinators, a bagged treatment group to exclude pollinators, and pollination service (open – bagged treatments). All *P*-values are significant at α = 0.05.

Yield metric	Treatment	Variable	Slope (95% CI)	$R^2_{marginal}$	*F* (df)	*P*[a]
Fruit set (%)	**Open**	**Honey bees / 100 flowers**	3.96 (0.60, 8.94)	0.10	5.49 (1, 8.4)	**0.05**
	Bagged	**Honey bees / transect[b]**	-0.56 (-0.77, -0.25)	0.49	30.89 (1, 12)	**<0.001**
		Honey bees / 100 flowers	-12.78 (-18.08, -5.29)	0.42	24.06 (1, 6.95)	**0.002**
	Pollination service	**Honey bees / transect[b]**	0.66 (0.45, 0.88)	0.61	35.83 (1, 22)	**<0.001**
		Honey bees / 100 flowers	16.97 (11.5, 22.45)	0.61	36.69 (1, 22)	**<0.001**
Berry wt. (g)	**Open**	**Honey bees / 100 flowers**	0.09 (0.01, 0.18)	0.16	4.37 (1, 22)	**0.05**
	Bagged	**Honey bees / transect[b]**	-0.005 (-0.009, -0.002)	0.31	10.17 (1, 22)	**0.004**
		Honey bees / 100 flowers	-0.17 (-0.25, -0.08)	0.38	14.3 (1, 22)	**0.001**
	Pollination service	**Honey bees / transect[b]**	0.009 (0.003, 0.015)	0.33	11.49 (1, 22)	**0.003**
		Honey bees / 100 flowers	0.27 (0.13, 0.4)	0.41	16.91 (1, 15.1)	**<0.001**
Seed set (count)	**Open**	**Honey bees / transect[b]**	0.31 (0.16, 0.42)	0.49	34.45 (1, 12.62)	**<0.001**
		Honey bees / 100 flowers	7.9 (5.11, 10.15)	0.54	46.74 (1, 10.31)	**<0.001**
	Bagged	**N/A**	N/A	N/A	N/A	N/A
	Pollination service	**Cluster count / colony**	1.65 (0.13, 3.17)	0.16	4.51 (1, 22)	**0.05**
		Honey bees / transect[b]	0.32 (0.17, 0.42)	0.53	45.17 (1, 12.03)	**<0.001**
		Honey bees / 100 flowers	8.19 (6.05, 9.95)	0.62	82.04 (1, 10.35)	**<0.001**

[a] Linear regression. [b] Sampling time = 10 minutes.

Table S2.7. Comparison of estimated yields between 2021 and 2022 across 12 fields of 'Bluecrop' blueberries in Michigan. *P*-value in bold is significant at α = 0.05.

2021 Mean (SD)	2022 Mean (SD)	Mean difference (95% CI)	*t* (df)	*P*[a]
3535.76 (588.61)	2734.31 (764.13)	801.45 (145.93, 1456.96)	2.69 (1, 11)	**0.02**

[a]Paired samples *t*-test.

Table S2.8. Significant results of linear regression analysis (without fixed effect of year) between estimated yield and measures of honey bee abundance during crop bloom across 12 fields of 'Bluecrop' blueberries in Michigan over 2021 and 2022. *P*-values are all significant at α = 0.05.

Variable	Slope (95% CI)	$R^2_{marginal}$	*F* (df)	*P*
Honey bees / transect[a]	16.02 (4.66, 27.7)	0.25	7.69 (1, 19.2)	**0.01**
Honey bees / 100 flowers	424.03 (147.75, 723.35)	0.27	9.27 (1, 12.56)	**0.01**

[a] Sampling time = 10 minutes.

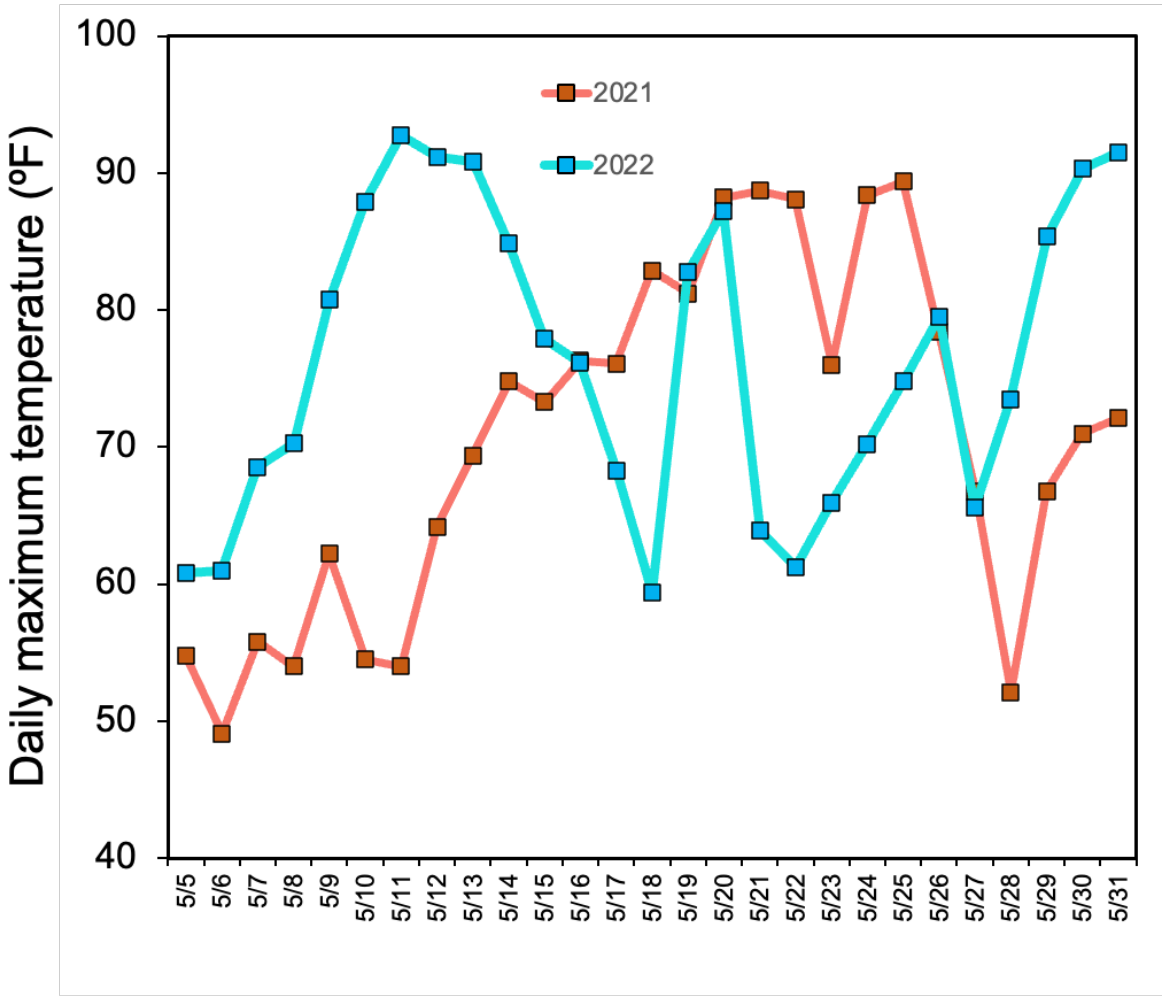

Figure S2.1. Average daily temperature in Grand Junction, Michigan during two highbush blueberry bloom seasons. Pink line: 2021; teal line: 2022. Data obtained from MSU Enviroweather weather station.

