HONEY BEE (*APIS MELLIFERA*) FORAGING PREFERENCES ARE NEGATIVELY CORRELATED WITH ALFALFA LEAFCUTTING BEE (*MEGACHILE ROTUNDATA*) PRODUCTIVITY IN VIRGINIAN LANDSCAPES

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ABSTRACT

Honey bees (*Apis mellifera*) may serve as bioindicators of habitat quality for themselves and also other insect pollinators because we can observe, decode, map, and analyze the information encoded in the waggle dance communication behavior, which allows us to know where and when bees are collecting high quality forage. Previously we measured honey bee foraging dynamics for two years (2018-2019) by waggle dance decoding at three geographically distanced sites in Virginia (Blacksburg, Winchester, Suffolk), consisting of different dominant landcover types. Here we use those data on where and when honey bees were finding profitable resources throughout the season to predict the success of a non-*Apis* bee in these same landscapes.

Alfalfa leafcutting bees (*Megachile rotundata*) are managed, polyleptic, solitary, cavity-nesting bees that are widely naturalized in North America. We selected *M. rotundata* as a model organism to validate the honey bee foraging data because they share some characteristics with other cavity nesting wild bees, but they are a tractable study system because they are commercially reared and can be purchased for study. At each of the three sites, we installed 15 nest box stations, each stocked with nesting materials and 160 *M. rotundata* cocoons, at varying distances and directions from the original honey bee hive locations. Most importantly, nest box stations were distributed across a range of honey bee foraging propensities, calculated as the mean foraging probability determined from our honey bee waggle dance decoding data, within a 300m buffer around each nest box. We hypothesized that honey bee foraging probability would positively correlate with *M. rotundata* cocoon production and survival.

For two years (2021-2022) from May-August, we monitored the nest boxes and also collected data on the relative abundance of floral resources at each of the 15 stations per site. At the end of each season, we collected nesting materials and counted both *M. rotundata* along with incidental (i.e., non-*M. rotundata*) wild bee cocoons. *M. rotundata* cocoon productivity varied by location (log-likelihood ratio test: $\chi^2 = 311.0$, df = 2, $p < 0.001$), with Winchester as the most
productive location (mean cocoon count (95% CI): 26.2 (23.7 to 28.9)), followed by Blacksburg (20.4 (18.2 to 22.9)), and Suffolk (4.4 (3.5 to 5.5)). The abundance of clover, both red and white, had a significant positive effect on ALCB productivity (log-likelihood ratio test: $\chi^2 = 778.36, < 0.001$). On the other hand, the number of ALCB cocoons decreased significantly with the count of *Trypoxylon* wasp cocoons present in the nest boxes (log-likelihood ratio test: $\chi^2 = 54.37, < 0.001$). Most importantly, we found that there was an overall negative relationship between honey bee foraging probability and alfalfa leafcutting bee cocoon productivity (log-likelihood ratio test: $\chi^2 = 55.42, < 0.001$), where areas of higher honey bee foraging probability were associated with lower levels of alfalfa leafcutting bee productivity. This surprising result is in the opposite direction to our original hypothesis that preferred honey bee foraging areas in the landscape, as indicated by decoded waggle dance data, would be positively correlated with alfalfa leafcutting bee productivity. These data demonstrate that while honey bees may indeed act as bioindicators to other insect pollinators, this indication will likely be species and context specific and may even specify the opposite direction.
HONEY BEE (*Apis mellifera*) FORAGING PREFERENCES ARE NEGATIVELY CORRELATED WITH ALFALFA LEAFCUTTING BEE (*Megachile rotundata*) PRODUCTIVITY IN VIRGINIAN LANDSCAPES

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GENERAL AUDIENCE ABSTRACT

Recent challenges facing the beekeeping industry have laid bare the fragility of honey bee pollination services, highlighted the role of other bee pollinators, and sparked widespread concern over the effect of declining bee populations on food security and continued ecosystem function and services. Both honey bees and wild bees face similar challenges including pesticides, parasites, pests, and poor nutrition from a lack of flowers in the landscape. Therefore, it is critical that we develop methods to evaluate the landscape’s ability to feed bees in order to help them and other pollinators continue providing essential pollination services.

There are many ways to measure the quality of a landscape for pollinators, but honey bees offer a unique opportunity to do the work for us: honey bees communicate the location of where they find food to their nestmates through a behavior called the waggle dance. Waggle dances can be observed and the dance language decoded so that we can determine the location of high-quality food sources. Previously, we used honey bee waggle dance data to map where bees are collecting food in three geographically distinct sites (Blacksburg, Winchester, and Suffolk, Virginia). These data allow us to understand where, when, and on what flowers the honey bees were feeding.

The goal of this project was to investigate the relationship between honey bee foraging and non-honey bee success across the same three landscapes to determine if honey bees can be used as bioindicators of habitat quality for other bees. We chose Alfalfa leafcutting bees (*Megachile rotundata*) as a model organism because they are solitary, cavity-nesting bees, like the majority of wild bees. However, as managed pollinators, Alfalfa leafcutting bees (ALCBs) can be purchased commercially and retained in nesting boxes to allow us to gather productivity and survival data, which we can then compare to what the dancing honey bees previously told us about where and when they can collect good food. We hypothesized that areas of the landscape that honey bees had indicated where higher quality would correlate to better ALCB cocoon production and survival.
We placed wooden nest boxes, 15 per site, stocked with ALCBs across the same landscapes for which honey bee data had been collected in the previous years and measured their productivity in terms of cocoons produced at each site. ALCB productivity varied by location, with Winchester as the most productive location (mean ALCB count (95% CI): 26.2 (23.7 to 28.9)), followed by Blacksburg (20.4 (18.2 to 22.9)), and Suffolk (4.4 (3.5 to 5.5)). The abundance of clover, both red and white, had a significant positive effect on ALCB productivity (log-likelihood ratio test: $\chi^2 = 778.36, < 0.001$). On the other hand, the number of ALCB cocoons decreased significantly with the count of a non ALCB nest box resident, Trypoxylon wasp cocoons, present in the nest box (log-likelihood ratio test: $\chi^2 = 54.37, < 0.001$). Surprisingly, we found that there was an overall negative relationship between honey bee foraging probability and alfalfa leafcutting bee cocoon production (log-likelihood ratio test: $\chi^2 = 55.42, < 0.001$). In this study, across three different field sites with varying landscapes in Virginia, areas of higher honey bee foraging probability were associated with lower levels of alfalfa leafcutting bee productivity.
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CHAPTER 1: INTRODUCTION

Bee pollination is ecologically and agriculturally essential

The health of our wild and agricultural North American ecosystems depends on a wide range of insect fauna to deliver pollination services (Rodger et al., 2021; Katumo et al., 2022). Most flowering plants (87.5%) depend on animal pollination, of which insects account for the vast majority (Ollerton et al., 2011). Butterflies, beetles, ants, wasps, and flies serve the critical role of ensuring the reproduction of the flowering plants that sustain us, but one group - the bees - buzz above the rest. Over 4,000 species of native bees call this continent home and are the primary pollen spreaders for most of our wild and crop plants (Cane & Tepedino, 2001). Bees and apoid wasps (Apoidea) descended from stinging wasps (Hymenoptera: Aculeata), switching from carnivory to pollenivory around 120 million years ago (Branstetter et al., 2017). Bees (Anthophila) coevolved with their plant allies, acquiring nutrition from the pollen and nectar they provide, and in turn, efficiently transferring pollen from one plant to another to facilitate reproduction. As bees diversified alongside plants, many of the relationships between bees and their flowers have become specialized, creating plant-pollinator networks that may be co-dependent (Zayed et al., 2005; Burkle et al., 2013). Therefore, it is essential that the greatest number and diversity of bees be conserved to maintain these relationships, many of which are not well characterized.

