IPM IN MIDWESTERN AGRICULTURE: IMPLICATIONS TO PESTS, POLLINATORS, AND YIELD

by

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To the family gained along this journey:

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

There is an existing conflict thrust upon U.S. food production systems; optimize crop yield to provide sustenance to a growing population while minimizing ecological impacts of high-intensity agriculture. This balancing act is experienced by Indiana's watermelon growers who must maintain marketability of a crop that is reliant on insect pollinators. The dependence on pollinators to produce yield means that growers have to consider the negative impacts pesticide applications may have on both the desired pest and non-target pollinators. Navigating these trade-offs must be considered not just in the watermelon fields, but the surrounding agricultural landscape that has become increasingly reliant on prophylactic insecticides to control pests. This dissertation work results from an intensive set of experiments replicating grower practices in experimental fields throughout Indiana to assess the relationships of pests, pollinators, and crop yield.

Beginning with a priming year in 2017, watermelon plots were planted within larger corn fields to replicate the agriculture landscape and provide a "snapshot" of typical environment. These plots were paired at multiple locations and provided a contrast between a conventional management system that replicates grower insecticide programs with an IPM approach that removes insecticide applications outside of those based on scouting recommendations. I found that, while pest abundance and damage was higher in IPM fields, the increased pollinator visits in IPM fields led to higher yields in watermelons while corn yield was unaffected by an absence of insecticide use. Managed pollinator hives were placed in these fields and IPM resulted in the colonies exhibiting greater weight gain, lower mortality, increased reproductive growth, and higher insecticide residue accumulation. Insecticide residues were found more frequently at higher levels in the leaf tissue, crop pollen, field soil, and honey bee-collected pollen taken from CM fields. Despite these findings, there was a variable effect of the surrounding land use on the quality of collected pollen or the insecticides gathered by pollinators. These experiments demonstrate that IPM is a viable set of practices for specialty crop growers in the Midwest; successfully monitoring insect pests and conserving pollination services from both managed and wild pollinators. These findings provide a comprehensive look at the effect of IPM practices not just on the a specialty crop, but to the surrounding agricultural landscape as well. An IPM approach can be implemented by growers to decrease non-target effects from insecticides while maintaining or even improving productivity and profitability.

CHAPTER 1. INTRODUCTION

1.1 Literature Review

1.1.1 Neonicotinoids, seed treatments, and a replacement of IPM

To control insect pests in US agricultural systems, the predominant management practice is the application of synthetic insecticides. Widespread adoption began with the conversion of war time chemicals into cost-effective insect control products that, by the 1990s, had ballooned into a nearly $9 billion worldwide market (Goulson *et al.*, 2013). The introduction of a new class of insecticides, neonicotinoids, revolutionized the market and became the most widely used insecticide group within 20 years of adoption (Douglas *et al.*, 2015; Simon-Delso *et al.*, 2015). The first synthesized neonicotinoid product, imidacloprid, replaced many of the older chemical classes that had grown less effective because of resistance evolving in many pests (Elbert *et al.*, 2008; Bonmatin *et al.*, 2015). The success of imidacloprid led to the development and commercialization of other neonicotinoids such as acetamiprid, clothianidin, and thiamethoxam, which are collectively registered for over 140 crops across 120 countries (Jeschke *et al.*, 2011; Bass *et al.*, 2015; Bonmatin *et al.*, 2015).

The unique chemistry and toxicology of neonicotinoids contributed to their rapid adoption. Neonicotinoids are synthesized using nicotine, and act as agonists to nicotinic acetylcholine receptors (nAChRs). These nAChRs are frequently found in the central nervous system of insects and even low quantities of neonicotinoids cause persistent excitation of the receptor leading to paralysis and death (Jeschke and Nauen, 2008). One of neonicotinoid's novelties is that the highly selective activity results in a much lower mammalian toxicity than earlier insecticides (Tomizawa and Casida, 2005; Bass *et al.*, 2015). The selectivity to insects also means that neonicotinoids can elicit lethal and sublethal effects at lower levels compared to other insecticide classes (Tomizawa and Casida, 2005).

1.1.2 Neonicotinoid versatility and systemic activity

One of the factors that has led to the widespread success of neonicotinoids is their high water solubility and systemic nature. The systemic and translaminar activity means that, regardless of how the insecticides are applied, neonicotinoids can be taken in by the plant and distributed