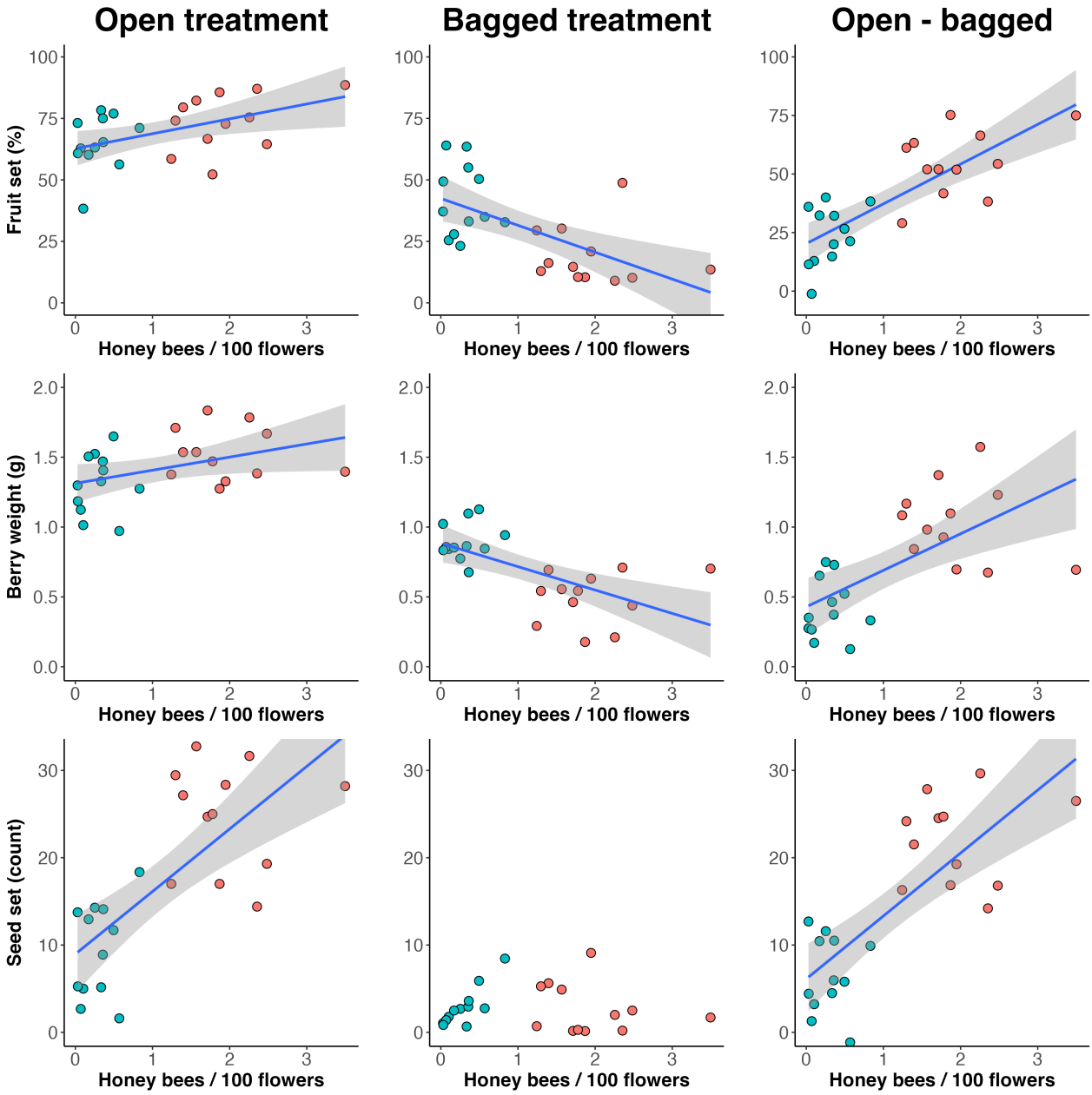

Figure S2.2. Relationship between honey bee floral visitation (honey bees per 100 flowers) during bloom and percent fruit set in open, bagged, and pollination service treatments (top), berry weight in open, bagged, and pollination service treatments (middle), and seed number per berry in open, bagged, and pollination service treatments (bottom) across 12 fields of 'Bluecrop' blueberries in Michigan in 2021 and 2022. Blue lines indicate significant results of linear regression without the effect of year included in the model. Grey shaded areas show 95% confidence intervals. Pink points: 2021; teal points: 2022.

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CHAPTER 3:

A comparison of three approaches for honey bee colony size assessment

Introduction

Bees are crucial to the pollination of many fruits and vegetables, providing pollen movement that facilitates crop pollination (Klein et al. 2007). Growers of pollination-dependent crops routinely stock their fields with honey bee colonies to help ensure pollination and high yields of their crop (Mayer & Delaplane 2000; Garibaldi et al. 2017). However, the supply of managed honey bee colonies is growing at a disproportionately slower rate than the demand for pollination services (Aizen et al. 2009). Thus, there has been an increased emphasis on the provision of strong honey bee colonies to meet pollination demands (Goodrich & Goodhue 2020).

Typically, a specific stocking rate, or number of hives per acre, is recommended for growers to meet their pollination demands (Rollin & Garibaldi 2019). However, the reliability of this approach has been called into question for almost a century (Farrar 1931) because the number of adult foragers in a single colony varies widely (Farrar 1937). In Chapter 2 of this thesis, my results revealed that colony strength as a measure of adult foragers was a more significant indicator of pollinator activity than the standard measure of hives per acre. This highlights the need for assessment of colony size to ensure that all colonies meet the required minimum size for pollination. To account for the differences in foragers, some extension documents suggest minimum colony sizes of six frames of bees for colonies used in crop pollination (Sagili & Burgett 2011).

The ability to estimate the size of honey bee colonies in a field accurately and rapidly could allow growers, beekeepers, and bee brokers to make informed economic decisions about colony rental to maximize profits (Eeraerts et al., in progress). Measuring honey bee colony size can be useful for growers because it provides assurance that the product they have paid for is at the expected colony strength (Goodrich & Goodhue 2020). This approach has been used in almonds (Goodrich 2019) and has recently been evaluated in blueberries in Oregon (Grant et al. 2021), where honey bee colony size was found to predict highbush blueberry yields when the number of frames in the colony and the rate of foragers returning to the hive are correlated (Grant et al. 2021). Beekeepers can also benefit from colony size estimation. The size of a colony can provide beekeepers with a measure of colony productivity and health (Taha and Al-Kahtani 2013), and an estimation of foragers available for pollination services (Harbo 1986). This suggests that by sampling colony size, the potential of commercial colonies for providing crop pollination services can be predicted, and a size-based payment structure could be created that rewards beekeepers for supplying stronger colonies.

Growers and commercial beekeepers often engage in pollination agreements or contracts to guide the provision of colonies to farms during crop bloom (Goodrich 2017). In this arrangement, a beekeeper agrees to rent out their colonies to be placed on the land of the grower during bloom of their crop with stipulations of price, pesticide safety, placement, and delivery timing. These agreements also typically include a minimum standard for colony size based on a certain number of frames covered with bees. Then, either the beekeeper or a third-party broker will open randomly selected colonies and validate their size for the grower (Goodrich 2017), typically via the cluster count method (Nasr 1990). This involves opening the colony, then each box is viewed from above and below and the observer identifies the cluster of bees and estimates

the number of frames covered in bees to the nearest half frame (Nasr et al. 1990). Although this method of assessing colony size can be done with a high level of accuracy (Chabert et al. 2020), there is still potential for estimation error (Nasr et al. 1990). Furthermore, any approach that requires opening the hive can be a practical difficulty from a time and experience standpoint.

Alternative approaches to estimating colony size could allow for gathering the needed information from colonies without opening them. Sagili & Burgett (2011) suggested the use of "returning forager counts" as a proxy for colony size, and this method was validated by Grant et al. in 2021. This involves viewing the hive entrance and counting the number of forager bees entering the hive for one minute to estimate the number of foragers available for pollination (Sagili & Burgett 2011). This can be done in real-time, but to get an accurate count when bee activity is high, it is recommended that hive entrances are recorded and the video is viewed in slow motion. Counts of returning foragers are strongly correlated with adult bee population in the colony (Grant et al. 2021) and require little experience to perform.

Infrared (IR) image analysis has also been suggested as a method of colony size estimation (Shaw et al. 2011; Fernandez et al. 2018). This technique employs an IR camera to capture a thermal heat signature from the colony, which can be used to estimate the bee population inside the colony. The Verifli system developed by The Bee Corp (Indianapolis, IN) can be used to quantify colony size using IR imaging. In this system, a hand-held camera captures an image of the colony, this is transmitted to a central database via a cellphone app, and information from this image is translated into a frame count score that is quantified and posted to a user's online account.

Non-invasive and rapid methods for assessing colony size have the potential to maximize pollination efficiency and hive rental revenue by improving identification of sub-standard

colonies and allowing them to be targeted for remedial management or removal prior to pollination contracts. In this two-year study on research colonies and commercial colonies at blueberry farms, I compared estimates of honey bee colony size using the standard cluster count to the two non-invasive approaches using returning forager counts and IR image assessment. This study also allowed for investigation of whether the non-invasive methods had predictable bias in their estimate of colony size.