In agricultural ecosystems, both wild bees and managed honey bees are crucial to the production of circa 75% of agricultural crops (Klein et al., 2007). Honey bees have long been the most economically important pollinator (Aizen & Harder, 2009) because they possess many workers (approx. 10,000-60,000 per colony) and can fly far to forage (usually >1 km, but sometimes as far as 10-12km) and provide pollination services (von Frisch, 1967; Gruter & Hayes, 2022). The fact that honey bees can be easily transported in large numbers; provide honey, wax, and propolis; and are a fascinating example of a eusocial superorganism all contribute to our reliance on and obsession with honey bees. However, in recent years, we have seen an increased understanding of how critically important a wide range of different bee species is to ensuring agricultural and wild plant pollination (Klein et al., 2007). Many crops are already pollination-limited, where they are currently not receiving the level of pollination needed to maximize production (Reilly et al., 2020). Therefore, managed (e.g. honey bees, bumble bees,
leafcutting bees, mason bees, alkali bees) and wild bee populations will need to be not just sustained, but increased, to meet the needs of growing demand for agricultural productivity while maintaining pollination services to natural ecosystems in the future (Aizen & Harder, 2009; Jordan et al., 2021).

**The global stock of honey bees is outpaced by the demand for their services**

From 1961 to 2017, the total number of managed honey bee colonies increased globally by 85%, with increases on every continent except for North America and Europe, which have experienced per capita declines over the same time period (Phiri et al., 2022). In North America, the total number of honey-producing colonies increased from a low of 2.3 million colonies in 2008 to 2.8 million colonies in 2018, despite the rise in colony loss rates (USDA, 2020). Although the number of colonies has nearly doubled globally, the human population has more than doubled over this time period, representing a per capita loss of colonies (Phiri et al., 2022). There have been regional declines in honey bee stocks in the USA, with a 59% loss of colonies between 1947 and 2005 (Natural Resources Council, 2006). Concurrently the need for insect-mediated pollination in agriculture has increased approximately three-fold in the last half century (Aizen & Harder, 2009), and the pollination services of honey bees alone is currently valued at over $250 billion annually in the US (Bond et al., 2021). The need for large-scale, managed honey bee pollination has thus taken an outsized role, where the production of crops such apples, almonds, cranberries, and citrus are now reliant on honey bee colonies supplied by commercial operations (Mader et al., 2010).

Honey bee-dependent crops have expanded rapidly and require very close to the total number of hives available both in the United States and globally (Aizen & Harder, 2009). For example, pollination of California almonds requires more than 68% of all U.S. commercial honey bee colonies (Goodrich et al., 2019). Commercial colonies used for pollination of almonds and other major crops such as citrus and cherries are subjected to a stressful combination of poor nutrition from limited flower resource diversity, exposure to pesticides, long-distance travel, and high stocking densities (Bond et al., 2021). These conditions lead to increased vulnerability to disease, as well as potential viral spillover into non-commercial honey bee stocks and native bees (Fürst et al., 2014). So, while commercial honey bee stocks are currently meeting, or close to meeting demand from growers, there is concern that may not be the case in the near future, due
to both the growing demand and vulnerability that comes from relying on a single species for this critical service. Therefore, improving pollination resiliency will rely on enhancing honey bee health, increasing the total number of colonies, and developing alternative pollination strategies using non-\(Apis\) managed bees and wild bees.

**Non-\(Apis\) managed bees and wild bees are important and declining.**

Native bees, as well as managed species such as bumble bees (\(Bombus impatiens\)), mason bees (\(Osmia lignaria\)), and alfalfa leafcutter bees (\(Megachile rotundata\)), are highly effective pollinators for a variety of crops, and their use can be synergized with honey bees (Garibaldi et al., 2013). For example, the presence of both honey bees and wild bees has been shown to maximize pollination of hybrid sunflowers (Greenleaf & Kremen, 2006), as well as cranberries (Evans & Spivak, 2006) and almonds (Brittain et al., 2013). In other words, the greater the abundance and diversity of bees, the greater the potential for successful pollination (Reilly et al., 2020). However, regions of the US with the largest areas of pollinator-dependent crops often correspond with the lowest bee abundance of wild bees, as intensive crop production areas are characterized by degraded habitat value (Scheper et al., 2014).

Growers spend millions annually on honey bee pollination services, yet recent studies have shown that it is in fact the wild bees that are responsible for most of the pollination work in some crops such as apples (Mallinger et al., 2015) and pumpkins (Petersen et al., 2013). Efforts to quantify the economic contribution of honey bees versus wild bees have shown that the relative importance of the two groups varies based on the landscape, crop, and scale. For example, in blueberry pollination, the relative contributions of honey bees and wild bees is dependent on the size of the field, but each is crucial to the overall system (Hoshide et al., 2018), though fruit set is impacted about 1.6 times more by wild bees than honey bees (Asare et al., 2017).

In natural ecosystems, wild plants may become pollination limited if their wild pollinators decline, resulting in lower genetic diversity and survival, which can lead to extinction and loss of plant diversity (Potts et al., 2010; Burkle et al., 2013). Likewise, the codependency between host plants and their pollinators means that a decline in plant populations or local extirpation of a plant species from an area can in turn result in a decline in their pollinator
(Scheper et al., 2014). Thus, wild bees and other native pollinators are keystone species, where their loss can result in cascading effects on the entire ecosystem (Menz et al., 2011).

**Wild and Managed Bees are declining due to multiple stressors**

The decline of honey bees in North America and Europe (Neumann & Carreck, 2015) has driven the awareness that they and other bees share similar stressors and thus are both imperiled by pesticides, pests, disease, and a lack of suitable flower forage (Goulson et al., 2015). Although honey bees are often used as model organisms for understanding the threats faced by other bees, it is essential to note that while these causes of decline may overlap at the individual level, they differ at the colony level (Wood et al., 2020), especially in terms of impact. Both managed and wild bees are experiencing declines, and there are global pollination deficits, but the risks to human well-being are not distributed evenly across the globe. Latin America is at the highest risk of impacts to human well-being associated with pollinator loss, owing to the severity of habitat degradation as well as high levels of pesticide use and genetically modified crops (Dicks et al., 2021). The causes and effects surrounding each stressor are described below, but the key caveat is that they do not function in isolation, but instead are synergistic (Grassl et al., 2018).

Pesticides, such as neonicotinoids, reduce bees’ ability to effectively forage, grow, and survive (Henry et al., 2012; Whitehorn et al., 2012; Muth & Leonard, 2019). Laboratory experiments showing these sublethal effects of neonicotinoids in honey bees and bumblebees has also been confirmed to have long-term impacts on bee diversity in oilseed rape fields (Woodcock et al., 2016). In another field study, neonicotinoid coated oilseed rape reduced wild bee density and nesting, as well as bumblebee colony growth, while not influencing honey bees in the same area (Rundlöf et al., 2015).

Synergistic negative effects of pesticide combinations are also possible, and their impact can be greater than that of individual pesticides alone (López-Uribe, 2021). For example, the neonicotinoid acetamiprid is toxic to bees as a stand-alone pesticide, but when combined with others, synergy results in even greater negative effects on honey bees (Wang et al., 2020). Synergistic effects between pesticides and other stressors have also been observed: for example, the neonicotinoid Thiamethoxam and the microsporidian gut pathogen *Nosema apis* impact individual and colony survival as stand-alone stressors, but when they are combined, it results in
the loss of the majority of males in each colony, as well as dramatically higher mortality and suppressed immunocompetence of workers (Grassl et al., 2018). Even pesticides that do not specifically target insect pests impact bees: for example, the herbicide glyphosate has been shown to directly influence bee survival, and the effect is even worse when combined with exposure to *Nosema* microsporidia (Faita et al., 2020) or opportunistic pathogens (Motta et al., 2018).

Bees and other pollinators face newly introduced pest species, along with the same natural enemies with which they have coevolved. For example, the introduced *Varroa destructor* mite feeds on honey bees’ fat bodies, while transmitting viral pathogens (Ramsey et al., 2019), and has had a dramatic impact on honey bee declines and the economics of the beekeeping industry. Like honey bees, other managed and wild bees are affected by naturally occurring and introduced parasites. For example, bumble bees in both managed and wild settings are affected by the bumble bee mite (*Parasitellus fucorum*), and several species of tracheal mites. Bumble bees have declined by as much as 96% since the 1990s, and this decline is attributed to increasingly fragmented and reduced habitat quality, and an increase in *Nosema bombi*, a fungal disease of bumble bees, along with pesticides (Cameron & Sadd, 2020). Additionally, there is a link between the expansion of commercial bumble bee rearing in the 1990s, increases in *Nosema*, and subsequent declines in local populations surrounding release sites (Otterstatter & Thomson, 2008). The case of the bumblebee is illustrative of the synergistic stressors which bees face: the initial stressor may be nutritional stress due to a pest, competition, or a lack of habitat, which in turn increases the bees’ susceptibility to other stressors.

Honey bees are non-native and there is evidence that their presence may negatively impact native bee populations (Geldmann & González-Varo, 2018), and the same is true for other managed pollinators, including Alfalfa Leafcutting Bees (*Megachile rotundata*) and the bumblebee *Bombus impatiens* when they are introduced into areas outside of their native range. Large populations of managed pollinators may directly compete for resources, impact local plant communities, and transmit pathogens; therefore, they represent an additional stressor that can contribute to declines of wild bee populations (Iwasaki & Hogendoorn, 2022). For example, several honey bee viruses and the microsporidian *Nosema ceranae* have been shown to also infect bumble bees, and the *Apicystis bombi* parasite of bumble bees can likewise infect honey bees (Graystock et al., 2015). Flowers serve as hubs for disease and parasite transmissions (Koch
et al., 2017) and vary in their propensity to transmit diseases, with smaller bees being generally at greater risk of infection (Adler et al., 2018). However, more research is needed to understand the full impact of managed bees on the sustainability of wild bee populations (Mallinger et al., 2017).

Lack of forage - that is, nectar and pollen - is a main factor contributing to insect pollinator declines (Klein et al., 2007; Kluser & Peduzzi, 2007). This stressor can act directly, where hungry pollinators are unable to meet their nutritional needs, or indirectly, where the resulting nutritional stress reduces the pollinators’ ability to cope with other stressors like diseases and pesticide exposure (Moret & Schmid-Hempel, 2000; Potts et al., 2010; Goulson et al., 2015). While each stressor is important, the overarching factor driving pollinator declines is the loss of habitat that provides both nesting sites and a diversity of flowering plants (LeBuhn & Luna, 2021). Land use change, particularly intensive agriculture, reduces abundance and diversity of wild pollinators, especially those with a higher degree of specialization (Weiner et al., 2014).

Climate change effects on pollinator biodiversity is likely exacerbated by monotonous and fragmented habitat and may compound the poor nutrition already being experienced by bees (Vasiliev & Greenwood, 2021). As the rate of global heating increases and climate change progresses, the effect is likely homogenization of pollinator assemblages due to fluctuations in winter weather conditions, increased frequency of extreme weather events, and changes in the length of the vegetational season (Nicholson & Egan, 2020; Vasiliev & Greenwood, 2021). The likelihood of plant-pollinator mismatches will continue to increase as earlier host plant flowering times may no longer overlap with the lifecycle of their pollinators, although the exact magnitude of the issue is not well understood (Gérard et al., 2020). Plant responses to climate change may include alterations to flower, nectar and pollen, and pollinator responses could include altered foraging activity and life span, which could modify plant-pollinator networks (Scaven & Rafferty, 2013). Elevated carbon dioxide levels also affect pollinators by reducing the nutrient levels of plants, for example, the protein content of goldenrod (Solidago spp.) has significantly declined over the past several decades (Ziska et al., 2016).

In summary, both managed and unmanaged bees are suffering the combined stressors of poor nutrition, pathogens, parasites, and pesticides. The very survival of our wild and agricultural ecosystem hinges on healthy populations of both categories of bees, and we must
therefore continue to investigate causes of declines, monitor populations of both pollinators and their host plants, develop solutions to increase managed pollination in agricultural settings, and implement conservation of wild bees across all landscape types.

Solutions to declining managed and wild bee populations

Protecting bee pollinators requires a multi-pronged approach that includes restoring habitat, limiting contaminant risks, and providing adequate nutrition (nectar and pollen) throughout the foraging season (Xerces Society, 2011). This approach should also include adopting biosecurity measures, enhancing bee population monitoring, and evaluating pesticide risks (Goulson et al., 2015). Specifically, there is a need for an international agreement that would include banning the prophylactic use of some neonicotinoids, improving the regulatory process for pesticide approval, and restoring the independence of regulatory science (van der Sluijs & Vaage, 2016).

Habitat monitoring and restoration are critical in both natural and human-dominated landscapes (Natural Resources Council, 2007; Kammerer et al., 2020). While pesticide regulation and disease prevention are regulatory issues, improving pollinator nutrition is the most actionable stressor to address. The Xerces Society (xerces.org) and NRCS (nrcs.usda.gov) have developed pollinator habitat assessment methods that are adapted for specific conditions and regions, with a goal of identifying current conditions and facilitating selection of management activities. However, assessing the conservation value of individual plant species used in habitat restoration and measuring management effectiveness on bee health is still needed. Unfortunately, many of these assessment methods are time consuming and require significant human effort (Couvillon & Ratnieks, 2015). As such, better indicators of habitat quality are needed to support healthy pollinator communities.