Methods

Study system

I conducted colony size assessments in 2021 and 2022 at a research apiary on the Michigan State University campus (East Lansing, MI). In the first year, I sampled 20 colonies representing a range of sizes commonly found in those used for blueberry pollination (one deep box to four deep and one medium box). In the second year, I sampled an additional 20 colonies at the same site using a similar distribution of sizes (one deep box to three deep and two medium boxes), including five control colonies with no honey bees. In 2022, I also sampled 10 haphazardly selected colonies rented for crop pollination at each of five commercial blueberry farms (n=50) near Holland, MI. These colonies were managed by different beekeepers and provided a smaller range of colony sizes than those at the MSU campus (one deep box to two deep and two medium boxes). Commercial hives were sampled once at the beginning (mid-May) and once at the end (early June) or blueberry bloom in 2022. Each colony in the study was tagged with a unique number to ensure the data were collected from the same colonies, and so that the data from cluster count, returning forager count, and IR samples could be compared.

Cluster counts and returning forager counts were done while the sun was shining, at temperatures above 12º C, when there was no precipitation, and windspeeds below 15 km/h. Infrared images were taken at least three hours after sunset when there was no precipitation. In 2021, cluster counts at the MSU research hives were performed between 8 A.M. and 10 A.M., and returning forager video assessments were taken later the same day between 3-4 P.M. Infrared images of the colonies were captured in the following night between 12-1 A.M. In 2022, a similar approach was taken at the MSU research colonies, where cluster counts were performed between 12-3 P.M., returning forager counts between 11 A.M.-12 P.M., and IR images were captured between 12-1 A.M. In the commercial hives used for blueberry pollination, cluster counts were performed between 10 A.M.-1 P.M., returning forager counts between 10:30 A.M. and 5:30 P.M, and IR images were captured between 12:30 -2:30 A.M.

Cluster counts

To estimate colony size manually using the cluster count method, each sampled colony was opened and observed visually without removing frames from the box (Figure 3.1A). The observer identified full seams of bees between frames in both the top and bottom of each box, to the nearest half frame. A full seam of bees was defined as the space between frames which is completely covered with bees when viewed from above or below (Figure 2B). The number of frames from above and below were averaged for each box, and the values from each box were summed to obtain the total frame count for each colony. Counts from medium and shallow sized boxes were multiplied by ¾ and ½, respectively, to account for the differences in frame size from a standard deep hive box.

Returning forager counts

The flight entrance count method (Sagili & Burgett 2011) was used to record the number of forager bees returning to the hive over a 1-minute period. Following the methods described by Grant et al. (2021), each hive entrance was video recorded with a smartphone camera for one minute, and recordings were slowed down post-production to count the number of honey bees that entered the colony (Figure 3.1C). Two observers independently counted bees for each recording, and the two values obtained from these counts were averaged to obtain one returning forager count for each hive.

Infrared imaging

To estimate colony size using IR image analysis, a hand-held FLIR E8-XT infrared camera (Teledyne, Wilsonville, OR) was used to capture an image of each hive 3 hours after sunset, within 24 hours of cluster counts and flight entrance counts. Images were taken at waist level, 4-5 feet away from the hive. All vehicle lights were turned off, and a headlamp was worn by the image-taker to illuminate the hive being imaged (Figure 3.1D). For each colony, the hive tag number, number of boxes per hive, size of hive boxes (shallow, medium, or deep), number of neighboring colonies on the same pallet (if applicable in commercial settings), hive location and image capture time were recorded in the image metadata. Each image was transmitted via the VerifliMobile app to The Bee Corp (Indianapolis, IN) where information from the image was quantified and translated into a frame count score using a proprietary algorithm.

Statistical analysis

All data were analyzed in R-Studio statistical software (v4.2.2; R Core Team 2022). Kendall's correlation coefficient was used to determine the correlations between cluster counts and returning forager counts, cluster counts and IR image analysis, and estimating returning forager counts and IR image analysis estimations of colony size. Kendall's correlation coefficient was used because the data did not always meet the assumption of normality required by Pearson's correlation coefficient. To produce confidence intervals for Kendall's Tau values, I used the kendall.ci function from the NSM3 package (v1.17; Becvarik et al. 2022). Data from the research colonies were analyzed separately for each year of data collection. Similarly, data from the commercial colonies were analyzed separately for each collection event (beginning and end of blueberry bloom). To determine if discrepancy between cluster counts and IR image analysis varied across the range of colony sizes, I plotted a linear model of the difference between cluster counts and IR estimations of frame count. I calculated the x-value where the best fit line intersected y=0 to obtain a point of inflection between under- and over-estimation of colony size (i.e., where the two methods provided the same estimation). I performed F-test analyses to compare the variances of cluster count and IR image analysis samples at the beginning and end of blueberry bloom, and a t-test to compare their respective mean values.

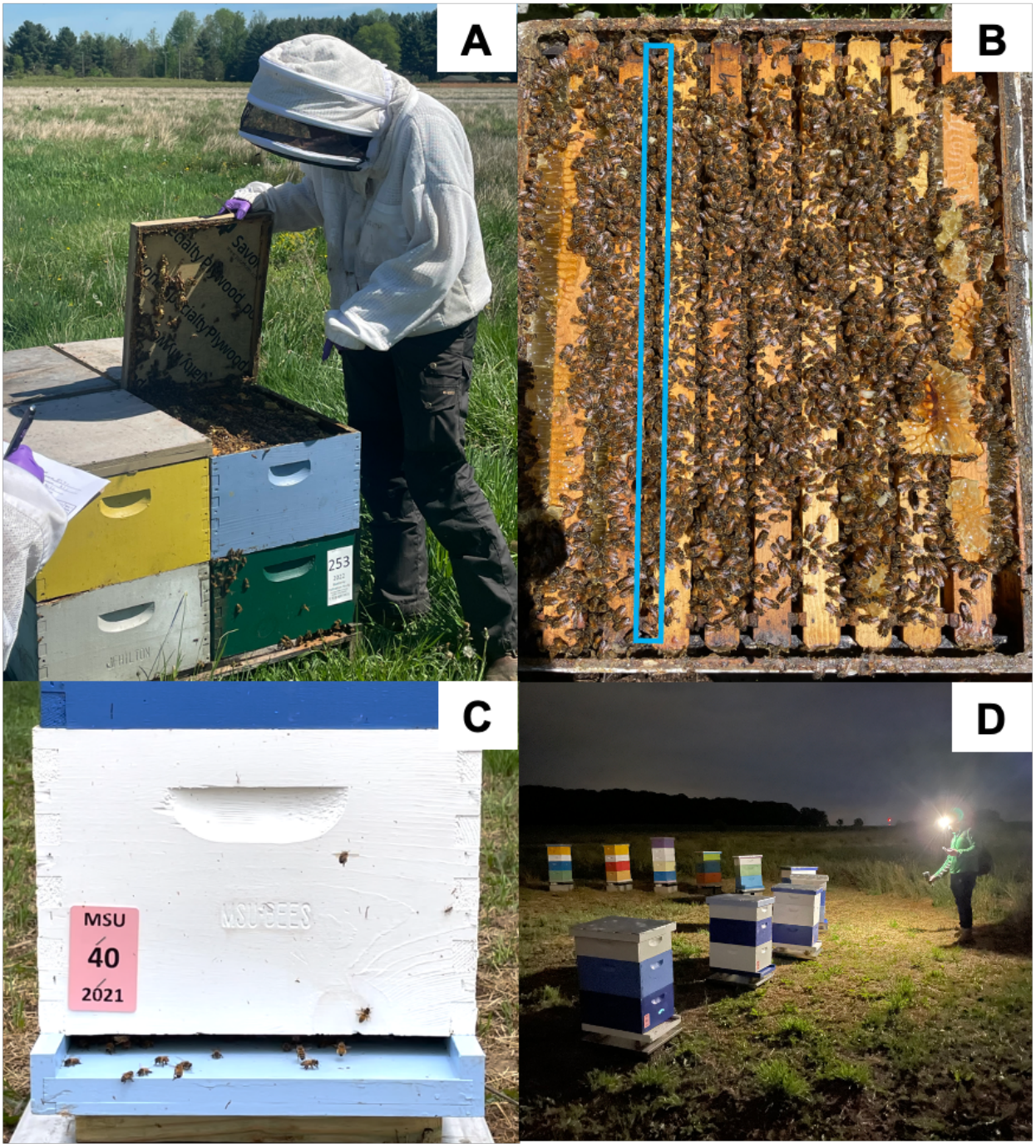

Figure 3.1. A) Observer performing a cluster count from above without removing frames from the hive. B) Point of view of observer performing cluster count. Blue box indicates one full seam of bees between frames. C) Ideal filming angle for returning forager count videos. D) Image taker uses a FLIR E8 infrared camera to capture the thermal signature of a hive at midnight.