Honey bees as bioindicators

One idea for a novel assessment of habitat quality is to let honey bees, which themselves would benefit from a healthy landscape, do the hard work for us. We have three a priori reasons that honey bees might be good indicators. Firstly, honey bees, in contrast to other bees, forage at long distances of several kilometers, potentially up to 10-12km, meaning they can survey wide areas of land (von Frisch, 1967; Seeley, 1994; Couvillon et al., 2014). Secondly, honey bees are
generalists in their foraging, so the flowers they visit are likely to be visited by other pollinators (Biesmeijer et al., 2006). Thirdly and most importantly, the honey bees have the waggle dance, a recruitment behavior that they perform for only good resources at any given time (von Frisch, 1967; Couvillon & Ratnieks, 2015). By definition, bees only dance for the best resources at any given time, so every day that we observe is for a “good” resource (Seeley, 1994). This behavior, which is visible and decodable to scientists, means that we could in theory deploy honey bees and let them do continuous surveying and reporting on the presence of valuable floral resources within the landscape. Therefore, honey bees have the potential to serve as bioindicators of habitat quality, not just for themselves, but maybe also for other insect pollinators given that they are generalists and that it is possible to know where, when, and on what flowers they forage through waggle dance observation.

In Phase 1 of this five-year study (2018-2023), we used methods that we piloted that analyzed honey bee waggle dances, a form of communication where successful foragers indicate to nestmates the vector to food (von Frisch, 1967; Couvillon et al., 2012). Waggle dances are visible to the eye and can be decoded (Couvillon et al., 2012) and mapped (Schürch et al., 2013, Schürch et al., 2019). Couvillon lab students Brad Ohlinger, Taylor Steele & Mary Silliman collected waggle dance data (n = 11,033 dances) from observation hives located at three sites in Virginia (n = 3 hives per site) that are unique in terms of geography as well as dominant land cover. The three sites were Blacksburg, VA, a mixed landscape of agricultural grasslands, developed residential areas, and other commercial and agricultural areas; Winchester, VA, which is dominated by fruit orchards, especially apples; and Suffolk, VA which is largely row crops (cotton, peanut, corn, and soybean).

In Phase 1, each location was its own field site and study. For example, these studies determined that honey bees focus their mid-summer foraging on nearby row crops, especially peanut, corn, and cotton but not soybean (Silliman et al., 2022). Additionally, honey bees located near apple orchards do forage in those fields when the apple trees are in bloom, but they visit the same orchards even more after tree bloom, preferring instead to collect food from the weedy understory between apple trees (Steele et al., 2022). Lastly, foraging in Blacksburg is mostly short-ranged and focused on agricultural grasslands, but only if they are nearby to the hive (Ohlinger et al., 2022; Ohlinger et al., Submitted). However, in addition to providing important data on honey bee foraging dynamics across three different landscapes, the previous work from
our lab also created a unique and powerful data set of over 11,000 decoded honey bee waggle dances from two years. These data are now available for use in our project and will provide a measure of honey bee foraging probabilities at each of the three locations (see Methods & Materials for more detail). Our project here extends the honey bee foraging data in new and exciting ways by validating it against a different data set that goes beyond honey bees and includes another bee, the Alfalfa leafcutting bee.

The goal of this study was to investigate how the information from the dancing honey bees relates to the productivity, survival, and foraging of a non-**Apis** bee, the Alfalfa leafcutting Bee (**Megachile rotundata**). If other bees have better survival and productivity success in areas indicated as preferred by honey bees, based on their decoded and mapped dance behavior, that would be evidence that honey bee waggle dance decoding could serve as bioindicators not just for them, but also for other bees as well. Therefore, our goal was to place this non-**Apis** bee across these same landscapes, monitor their health and compare that to the honey bee ‘preference’, or foraging probability for that area to validate this hypothesis.

**Alfalfa leafcutting bees as a model organism for validating honey bees as bioindicators**

The Alfalfa leafcutting Bee, **Megachile rotundata**, was introduced to the United States from Europe in the late 1930s and established across much of the US by the 1940s. Alfalfa leafcutter bees (**ALCBs**) dramatically increase the yield of alfalfa seed and are highly effective pollinators for alfalfa and a variety of other crops, especially legumes within Fabaceae (Pitts-Singer & Cane, 2011). The **ALCBs** quickly became a key commercial pollinator of alfalfa for seed production in Canada and the United States, only to be decimated in the 1970s by chalkbrood, a common fungal infection (Richards, 1987). Since then, rearing **ALCBs** in the United States has become increasingly difficult due to this disease, along with various parasitoid wasps and other pests (Mader et al., 2010). Today, the US imports its annual supply of **ALCBs** for alfalfa pollination from Canada because the cooler northern climate not only limits fungal infection spread, but it also results in a greater number of diapausing bees versus those which produce a second generation within a single season. A second generation emerging within the same season results in lower yield of offspring due to lower quality foraging resources later in the season, along with mortality caused as emerging bees damage their diapausing nest mates (Kemp & Bosch, 2001). As a result of lower rates of diapause and higher rates of disease at
lower latitudes, ALCBs in the northern United States have a 50% cocoon return rate; in this way, the alfalfa seed and ALCB industry in America is unsustainable and is dependent on Canada (Pitts-Singer & Bosch, 2010).

ALCBs are similar to the majority of wild bees, given that they are solitary, cavity-nesting, and capable of traveling only short distances from their nest to find resources (100 m–300 m), compared to 1-12 km for honey bees (Tasei et al., 1984; Ohlinger et al., 2022). The active season for ALCBs is May-August, which is within the longer honey bee foraging season for which we collected data in Phase 1. After emergence, ALCB females mate and then construct a nest within a hollow reed or existing hole in wood and use these cavities to gather provisions and lay their eggs. They may lay up to 35 eggs in their lifetime of two months (Guedot et al., 2006). Each thimble-shaped cell within the tube consists of a liner made from a cut leaf, a pollen provision, and an egg, which will develop into an adult within this cell. Leafcutters cut their leaves from a wide variety of native and non-native plant species, preferring those that are softer, lack latex, and have a minimal waxy cuticle (MacIvor & Packer, 2015). Like most wild bees, ALCBs have several natural enemies, including cuckoo bees, clerid beetles, blister beetles and cleptoparasite pollen beetles (Eves et al., 1980). The most important enemies are the parasitoid Chalcid wasps (Pteromalus venustus), which may decimate wild and managed leafcutter populations if not mitigated (Pitts-Singer & Cane, 2011).

As their name implies, Alfalfa leafcutting bees' most preferred host plant is alfalfa (Medicago sativa), and more generally, members of the Fabaceae. Although leafcutters are indeed extremely effective pollinators of alfalfa, they are generalists (polylectic), like honey bees, and gather nectar and pollen from a diverse array of crops and wild plants. Important crops pollinated by leafcutters also include canola, blueberries, carrots, and melons (Pitts-Singer & Cane, 2011). Potential for pollination in other crop systems is currently being investigated, and there is a critical need to better understand ALCB foraging ecology in natural systems. In a study of ALCB pollen provisions across a land use gradient in Utah, 85% of the provision was fabaceous, and other pollen species included Asteraceae, Rosaceae and Convolvulaceae (Dunn et al. in progress 2021).