Results

Research colonies

In 2021, cluster counts ranged from 1.25 to 12.5 frames per colony. The returning forager counts ranged from 16.5 to 81.5 bees per minute and the IR estimations of frame count ranged from 5 to 11.5 frames per colony. In 2022, cluster counts ranged in size from 0 to 22.33 frames per colony, returning forager counts ranged from 0 to 115 bees per minute, and IR estimations of frame count ranged from 2.2 to 18.1 frames per colony.

In both years, returning forager counts were positively correlated with cluster counts (2021, $\tau=0.52$, $p<0.01$; 2022, $\tau=0.72$, $p<0.01$; Figure 3.2). In 2021, there was no significant correlation between cluster counts and IR estimations of frame count ($\tau=0$, $p=1$, Figure 3.3, top left). Using cluster counts as the baseline for comparison, analysis of the difference between cluster counts and IR estimations showed that IR image analysis tended to over-predict frame counts in colonies that were manually estimated to be small, and under-predict frame counts in colonies that were manually estimated to be large, with an inflection point of ~9 frames (Figure 3.3, bottom left).

In 2022, after data scientists at Verifli made an adjustment to the proprietary algorithm used to estimate frame count from the IR images, there was a significant positive correlation between cluster counts and IR estimations of frame count ($\tau=0.62$, $p<0.01$, Figure 3.3, top right). The pattern seen in the previous year remained, with IR estimations over-predicting frame counts in colonies that were manually estimated to be small, and under-predicting frame counts in colonies that were manually estimated to be large, with an inflection point of ~12 frames (Figure 3.3, bottom right). Additionally, IR estimations for the control hives, which contained zero bees, were all above zero (range: 2.2 to 5.9 frames per hive). In 2021, there was no correlation

between returning forager counts and IR estimations of frame count (τ=0.01, p=0.97; Figure 3.4, top), but we did find a positive correlation in 2022 (τ=0.58, p<0.01; Figure 3.4, bottom).

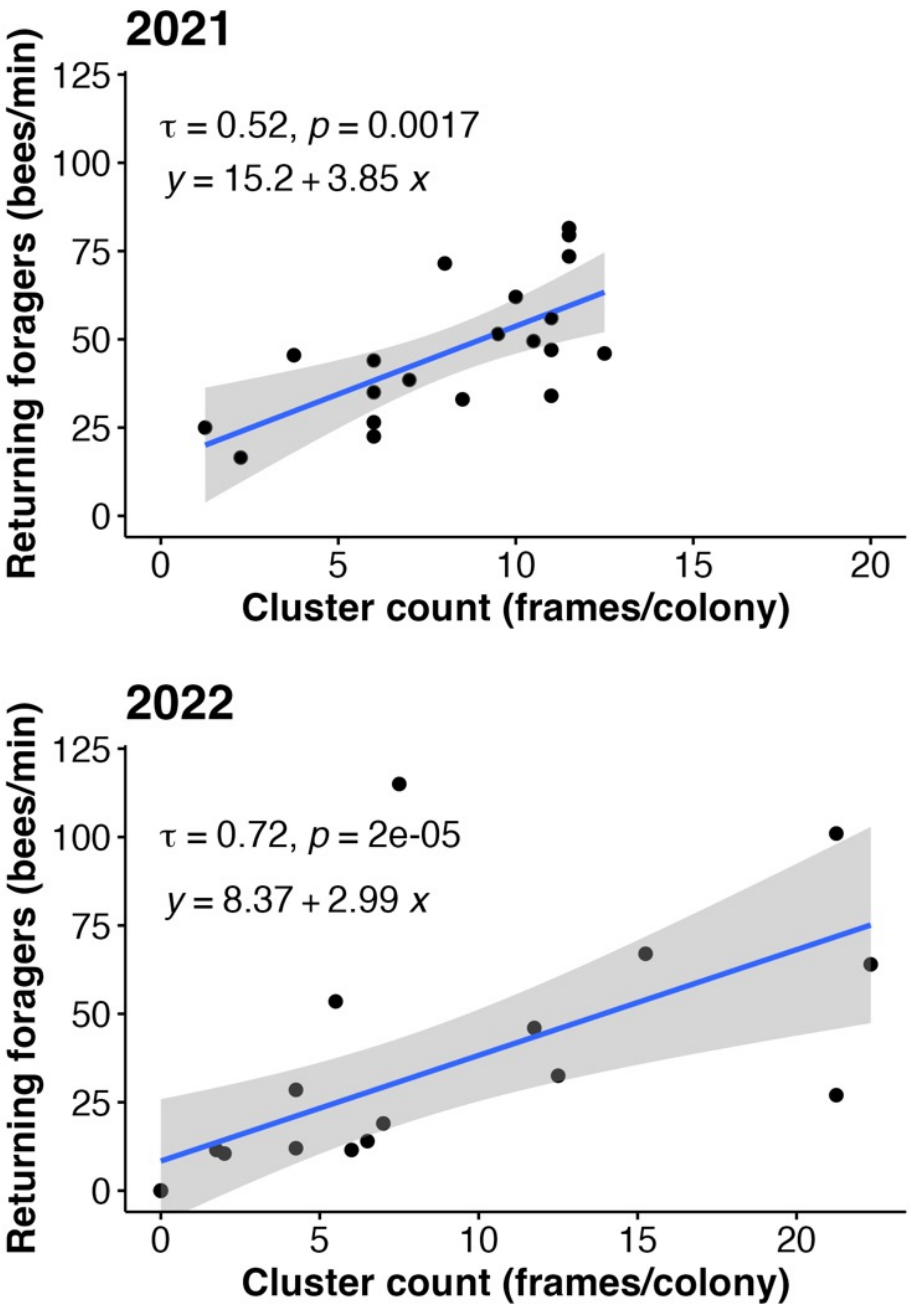

Figure 3.2. The relationship between the cluster count estimation of a colony's size and the rate of foragers returning to its entrance (n = 20 colonies) in 2021 (top) and 2022 (bottom). Blue lines indicate significant relationships, grey shaded areas show 95% confidence intervals, and each dot represents one colony.

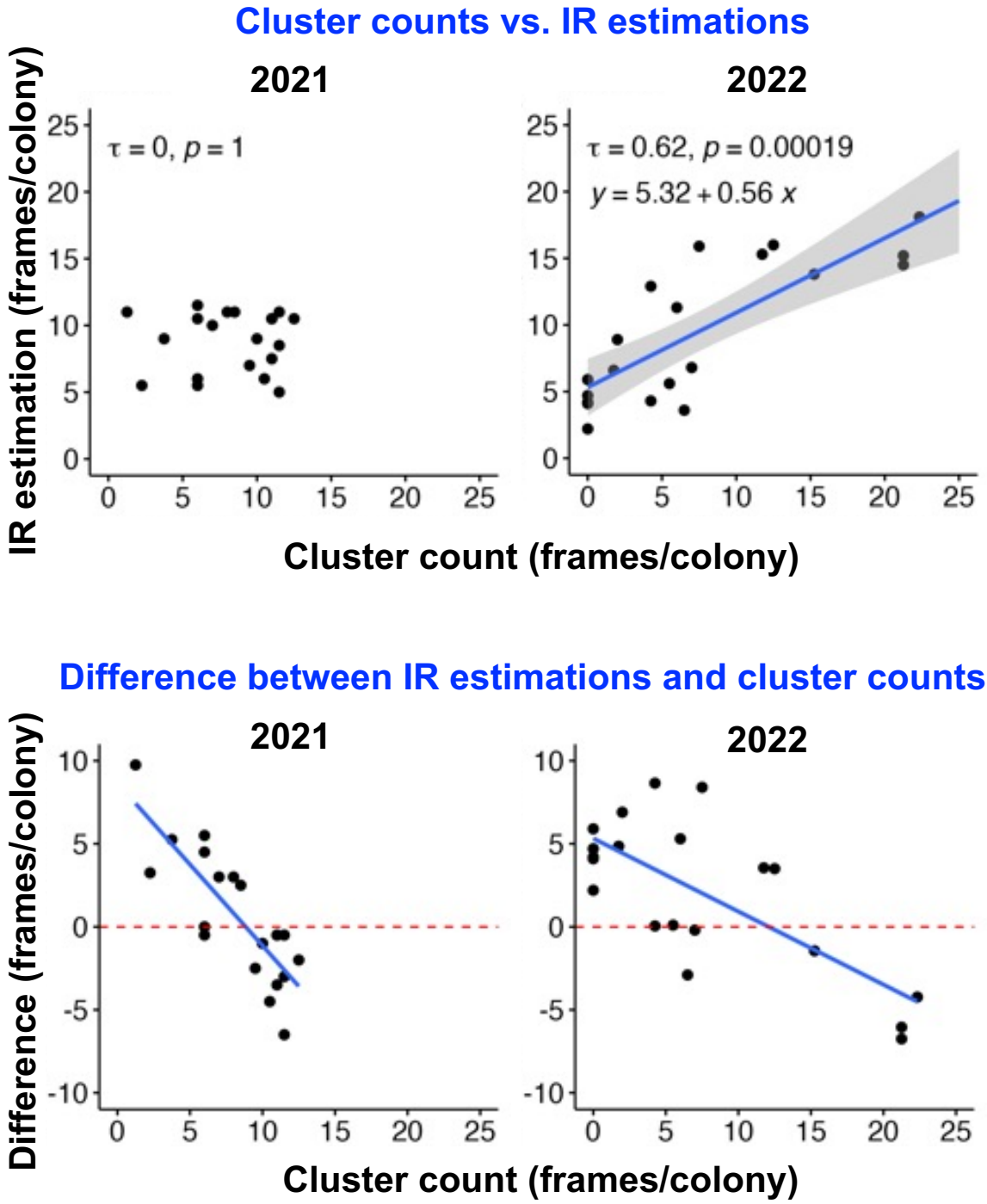

Figure 3.3. The relationship between the cluster count and IR estimation of a colony's size (n = 20 colonies) in 2021 (top left) and 2022 (top right) as well as the discrepancy between the two methods (IR estimation – cluster count) in 2021 (bottom left) and 2022 (bottom right). Blue lines indicate significant relationships, grey shaded areas show 95% confidence intervals, each dot represents one colony, and dashed red lines indicates a difference of zero between the two sampling methods.

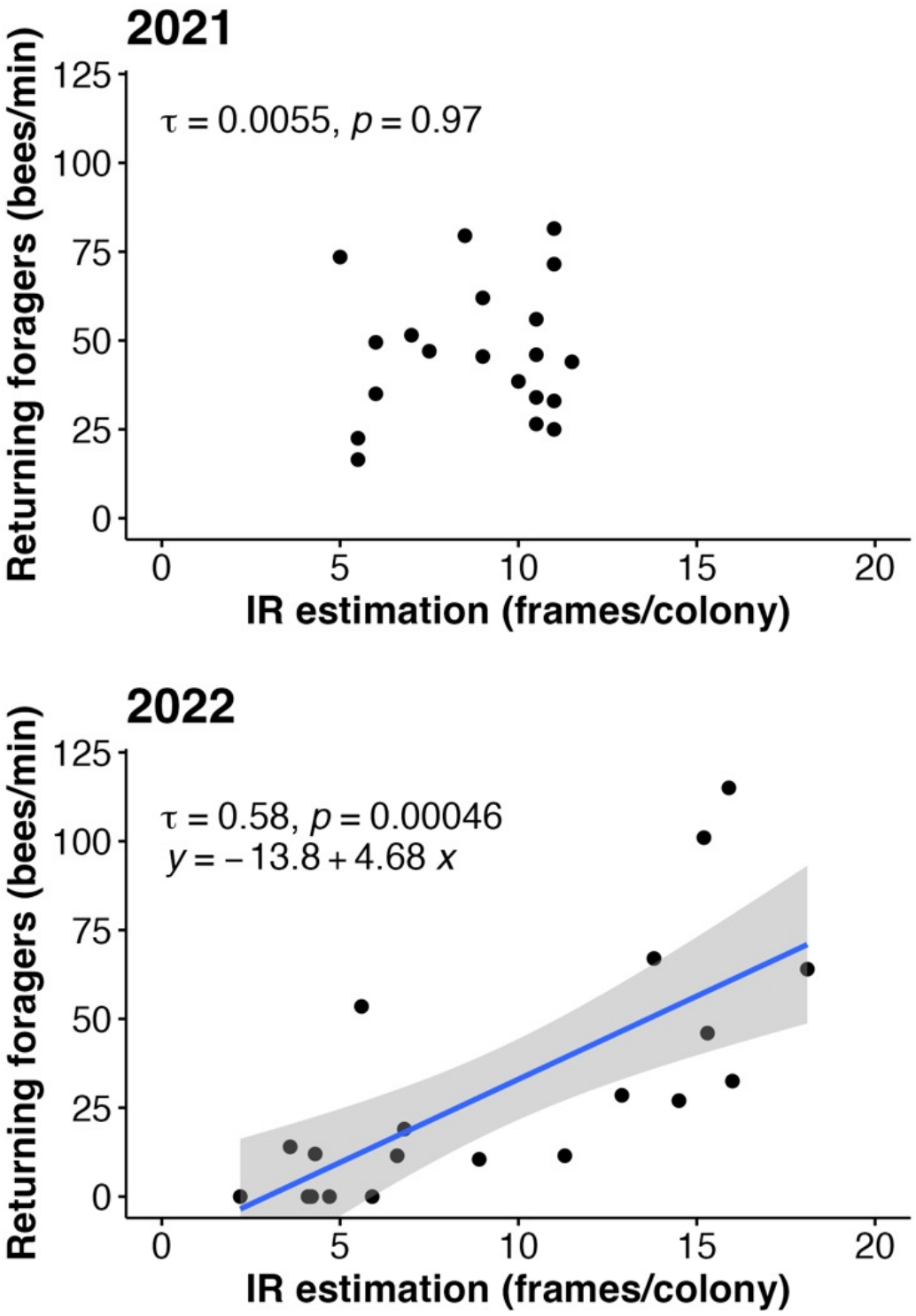

Figure 3.4. The relationship between the IR estimation of a colony's size and the rate of foragers returning to its entrance (n = 20 colonies) in 2021 (top) and 2022 (bottom). The blue line indicates a significant relationship, the grey shaded area shows the 95% confidence interval, and each dot represents one colony.

Commercial colonies

At the beginning of bloom, cluster counts ranged in size from 2.75 to 19.3 frames per colony, returning forager counts ranged from 1 to 146 bees per minute, and IR estimations of frame count ranged from 7.4 to 20.3 frames per colony. At the end of bloom, cluster counts ranged in size from 3.25 to 21.17 frames per colony, returning forager counts ranged from 16.5 to 164.5 bees per minute, and IR estimations of frame count ranged from 5.3 to 16.9 frames per colony.

At these commercial blueberry farms, returning forager counts across the beginning and end of bloom were positively correlated with cluster count estimates of frame count (beginning, $\tau=0.39$, $p<0.01$; end, $\tau=0.44$, $p<0.01$; Figure 3.5). There was a significant positive correlation between cluster counts and IR estimations of colony size at the beginning and end of bloom (beginning, $\tau=0.61$, $p<0.01$; end, $\tau=0.32$, $p<0.01$; Figure 3.6, top), and we also found a significant interaction ($t=-6.22$, $p<0.01$) between sampling round (beginning or end of blueberry bloom) and sampling method (IR or cluster count estimation of frame count).

Analysis of the difference between cluster counts and IR estimations showed that IR estimations over-predicted frame counts in all but two colonies at the beginning of bloom, while continuing to over-predict frame counts in colonies manually estimated to be small and under-predict frame counts in colonies that were manually estimated to be large, with an inflection point of ~12 frames, at the end of bloom (Figure 3.6, bottom). At the beginning and end of blueberry bloom, there was a significant positive relationship between returning forager counts and IR estimations of colony strength (beginning, $\tau=0.38$, $p<0.01$; end, $\tau=0.26$, $p<0.01$, Figure 3.7).