In conclusion, we selected leafcutter bees for this project because they are generalist, solitary, cavity nesters that forage for a short distance from their nest site, and are active during May-June, a time of year for which we have honey bee waggle dance data. Therefore, while their
lifecycle and foraging behaviors match those of native bees, they can be purchased, managed, and observed, similar to honey bees. ALCBs are considered naturalized in this area and most of the United States and therefore pose low risk for affecting native bee populations through new invasion or disease introduction compared to commercially reared bumble bees (*Bombus impatiens*) (Xerces Society, 2011). By placing stocked leafcutter bee houses into the landscape and monitoring their productivity, leafcutter bees may themselves serve as bioindicators of the floral resources impacting a wide range of native bees. Importantly, for the purposes of this project, we are then able to compare these data on ALCB well-being to both the previously collected honey bee data and, additionally, native bee abundance and diversity from these same sites, which is the work of another Ph.D. student, Rob Ostrom from the Schürch lab.

**Specific Objectives and Hypothesis Tested**

Previous research in our lab studied honey bee foraging through waggle dance decoding at three geographically distinct sites in Virginia, consisting of different dominant landcover types: (1) Blacksburg, VA: Mixed use landscape of pastureland, forest, suburban/residential areas (Ohlinger et al., 2022) (2) Winchester, VA: Fruit orchards, especially apples, and other value-added crops that depend on insect pollinators (Steele et al., 2022), and (3) Suffolk, VA: Row crop environment containing cotton, corn, and soybeans, crops that are largely wind or self-pollinated but produce pollen (and even nectar) nonetheless and attract insect pollinators (Silliman et al., 2022). Our lab previously used these data to determine when, what, and from where honey bees collect their food. However, in the answering those questions, we also amassed a data set that then can be used as a foundation for this project. In particular, is it possible that the data from decoding honey bee waggle dances might also be applicable to a wide range of pollinating insects, including other bees?

The overall goal of this project was to determine if non-*Apis* bees, in particular Alfalfa leafcutting Bees, would display higher cocoon production in areas that have been previously indicated as profitable for forage by dancing honey bees. In other words, we hypothesized that, given habitat preference overlap in flowers potentially used by honey bees, which are generalists, and wild bees, we would predict that ALCB nest boxes should be more productive in terms of cocoons produced at sites that dancing honey bees had indicated were of better forage quality. This is an important step in understanding the relationship between honey bee foraging
preference as indicated by waggle dance data, and the well-being of other bees. More broadly, these data may help establish honey bees as bioindicators for them and other insect pollinators.
CHAPTER 2: MATERIALS AND METHODS

Site Selection

At each site in Virginia (Blacksburg, Winchester, Suffolk), we installed a total of 15 leafcutter bee stations in May 2021 and again in May 2022. We selected station locations using the honey bee waggle dance data (honey bee residual foraging probabilities, see Statistical Analysis section below) from Phase 1 and based on the following criteria:

1. **Direction.** Nest box stations were located in all directions from the central observation hive location, with the goal of transecting the study area.

2. **Distance.** Nest box stations are at a range of distances, but within the outermost range of the 90% confidence hotspot range, and are at least 300m apart, meaning that ALCBs at each nest station would forage in a non-overlapping manner (Pitts-Singer & Cane, 2011) with ALCBs from adjacent stations.

3. **A range of honey bee residual foraging probability.** A map of honey bee residual foraging probabilities was used to select nest box station locations possessing a range of values, representing ‘hotter’ and ‘colder’ honey bee foraging zones.

4. **Landowner permission and site accessibility.** Once potential nest box station locations were identified, we used county GIS data to identify land owners, whom we then approached if the location had reasonable access to the road. Landowners had to agree to participate in the study and allow access to their land for two years.

5. **A balance of cover types** (based on a 300 m foraging radius). In combination with the above criteria, we aimed to find station locations representing the various land cover types in the area. For example, in Blacksburg, we sought to find some locations that were more forested, with others in more open landscapes.
We first visually inspected and annotated the maps created by Brad Ohlinger in Phase 1 of the study (Ohlinger et al., 2022) to identify ‘hotter’ and ‘colder’ areas of the landscape (See Fig 1, Honey Bee Foraging Probability Maps, and statistical analysis section for a description of the waggle dance data analysis protocol). Next, we used those general areas to identify potential study sites and landowners by County GIS data and personal contacts from local cooperative extension agents. We created a brochure (See Appendix A), which we shared electronically and in person as we approached people to request permission to access their property for placing the nest boxes. Participating landowners agreed to allow us access over two years to collect data at their site.

![Honey Bee Foraging Probability Maps](image)

**Figure 1:** Honey Bee Foraging Probability Maps which show ‘hotter’ areas in red and ‘colder’ locations in blue, based on phase 1 honey bee waggle dance data at three locations.

**Establishing Alfalfa Leafcutting Bees at the Sites**

**Nest box design and management**

Based on existing designs (Cane 2006; Pitts-Singer 2013; MacIvor 2016) and in consultation with experts in the field (Theresa Pitts-Singer, USDA-ARS & Dave Hunter, Crown Bees, LLC), we designed and constructed 45 (15 per site * 3 sites) 20cm x 20cm x 20cm wooden nest boxes (Fig 2, next page). Each box was protected from birds by 2cm plastic mesh and was deep enough to provide protection from sun, wind, and rain. Each box was provided with 8
bundles of 20 natural reed tubes from Crown Bees that were approximately 6mm in diameter. We placed all nest boxes with the opening facing north to avoid direct sun exposure. All tubes were removed at the end of 2021 and replaced with new tubes in spring 2022. Tubes were removed in December 2022, along with the nest boxes at the completion of the project.

**Purchasing, installing, and monitoring of Alfalfa leafcutting Bees in the field**

In year 1, we purchased 9000 partially incubated ALCB cocoons (www.crownbees.com, Woodinville, WA), which arrived in May 2021. 160 cocoons were selected with a balanced gender ratio (approximately 80 males, 80 females) per site based on size of the cocoons, and we released the cocoons at each site by placing them in a small, open box at the back of each nest box. Unfortunately, and ultimately quite consequentially, the cocoons had been delayed in the mail for nearly seven days, which caused concern about emergence rates, and unfortunately no cocoons emerged, presumably due to chilling in the mail after being incubated. Another round of cocoons was ordered (from MidasBees.com, Hermosa, SD), sorted, and released on June 15, 2021. The emergence rate, 72%, was within industry standards of expected emergence rate. However, it is important to note that cocoon release was therefore 6 weeks later (Table 1) in year 1 than in year 2.

The same procedure was followed in 2022, except that, given the challenges of the previous year, we opted for the bees to be sent without incubation in the first week in April, and we released them to the nest boxes in May. These bees emerged beginning in the first week of June, with again a mostly successful emergence rate of 71%.

After successful installation of the ALCB stations at each site, we performed three monitoring visits to each field site across the season. The purpose of these visits was to inspect the nest boxes for activity and damage and to remove any pests, such as paper wasps or spiders, that had taken up residence in the boxes. The timeline of sampling and floral surveys (Table 1 below) was as follows:
<table>
<thead>
<tr>
<th>Date</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>May 1, 2021</td>
<td>Cocoon release 1</td>
</tr>
<tr>
<td>June 15, 2021</td>
<td>Cocoon release 2, Floral survey</td>
</tr>
<tr>
<td>July 15, 2021</td>
<td>Monitoring visit, Floral survey</td>
</tr>
<tr>
<td>August 15, 2021</td>
<td>Materials collection, Floral survey</td>
</tr>
<tr>
<td>May 1, 2022</td>
<td>Cocoon release</td>
</tr>
<tr>
<td>June 15, 2022</td>
<td>Monitoring visit, Floral survey</td>
</tr>
<tr>
<td>July 15, 2022</td>
<td>Monitoring visit, Floral survey</td>
</tr>
<tr>
<td>August 15, 2022</td>
<td>Monitoring visit, Floral survey</td>
</tr>
<tr>
<td>December 15, 2022</td>
<td>Final materials collection</td>
</tr>
</tbody>
</table>

**Table 1**: Timeline of sampling. Note that we had to release cocoons twice in the first year because a mailing delay caused the first batch of cocoons to be dead upon their arrival.