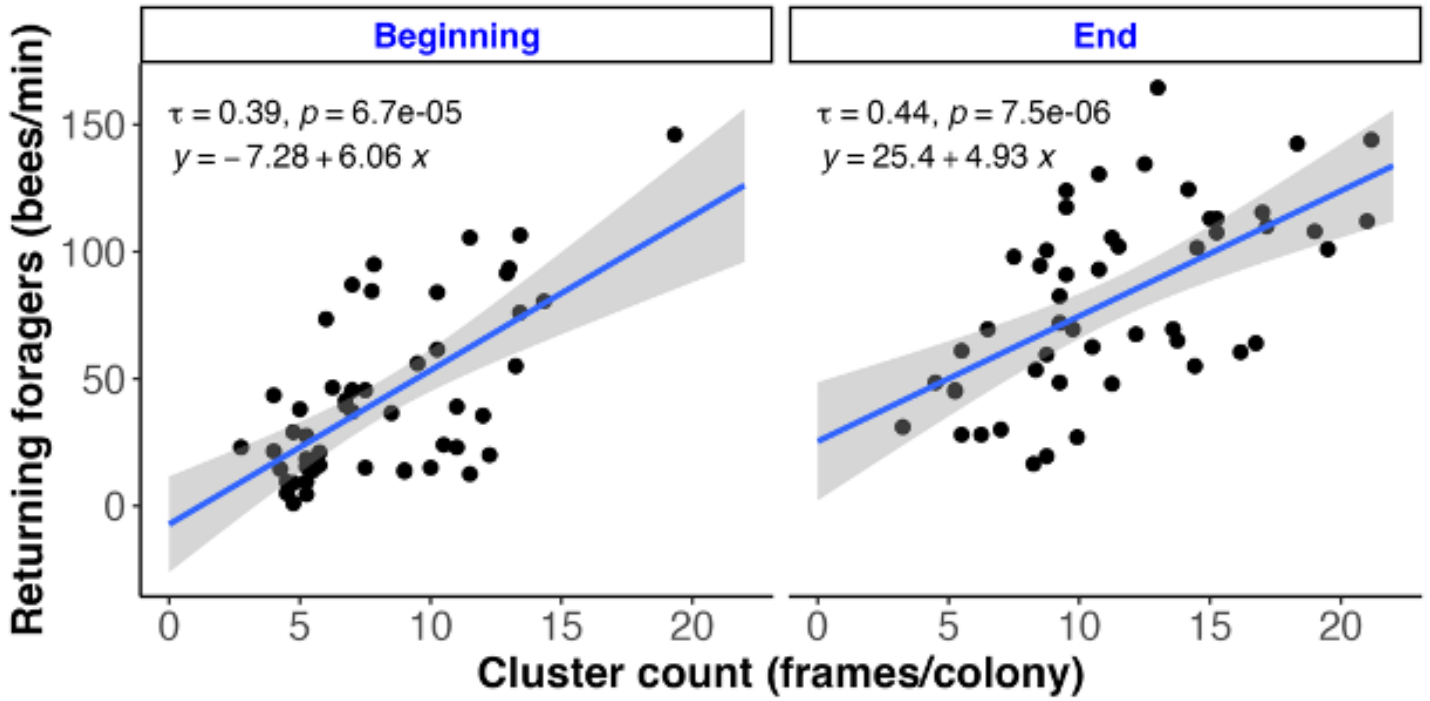

Figure 3.5. The relationship between the cluster count estimation of a colony's size and the rate of foragers returning to its entrance (n = 50 colonies) at the beginning (left) and end (right) of blueberry bloom. Blue lines indicate significant relationships, grey shaded areas show 95% confidence intervals, and each dot represents one colony.

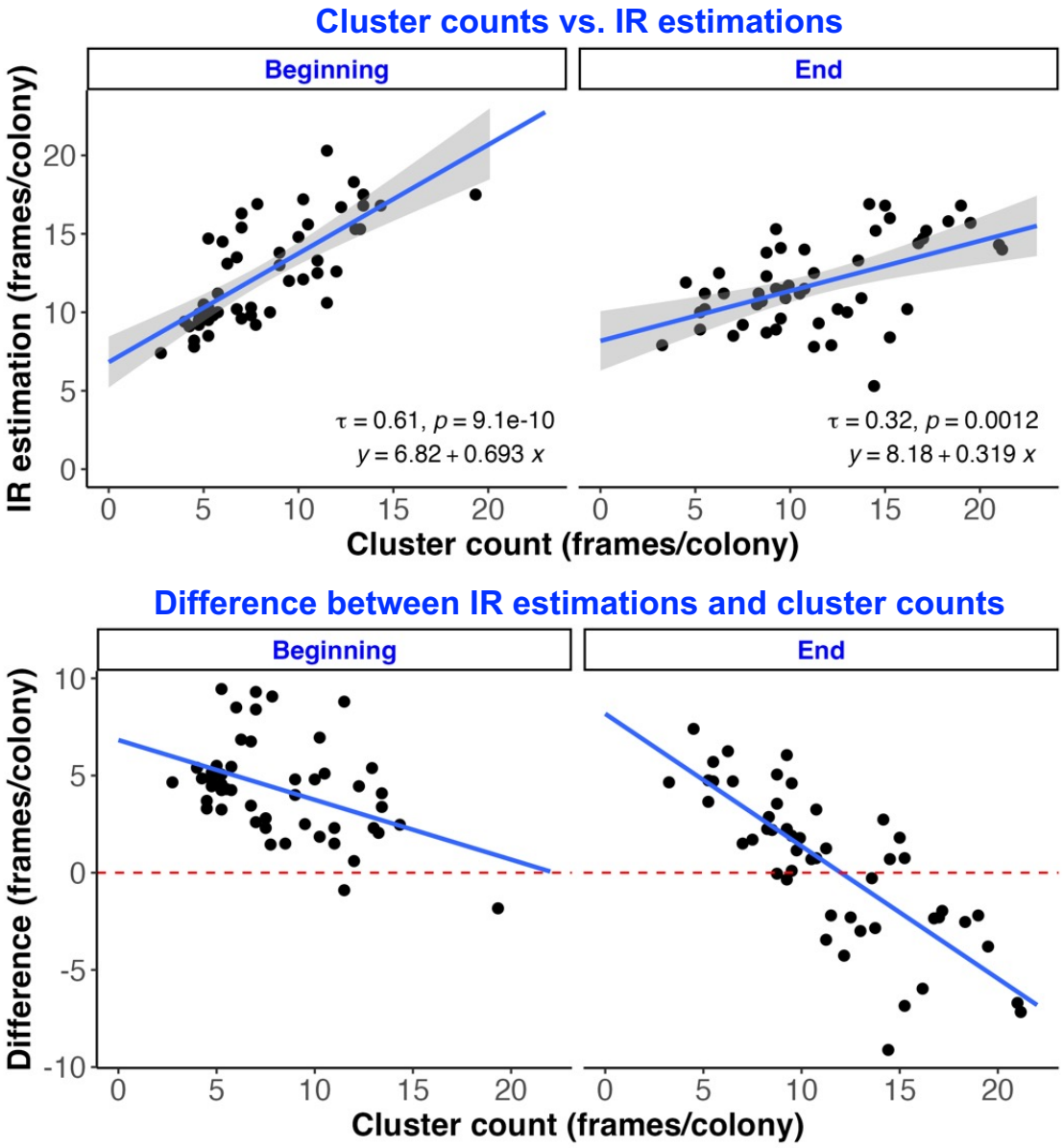

Figure 3.6. The relationship between the cluster count and IR estimation of a colony's size (n = 50 colonies) at the beginning (top left) and end (top right) of blueberry bloom, as well as the discrepancy between the two methods (IR estimation – cluster count) at the beginning (bottom left) and end (bottom right) of bloom. Blue lines indicate significant relationships, grey shaded areas show 95% confidence intervals, each dot represents one colony, and dashed red lines indicate a difference of zero between the two sampling methods.

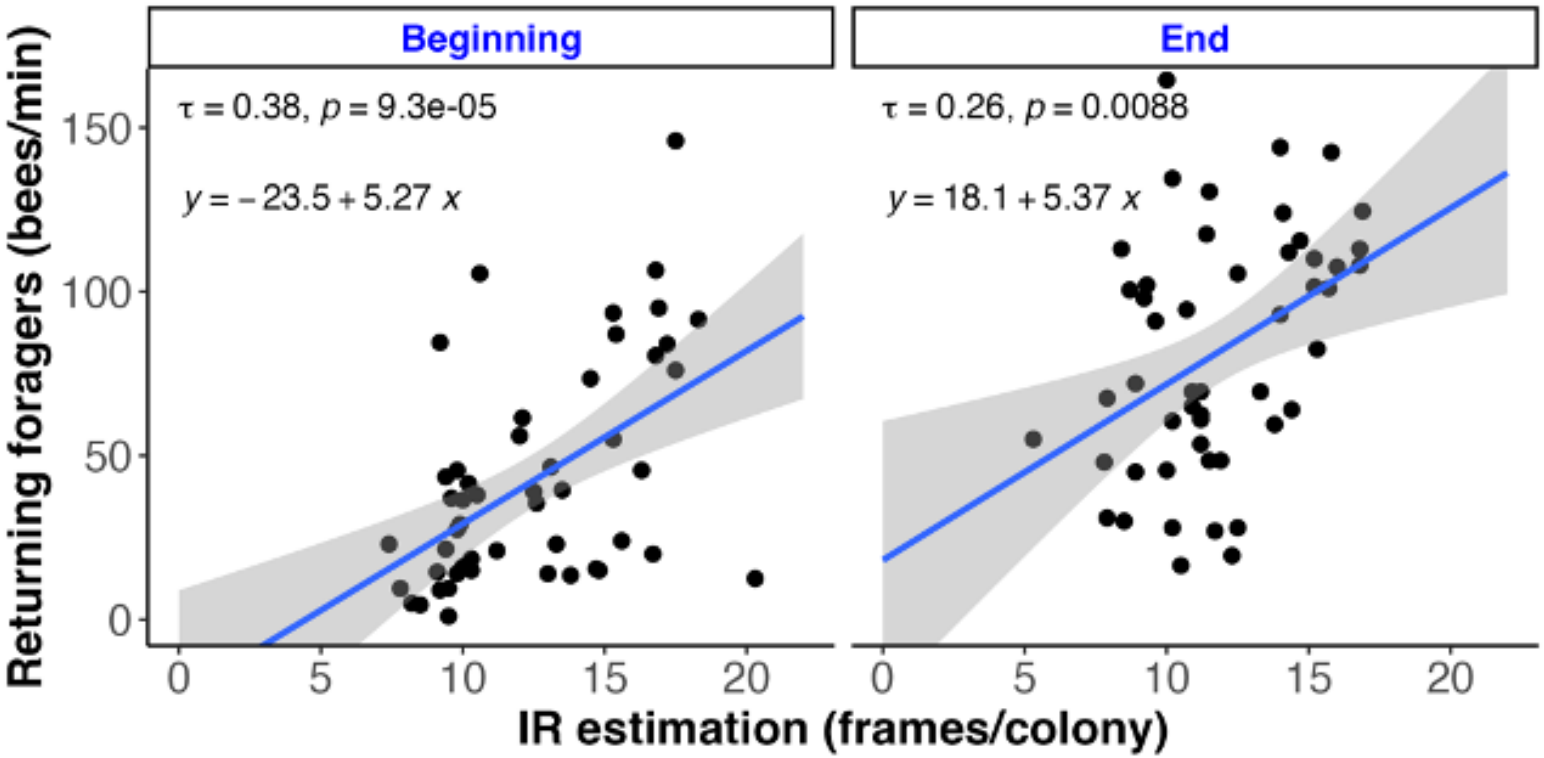

Figure 3.7. The relationship between the IR estimation of a colony's size and the rate of foragers returning to its entrance (n = 50 colonies) at the beginning (left) and end (right) of blueberry bloom. The blue lines indicate significant relationships, the grey shaded areas show 95% confidence intervals, and each dot represents one colony.

Discussion

This study has demonstrated that both non-invasive methods tested here - returning forager counts and IR sampling of individual honey bee colonies - can provide a rapid and non-invasive method for assessing colony size in honey bee colonies. The results of both of our small-scale studies as well as our commercial-scale study validate the findings of Grant et al. (2021), showing a strong positive relationship between cluster counts and counts of returning foragers estimated using video recordings. This indicates that returning forager counts can be reliably used as a proxy for colony size; however, to ensure accurate counts, this method requires extra equipment and resources including a video camera, multimedia player software, and time to watch videos. Future methods development may be able to automatically assess videos for bees returning into the hive entrances, increasing ease of use, and potentially improving speed and accuracy of data collection. Additionally, practice videos of colonies with different activity levels could be used to train observers to what a small, medium, or large colony should look like during good foraging weather.

After the first year of the small-scale study when we did not find any relationship between cluster count and IR estimations, the algorithm for image analysis was adjusted, and this resulted in a significant positive correlation between cluster counts and IR estimations in both the research and commercial colonies in 2022. The IR analyses still resulted in overestimation of small and underestimation of large colonies. The consistent discrepancy in colony size estimation suggests that algorithm adjustment could improve the correlation and similarity in colony size estimates from IR sampling method. Additionally, the IR camera must have detected some level of heat emanating from the empty hives, as IR estimations for the control hives in the 2022 small-scale study all fell above zero (Figure 3.3). This may have been caused by leftover wax

comb or propolis in the hives, potentially interfering with the thermal signature due to heat retention.

Many of the colonies in the commercial study were equipped with an additional hive box, known as a super, during bloom, to accommodate for the honey surplus during nectar flow (Delaplane 1997, 2010). This added space is generally associated with the increase, or at least the stability, of colony population size (Delaplane 1997). The expected growth in colony size throughout the bloom period was reflected by the cluster count method and validated by the returning forager count method. However, IR image analysis of frame count indicated a decrease in average colony sizes from the beginning to the end of bloom (Table S3.1, Figure S3.1), suggesting that the analyses may be over-compensating for the larger hive size due to additional boxes. An alternative interpretation of this result is that there may not have been an increase in colony population, but that cluster counts were biased toward an increase in size due to the addition of hive boxes. This interpretation supports beekeeper concerns about decreased colony strength after blueberry pollination due to a combination of poor nutrition and pesticide exposure, a claim which has been substantiated by numerous studies in the literature (Girard et al. 2012, Colwell et al. 2017, Topitzhofer et al. 2019, Grant et al. 2021), as well as the results of the IR image assessments in this study.

This study compared practical approaches to colony size estimation, acknowledging that we did not count the bees directly. The most accurate way to determine the adult bee population of a colony is by shaking out and weighing all the adult bees in the colony (Chabert et al. 2021). While this is disruptive and not possible with commercial colonies being rented for commercial pollination, this method could be used in future research to provide a baseline for comparison against cluster counts, returning forager counts and IR estimations to understand which method

best captures the true adult population in the colony. The purpose of this study was to compare against each other the current methods of estimation that can feasibly be used by beekeepers and growers in commercial pollination settings, but more research is required to determine which of these methods is most accurate. It would also be useful for a cost-benefit analysis to be conducted to determine whether the value added of this service is enough to offset its cost.

IR image analysis as a rapid, non-invasive method of colony size assessment has the potential to improve the practices of beekeepers and growers alike. Beekeepers can benefit from the identification of sub-standard colonies for remedial management in the spring before commercial pollination, or at the end of summer before returning to overwintering sites. Furthermore, by selecting only strong colonies for pollination contracts, beekeepers can ensure that colonies provided to growers are at or above the minimum size for pollination. The use of IR image analysis as a tool for colony size validation shows promise due to correlation with the standard cluster count method, however, more research is needed to understand and adjust for the discrepancy between IR estimations of frame count and cluster counts and returning forager counts. Additional research should determine how these methods relate to the true number of foragers available for pollination services.

APPENDIX

Table S3.1. Comparison of cluster count and IR image analysis predictions of honey bee colony size between the beginning and end of highbush blueberry bloom. *P*-values in bold are significant at α = 0.05.

Method	**Beginning mean (SD)**	**End mean (SD)**	**Mean difference (95% CI)**	***t* (df)**	***P*[a]**
Cluster count	8.20 (3.53)	11.39 (4.48)	3.19 (-3.90, -2.48)	-9.03 (49)	**<0.001**
IR estimation	12.50 (3.30)	11.81 (2.78)	-0.69 (-0.24, 1.62)	1.50 (49)	0.14

[a] Paired samples *t*-test.