**Surveying Floral Resources at each Site**

We conducted flower resource availability surveys at each site (n = 15 per location, for total of N=45), for a total of three floral survey events during the active season of ALCBs (May-August). We quantified the occurrence and abundance of insect-pollinated flowering plants using the visual scanning and abundance estimation method (Szigeti et al., 2016). Briefly, we established a four-part transect at each site, which covered four directions from the nest box center point. Each transect was 50m, first going north, then southwest, then east across the center point, and then southwest. We recorded a list of blooming plants at each sampling, and estimated the abundance of each species having open, non-wilted flowers on a rank scale for the
entire area: 1: very scarce; 2: scarce; 3: more or less scarce; 4: more or less abundant; 5: abundant. The abundance estimate was calibrated across sites (Szigeti et al., 2016) so that each species is assessed in the same way relative to the other sites. We were particularly interested in two metrics. Firstly, we wished to calculate a measure of plant species richness, which is defined as a count of the total species of flowering plants present at each location. We then would use this measure per location within the site to determine if it would explain our response variables (see below). Secondly, we were particularly interested in clover (*Trifolium* spp.), both white and purple, because clovers are important resources for both honey bees and ALCBs. We had an *a priori* expectation that clover might have an impact on ALCB fecundity.

**Measuring Proximity to Intensive Agriculture**

Many of the nest box locations were located alongside intensive agriculture fields, where habitat may be highly variable, and most importantly, where pesticide exposure risk is extremely high. This includes drifting directly into the nest box. Nest box stations that were directly adjacent to row crop or apple orchard production were assigned either a 1 or a 0 for the presence or absence respectively of intensive agriculture. This enabled us to test the impact of intensive agriculture, due to habitat deficiency, pesticides, or a combination of both, on ALCB productivity.

**Statistical Analysis**

**Data Counts**

We opened each nest tube and counted the number of ALCB cocoons. Individual cocoons were inspected for signs of emergence, where a hole had been chewed in the end of the leaf covering and there was no bee or larvae present. In addition, we inspected for signs of damage caused by parasitization, where a smaller hole was chewed in the side of the leaf cocoon. If the cocoon was intact with no sign of emergence or damage, it was counted as a diapausing ALCB adult. We also counted all cocoons from other species as Non ALCB cocoons, and counted the number that either emerged or were damaged. While the species of each Non ALCB bee or wasp that took up residence in the nest box is difficult to identify until they emerge, it is, in some cases, possible that the Genus can be identified, such as that of the *Trypoxylon* wasp. *Trypoxylon* wasps, likely *Trypoxylon collinum*, were common in the nest boxes, and we hypothesized that
they may have had a particularly negative impact on ALCB nest box retention due to their prevalence and size. Therefore, we noted especially the presence and number of these wasp cocoons.

We calculated floral resource species richness by counting the presence of individual blooming flower plant species at each nest box station across the season. We hypothesized that higher species richness would be correlated with higher ALCB productivity. Additionally, we calculated clover count by adding up the presence or absence of both white and red clovers at each location. Given that ALCBs favor members of the Legume family (Fabaceae) and are known to pollinate clover, we hypothesized that the presence of any type of clover (red or white) would have a positive effect on ALCB cocoon counts.

**Honey Bee Foraging Probability Data Preparation**

Our 15 nest box locations at each site were chosen, in part, to represent a range of honey bee foraging probabilities, as indicated by waggle dance decoding from the previous Phase 1 of the larger project. Previous graduate students (Silliman, Steele, Ohlinger) established and maintained 9 honey bee observation colonies, three per site, at Blacksburg, Winchester, and Suffolk. Honey bee workers foraged freely at each site, and we filmed honey bee waggle dances approximately three times per week from April until late October for two years. These dances were then decoded using a modified version of Couvillon et al 2012, and the data were analyzed for stand-alone projects at each site (Silliman et al., 2022; Steele et al., 2022; Ohlinger et al., 2022).

For the purposes of our work here, we used this data set to provide honey bee foraging probabilities. Briefly, the average duration and angle of each dance was then used to simulate the dance 1000 times each according to the universal calibration model (Schürch et al., 2019) for each year. The simulated dances from both years were then used to create a 50 m resolution raster, where each individual pixel in the raster was assigned a foraging probability. This value represents the probability that at least one dance signaled that location. We fit a linear model with distance from the colony as the independent variable and the raw foraging probability as the dependent variable and then rasterized it. This resulted in the residual foraging probability maps for each location (Fig 1, Materials and Methods section).
We used these maps first at the beginning of the project in site selection to visually select target site areas that represent a range of foraging probabilities for the 15 ALCB nest box sites. For final data analysis, we used the standardized DHARMa residuals (using the simulate function from the DHARMa R package) from our linear model to calculate honey bee foraging probability within each nest box area (see below for more detail). Residual values ranged from 0 to 1, where values closer to 0 are on the lower end of simulated predicted values, and values closer to 1 are on the higher end of predicted values.

Alfalfa leafcutting Bee Data Preparation

All statistical analyses were performed using the current version of R (Version 2022.12.0+353). To obtain a value that represents the honey bee foraging probability over the ALCB foraging area surrounding each nest box, we first calculated a 300-m buffer, the maximum foraging distance of ALCBs. Then, we calculated the mean DHARMA residual honey bee foraging probability within that buffer. ALCB cocoon count data was used from 2022 alone, as the 2021 season was significantly different and contained very low numbers due to the late start described in the previous section.
CHAPTER 3: RESULTS

**Alfalfa leafcutting bee productivity is not correlated across years**

Given that we maintained the same sites within locations, we would expect the productivity of ALCBs, as measured by number of cocoons in the same site within locations, to be correlated across years. On the other hand, we also considered that the six-week delay in the delivery (Table 1) of the ALCBs in 2021 may negatively impact that first year such that the data would then not be comparable. Therefore, it was important to begin by determining whether year 1 (2021) and year 2 (2022) correlated, which we found not to be the case (Spearman’s rank correlation: $S = 386.3$, $p = 0.31$, $p = 0.261$). Because of the delivery delays, we predicted that the bees would consistently do better in the second year, when the cocoons arrived on time. Indeed we found that there was an increase by 1.4 (1.3 to 1.6) times in mean number of ALCB cocoons from year 2021 to 2022 (2021: 0.9 (0.3 to 2.6); 2022: 1.3 (0.4 to 3.7); $< 0.001$). Because of this, all subsequent analysis were restricted to data from 2022.