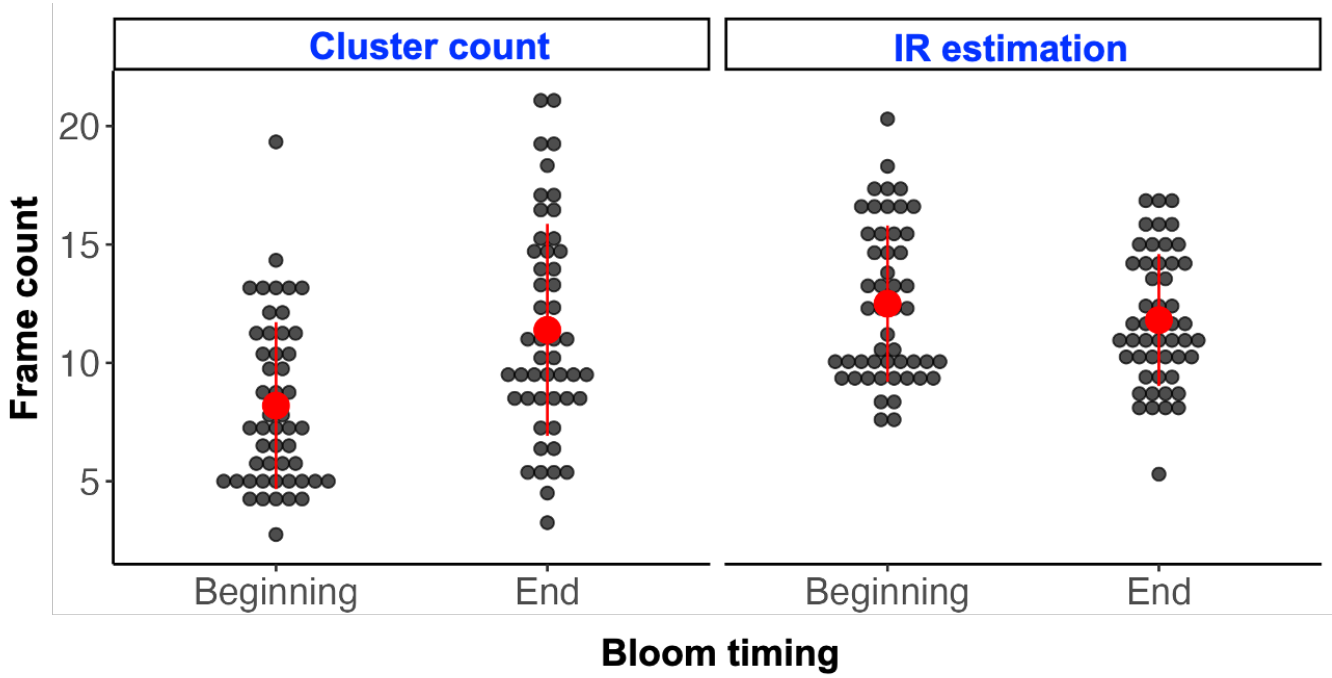

Figure S3.1. Predicted frame counts at the beginning and end of blueberry bloom by cluster counts (left) and IR estimations (right). Each grey dot represents one colony (n=50 colonies). Red dots indicate sample means, and red lines indicate standard deviation from the mean.

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CHAPTER 4:

Conclusions and future directions

Given the high input costs of blueberry production, it is crucial that growers invest in pollination to ensure profitability. The purpose of this thesis research was to investigate the role of honey bee hive stocking density and colony strength for highbush blueberry pollination. No study before this has revealed the contribution of a combination of colony stocking density and strength on highbush blueberry pollination outcomes, and I found that understanding how these factors combine to determine on-farm honey bee abundance and subsequent pollination service is necessary to inform colony stocking decisions.

In the Chapter 2 of this thesis, I investigated how colony stocking density and strength contribute to honey bee abundance and subsequent pollination services on highbush blueberry farms. The results of this research indicate that increasing colony stocking density from 2-4 to 4-6 hives per acre had no observable effect on honey bee abundance in blueberry farms. However, increasing average colony strength did contribute to increased bee abundance in blueberry farms. This pattern was only observed if measured as a count of returning foragers, rather than a cluster count. This is likely because a count of returning foragers represents a more accurate depiction of the foraging force supplied by a honey bee colony, whereas a cluster count may be accounting for younger bees not yet of foraging age. Thus, growers should ensure that rented colonies are of sufficient strength by performing returning forager counts. What's more, a measure of "stocking strength" taken by multiplying the average strength of rented colonies by the number of colonies per acre, was found to be the strongest predictor of honey bee abundance on farms. This result suggests that growers can directly influence pollinator activity on their farms by manipulating

stocking density and strength in combination, and that if colony density will be increased, it should be done with strong colonies.

My results in Chapter 2 emphasize the importance of stocking highbush blueberry farms with strong colonies. As such, colony strength assessment can be a valuable tool for growers to ensure a strong enough foraging force to pollinate their crop. Since honey bee colonies should not be handled without the assistance of a beekeeper, growers should assess colonies using a method that does not require opening the hive. In Chapter 3 of this thesis, I compared the standard cluster count method for evaluating honey bee colony strength to two rapid, non-invasive methods: the count of foragers returning to the hive, and infrared image analysis. Results from this study revealed that counts of foragers returning to the hive were consistently correlated with cluster counts, indicating that growers can successfully perform colony strength assessments at the hive entrance without opening the colony.

Infrared estimations of colony strength were not correlated with cluster counts in 2021. However, after The Bee Corp (Indianapolis, IN) adjusted their model's algorithm, infrared estimations of colony strength were correlated with cluster counts in 2022, suggesting that the Verifli product has potential for continued improvement as more data are collected. A current drawback of the product lies in its inability to identify empty colonies with zero bees. While additional improvements are needed to mitigate this discrepancy, the results of this study indicate that infrared image analysis is currently a promising approach to estimate colony strength. Of all three methods, the infrared image analysis is the fastest, potentially allowing for the rapid sampling of all colonies on a farm, however the cost of the service may be prohibitive. To maximize return on investment, more research is needed to determine if this approach to

estimating colony strength is predictive of honey bee abundance and subsequent pollination services.

I found that pollination services to 'Bluecrop' blueberry fields were best predicted by honey bee abundance in those fields, rather than colony stocking density or the strength of the colonies. Specifically, the number of bees per flower was predictive of all yield components, including fruit set, berry weight, seed set, and partial estimated yields. The number of honey bees per 10-minute transect sample was positively correlated with seed set and partial estimated yields. Although the number of bees per flower had the strongest association with yield components, this method of evaluating pollination is challenging to perform, because accurately sampling flower density is time-consuming. Instead, growers may still evaluate pollination by counting the number of honey bees visiting open flowers on the half bush facing them in a 100 m transect sample along the row. In general, a count of fewer than 50 bees per 10-minute sample is more likely to have poor to moderate levels of pollination, whereas fields with counts above 50 bees per 10-minute sample are more likely to have moderate to high levels of pollination.

This study focused on the abundance of honey bees on farms, colony stocking density, and strength, but there is still a need to better understand the other potential factors influencing the pollination of highbush blueberry (DeVetter et al. 2022). The results of this study across two very different pollination years suggest that weather is a key factor influencing bloom phenology and subsequent honey bee floral visitation. Further, there is evidence that pollen exposure to extreme heat reduces floral attractivity to pollinators (Descamps et al. 2021, Russel & McFrederick 2022). In addition, extreme heat can have detrimental effects on honey bee colony health and activity (Bordier et al. 2017, Medina et al. 2018). Therefore, future studies should determine if increasing honey bee colony stocking strength can be used to improve pollination

outcomes in growing seasons with poor weather. There is mounting confirmation of climate warming in the scientific literature, with crop production at high risk of experiencing detrimental effects (Walters et al. 2022). Future research should prioritize the study of the impact of extreme heat events on pollination outcomes to inform pollinator management in a warmer world. In addition, there are many other factors which likely influence pollination on commercial blueberry farms, including nutrition (Girard 2012, Topitzhofer 2019, Toshack 2019), disease susceptibility (Wardell 1982, Grant et al. 2021), pesticide exposure (Graham et al. 2021 & 2022), colony density of the surrounding landscape (Eeraerts et al. 2022), and more. Future studies should aim to determine the interactions between honey bee abundance, colony health during bloom, and landscape factors to further optimize the pollination of highbush blueberry.

The results of this study revealed a potential flaw in a commonly used experimental approach to estimate pollination service, or the realized contribution of pollinators to crop yield (Garratt et al. 2021). Excluding pollinators from flowers using mesh bags is a common experimental control used to estimate pollination service in these experiments, but the results of this study indicate that this methodology may lead to spurious results. Fruit set and berry weights in bagged branches declined with increasing honey bee visitation to the unbagged flowers, leading to increasing estimates of pollination service as the bee density increased. I hypothesize that resources were shifted away from the poorly pollinated branches in bags, with reallocation increasing towards unbagged branches on the rest of the bush as honey bee abundance and subsequent pollination increased. This theory is substantiated by similar findings in pollen limitation experiments (Zimmerman & Pyke 1988). Future studies should test this hypothesis by excluding branches or even whole bushes from pollinators at varying scales to determine if resource reallocation is indeed occurring. Results from previous pollination service experiments

should be interpreted with caution if similar methodologies to this study were used to exclude pollinators.

The results of this thesis can be used to guide the strategies used for stocking honey bee colonies during highbush blueberry bloom in Michigan. Future research should focus on the economics of pollination to determine whether additional investment in stronger colonies results in increased profits. In addition, multiregional, crop-specific research is needed to apply these pollination strategies to other systems. In conclusion, the importance of honey bees to commercial highbush blueberry pollination in Michigan is abundantly clear, and this relationship will likely only strengthen as the acreage of blueberries expands, horticultural developments and plant breeding increase the density of flowers produced by this crop, and the demand for pollination continues to grow.

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