**Alfalfa leafcutting bee productivity varied by location, with Winchester as the most productive site, followed by Blacksburg and Suffolk**

ALCB cocoon production varied by location (log-likelihood ratio test: $\chi^2 = 311.0$, df = 2, $p < 0.001$), with Winchester as the most productive location (mean ALCB count (95% CI): 26.2 (23.7 to 28.9)), followed by Blacksburg (20.4 (18.2 to 22.9)), and Suffolk (4.4 (3.5 to 5.5)) (Fig 3). The mean separation tests show that there is a significant difference between Blacksburg and Suffolk (ratio (95% CI): 4.7 (3.5 to 6.3), $z = 12.1$, $p < 0.001$), as well as between Winchester and Blacksburg (1.3 (1.1 to 1.5), $z = -3.2$, $p = 0.004$) and Winchester and Suffolk (6.0 (4.5 to 8.0), $z = -14.5$, $p < 0.001$). Interestingly, Blacksburg had the highest maximum cocoon count at any one site (191 cocoons), whereas Suffolk had the most zeroes (no ALCB cocoons at a site), in addition to its overall low numbers.
Alfalfa leafcutting bee productivity is negatively correlated with honey bee foraging preference

Alfalfa leafcutting bee productivity was negatively correlated with honey bee foraging preferences, as measured by waggle dance recruitment (log-likelihood ratio test: $\chi^2 = 55.42, < 0.001$) (Fig 4). Over the range of the DHARMa residuals from 0 to 1, we would expect the count of ALCB to decrease 0.2 (0.1 to 0.3) times, or from an expected count of 30.0 (11.3 to 79.6) ALCB cocoons to 6.2 (2.3 to 16.4) cocoons. In other words, dancing honey bees do serve as bioindicators, but in the opposite direction from our original prediction, where areas most likely to be indicated as good foraging locations are least likely to support ALCB productivity.
Alfalfa leafcutting bee productivity is positively impacted by the presence of clover and negatively impacted by the number of *Trypoxylon* wasps

We were interested in exploring the contribution of other factors to the variation in Alfalfa leafcutting bee productivity that we observed. In particular, we hypothesized that the number of parasitic wasps (genus *Trypoxylon*) present in our nesting boxes; the abundance of both red and white clover; the plant species richness; and whether the site was next to intensive agriculture (no/yes) may influence the ALCB productivity.

We began by testing these factors in a series of unadjusted, single fixed effect, models. All of these factors were highly significant (likelihood ratio tests, all $p < 0.001$). Therefore, we next created a fully adjusted model with honey bee residual foraging preference (see above), number of *Trypoxylon* cocoons, abundance of red and white clover, plant species richness, and presence of intensive agriculture (no/yes) as predictors. After single-term deletion of non-significant terms, we arrived at a final model with residual honey bee foraging preference, count
of *Trypoxylon* cocoons, and red and white clover as fixed effects. Both species richness and whether the site was adjacent to intensive agriculture were non-significant (*p* > 0.05).

Therefore, in our final model, ALCB cocoon count remained negatively correlated with honey bee residual foraging preference (log-likelihood ratio test: \( \chi^2 = 143.66, < 0.001 \)) with a 0.06 (0.04 to 0.10) times decrease over the range of dharma residuals. Number of ALCB cocoons also decreased significantly with the count of *Trypoxylon* cocoons (log-likelihood ratio test: \( \chi^2 = 54.37, < 0.001 \)), with a 0.96 (0.95 to 0.97) times decrease for every wasp cocoon present. In other words, in the absence of wasps, we predict 9.3 (7.7 to 11.2) ALCB cocoons, whereas at the maximum observed number of *Trypoxylon* cocoons we expect on average 0.4 (0.1 to 1.1) ALCB cocoons.

The abundance of clover, both red and white, had a significant positive effect (log-likelihood ratio test: \( \chi^2 = 778.36, < 0.001 \)), with a 2.02 (1.90 to 2.16) fold increase for each unit increase in abundance. In other words, with no clover, we would predict 0.4 (0.2 to 0.5), whereas at the maximum observed clover abundance we would predict on average 100.3 (79.9 to 125.9) ALCB cocoons (Fig 5).

**Fig 5:** Relationship between the three significant variables (honey bee foraging probability, left; *Trypoxylon* wasp cocoons, center; clover, right) and ALCB Cocoon Count.
CHAPTER 4: DISCUSSION

Here we have shown that ALCB productivity varied significantly by location (Fig 3), with Winchester as the most productive site, followed by Blacksburg, and then Suffolk. Most importantly, we found that ALCB productivity is negatively correlated with honey bee foraging probability (Fig 4). Additionally, we found that the abundance of red and white clover had a significant positive effect, while the number of *Trypoxylon* wasp cocoons present in the nest boxes had a significant negative effect on ALCB productivity (Fig 5). These results are both surprising, in that they are in the opposite direction of our original hypothesis regarding honey bee foraging preferences, and important, as they show factors that affect ALCB productivity, survival, and maintenance.

ALCB productivity, as measured by ALCB cocoon count, was not correlated across years. This may be due to differences in climate or vegetation (especially crop rotation and new residential development), but most likely is due to the late cocoon deployment in the first season due to the emergence failure caused by the shipment delay. The fact that the ALCBs were not active in the landscape until late June in 2021 versus in early May in 2022 makes the seasons difficult to compare and impossible to use as a two-year data set, as the lower numbers in 2021 were likely due to the shorter season and the bees missing the May blooms. Indeed, we had significantly lower total cocoon counts in 2021 versus 2022, and we had many more zeros (i.e., nest box locations where not a single cocoon emerged) in 2021 versus 2022. Ultimately the non-correlation between the two years led us to present here only the data from the second year.

We found that ALCB productivity varied across our three locations, with Winchester as the most productive, followed by Blacksburg, and then Suffolk (Fig 3). Although Blacksburg is a mixed landscape of pasture, forest, and residential land, and Winchester is dominated by apple orchards and forested land, they have similar climate, terrain, non ALCBs, and plant species present (data not shown), and this may explain the similarity in ALCB productivity between these two sites. In contrast, Suffolk is hotter and dominated by different dominant wild plant species, as well as rotational row crop agriculture, and had much lower ALCB success. This may be due to pesticide exposure and/or a lack of habitat associated with this environment, but based on our data, we can only conclude that Suffolk is more inhospitable to ALCBs than Winchester and Blacksburg.
The key finding of this study was that ALCB productivity is negatively correlated with honey bee foraging preference, the opposite of our hypothesis. Honey bees and ALCBs are both generalist bees, non-native, but widely introduced and naturalized, to North America, and both forage on a wide range of flowers, including clover. ALCBs tend to focus on alfalfa and other legumes in addition to clover, but their habitat certainly overlaps with honey bees, which is why we chose them as a model organism. Therefore, the negative relationship between honey bees foraging and ALCB productivity is even more surprising. However, this result is corroborated by the other component of Phase 2 of this study, conducted by Ph.D. student Rob Ostrom, who studied wild bee abundance and diversity in these same landscapes over the same time period, and found that there is a negative relationship between honey bee foraging probability and wild bee richness (Ostrom et al. in prep). The fact that there was a negative relationship between honey bees and both ALCBs and wild bees is important, which leads to the question of whether this is due to direct competition, or instead highlights differences in foraging behaviors. In our study, the honey bee observation hives were in position and active in 2018-2019, and the leafcutting bees were in the field in 2021 and 2022. Therefore, there was no direct temporal overlap, so there was not direct competition, but it is possible that other managed or feral honey bee colonies focus on those areas and outcompete ALCBs and wild bees there. At least in Blacksburg, the presence of our university apiary (i.e., many honey bee hives not involved with our project) adjacent to the original location of our observation hives means that there were indeed many honey bees in the area when the ALCBs were active. Other possible explanations for the unexpected inverse relationship between honey bee foraging and ALCB success in the landscape include:

1. **Scale:** The long foraging range and high numbers of honey bees means they are covering a wide area of the landscape in search of food and communicating that to their nestmates. Therefore, decoded waggle dance data may not be useful for comparison to ALCBs and wild bees, who only travel short distances from their nest site to gather resources. For example, in Blacksburg, a highly productive ALCB nest box station was located in the middle of a large honey bee cold spot. In this case, the whole area may indeed not be generally favorable habitat for honey bees nor ALCBs, but smaller, productive habitat areas within that may be high quality may have a huge impact on ALCBs with their smaller foraging range. Additionally and also involving scale, we made our buffers
300m, which is the reported maximum foraging range of ALCBs (Pitts-Singer & Cane, 2011). It may be that most of their foraging happens much closer by, and so important variation in habitat might be lost at our current resolution.

2. Interpretation of honey bee hot spots versus cold spots: It is possible that while honey bee “hot” spots are extremely attractive to honey bees, “cold” spots might represent an ok, but not top, preference. In other words, a cold spot may not necessarily mean that the habitat quality is low, but that it is just not as ideal as another area. Also, there is some stochasticity to where honey bees find food in the landscape (Seeley, 2019) and it is possible that “cold” spots are not low quality but are unexplored.

3. Temporal overlap of honey bee data and ALCB data: While we originally wanted to use the honey bee data from only June-August when the ALCBs were active in the landscape, we ultimately needed to use the entire honey bee data, set which spanned from May-October. This was because of the effect of reducing the honey bee foraging data set to only two months reduced the sample size and correlation of the data set too far, removing the power that we needed to make our calculations on residual foraging probability. For example, one cold spot in Blacksburg was a forested area dominated by tulip trees, which are highly attractive to honey bees, but only during May. Honey bees may have danced for that area at the time, but the overall number of dances for that area would therefore be diluted by other hotter spots, representing more dances over a longer period of the season.

Besides being affected by year and location, ALCBs were positively affected by the presence of red and white clover, which supported our hypothesis that clover may be an important driver of ALCB success. Clover was most common in Blacksburg and Winchester, where the ALCBs saw the most success overall, versus Suffolk, where clover was less common. The final variable that was significant in our model was the presence of Trypoxylon wasps. While Trypoxylon wasps are spider hunters and not directly predators or parasitoids of ALCBS, we hypothesized that their presence would negatively impact the bees’ willingness to occupy a nest box, as their size alone would likely deter the ALCB foundresses. Furthermore, they may be directly aggressive towards ALCBs, although this idea of nest box competition would require further investigation. Overall, these data demonstrate that the survival and success of ALCB is a
complex, multifaceted system impacted by several interacting factors, and that honey bee foraging proclivities are in fact indicative of ALCB success, although in the opposite direction of our original prediction.

The presence of nearby intensive agriculture did not have a significant effect on ALCB success, but this may be due to our current way of analyzing it as a binary variable versus a continuous scale, which would be able to characterize the intensive agriculture within 300 m of ALCB nest box stations. Given the negative, albeit non-significant, direction of the effect, it is likely that intensive agriculture is important in determining the productivity of ALCBs, and that needs further investigation in a future study.

Total species richness was not a significant factor determining the success of ALCBs in our study, which may indicate that the total number of flowering species may be less important than the presence of a few key species. Since we know that clover was a significant driver of ALCB success, it is possible that although they have been observed foraging other plant species, ALCBs really focus on legumes and alfalfa, and are not as polylectic as we originally expected, although this is also likely context and location specific.

In conclusion, these data demonstrate that the survival and success of ALCB is a complex, multifaceted system impacted by several interacting factors, and that honey bee foraging proclivities are in fact indicative of ALCB success, although in the opposite direction of our original prediction. Therefore, it may be that honey bees cannot serve as bioindicators of habitat quality for other bees due to key differences in their foraging behaviors. Honey bees, with their long flight distances, eusocial behavior, and large forager numbers may not be comparable to ALCBs or wild bees, with their shorter flight distances and solitary life style. Of course, it also may be that honey bees may still bioindicate for themselves and certain other bees, but that this may not be a general rule, as suggested by the data we present here. More research is needed to determine the usefulness of dancing honey bees in assessing habitat quality. Additionally, we have also now contributed to our general understanding of ALCBs, including important factors that might contribute to their successful management. We now know that ALCBs can potentially be quite productive in Virginia landscapes, especially if there is an abundance of clover nearby. Also, our study demonstrates the challenges ALCBs face from non-parasitoids such as *Trypoxylon* wasps. Overall, our work here provided insight into the relationship between honey bees and solitary bees, with implications more broadly for other pollinators.
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APPENDIX A: Landowner Recruitment Brochure

Our Research Helps Bees
The Couvillon and Schurch Entomology Labs at Virginia Tech investigate insects in the landscape. In 2018 & 2019, we placed honey bee hives in Blacksburg, Suffolk, and Winchester and allowed the worker bees to feed freely across the area. We looked at when, where, and upon what they ate. Now we want to see how other bees are doing.

Starting in April 2021, we wish to place a small nest box of alfalfa leafcutting bees on your land. These bees are a gentle and effective pollinator. Then, over the next 5 months, we will return periodically to monitor them. We also will survey the other bees to find out how many different bee species are present and what plants they are using for food.

Our goal is to be courteous, quick, and not harm your property while we work. Should you agree to participate, we will clearly communicate when we will stop by and will leave no trace of our visit. Please contact us with any questions, and we look forward to working with you. Thank you for helping our key allies, the bees.

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Virginia Tech Bee Study
Our research focuses on how, when, and where bees get their food - nectar and pollen - from the flowers in the Virginian landscape. Our study sites include Blacksburg, Winchester, and Suffolk.

Want to help us help bees?
Your property is within our study area. We are interested in conducting quick surveys for Spring, Summer, and Fall 2021-2022. Please contact us if you are interested in participating with this QR code or with our contact information on the back of this leaflet.

We Need Bees and Bees Need Us
When most people think of bee pollinators, they picture honey bees (top left). Although honey bees are very important to food production, wild bees also play a critical role. Most of the more than 4,000 species in North America are wild bees (middle, bottom left). Perhaps you have heard that pollinators are in trouble, which is worrisome for our food security. Both honey bees and native bees face similar challenges including pesticides, parasites, pests, and poor nutrition from a lack of flowers in the landscape. We are committed to understanding the factors causing pollinator declines to help reverse these trends.

Plants and bees need each other: the plant feeds the bees with nectar and pollen from its flowers, and the bee helps the plant to reproduce by transferring pollen between flowers. Wildflowers and crops like blueberries, cucumbers, peaches, almonds, and apples all rely on these pollinators.

Our goal is to investigate how bees find food, and to develop methods for measuring the ability of landscapes to provide what they need.

Learn more about our work at: www.freeflyingbees.com