throughout the tissues (Elbert *et al.*, 2008; Jeschke *et al.*, 2011). Expression throughout the plant tissue allows neonicotinoids to protect the young and vulnerable stages of crops (Elbert *et al.*, 2008). As the plant grows larger, neonicotinoid concentrations decrease in the tissue as the perceived risk to the plant decreases as well (Jeschke and Nauen, 2008). Neonicotinoids can therefore be applied in a variety of ways including foliar sprays, soil drenches, and most predominantly, seed treatments (Jeschke *et al.*, 2011). The ease of purchasing a crop seed pre-treated with neonicotinoids gained so much popularity that by 2008 neonicotinoid seed treatments covered 80% of the worldwide seed treatment market dominated by corn, soybean, wheat and cotton (Sparks, 2013). In pest-intensive regions, such as the southern U.S., the use of neonicotinoid seed treatments provided added value to farmers in both corn and cotton crops compared to a fungicide-only seed treatment program (North *et al.*, 2018a; North *et al.*, 2018b). The limitation of these studies is that they were conducted on small plots looking at yield only, and not the direct impact of pest insects in the absence of neonicotinoids. Ease of neonicotinoid use, along with relatively low costs, has led to a mentality of "early-season insurance" where the application is prophylactic and not directly responding to pest pressures (Jeschke *et al.*, 2011; Smith *et al.*, 2020). In fact, the neonicotinoid use increase over the last decade has occurred without any increase in pest pressures (Douglas and Tooker, 2016; Labrie *et al.*, 2020; Smith *et al.*, 2020).

1.1.3 Expression in non-crop sources

Asking "how effectively?" and "how long?" neonicotinoids control pests led to evaluations of the fate and concentrations of neonicotinoids applied as seed treatments. Of the active ingredient applied to crop seeds, < 5% has been found to be taken into the developing plant (Alford and Krupke, 2017). This means that most of applied seed treatments do not enter the plant to control pests, and instead may travel through the environment using a variety of pathways. Neonicotinoids can move in agroecosystems through the air (via planter dust) (Krupke *et al.*, 2012), soil (Bonmatin *et al.*, 2015), and groundwater (Main *et al.*, 2014). This movement results in neonicotinoids drifting from the applied crop to plants in margins or adjacent fields (Pecenka and Lundgren, 2015; Botias *et al.*, 2016). The persistence of these chemicals in water and soil can extend to over 1000 days and accumulate in areas where repeated use occurs (Main *et al.*, 2014; Bonmatin *et al.*, 2015). The risks of applied neonicotinoids and their metabolites moving between fields has led to a body of work examining the fate of these compounds in the pollen and nectar of both crops they are

applied to, as well as adjacent nontarget plants (Schmuck *et al.*, 2001; Goulson, 2013; Douglas *et al.*, 2015). Neonicotinoids have been frequently found in floral resources, which has led to research questioning the risk to pollinating insects in agricultural systems (Stokstad, 2007; Sanchez-Bayo, 2014).

1.1.4 Non-target insect exposure

The combination of movement of active ingredients from target plants throughout agroecosystems and high specific toxicity to insects makes neonicotinoids a potent risk to non-target insects living in the environment. Pest insects rarely represent a majority of the insect community in agriculture and reductions in insect biodiversity exacerbate pest problems (Lundgren and Fausti, 2015). Neonicotinoid seed treatments may even reduce yield due to non-target effects to beneficial insects. Across several major crops including soybeans (Seagraves and Lundgren, 2012; Douglas *et al.*, 2015), corn (Alford and Krupke, 2018; Labrie *et al.*, 2020; Smith *et al.*, 2020), and sunflowers (Bredeson and Lundgren, 2015), there were neutral or negative yields associated with neonicotinoid seed treatments due to the simultaneous suppression of beneficial insects; including both pollinators and natural enemies.

While neonicotinoids may still be controlling pests, the disruption of beneficial insects is increasingly problematic in crops that rely on insect pollination (Garibaldi et al., 2013). Pollination is an ecosystem service that requires consideration when deciding whether neonicotinoids are an appropriate management tool. Research has been conducted on a variety of the most common neonicotinoid products and their acute toxicity to non-target pollinators (Blacquiere *et al.*, 2012; Goulson, 2013). The acute oral toxicity of neonicotinoids to pollinators has enormous variation; different neonicotinoid products caused mortality to 50% of honeybees (LD_{50}) at values ranging from 3 to 17 µg/bee (Iwasa *et al.*, 2004; Decourtye and Devillers, 2010). Different bee species, both managed and native, have varying tolerances to the same neonicotinoid product, making risk assessments even more difficult (Cresswell *et al.*, 2012). The rapid adoption of neonicotinoids has been followed by observations of high mortality, reductions in bee foraging, and decreased honey production, leading to damaging economic losses to beekeepers worldwide (Chauzat *et al.*, 2009; Carreck and Ratnieks, 2014). While some cropping systems have shown connections between neonicotinoid use and hive losses (Henry *et al.*, 2012; Budge *et al.*, 2015), results have been mixed

with studies failing to make these connections to honeybee mortality (Cutler and Scott-Dupree, 2007; Chauzat *et al.*, 2009; Nguyen *et al.*, 2009).

Neonicotinoids have been detected in the pollen of crops they are applied to (Stoner and Eitzer, 2012), as well as soil and even floral tissues of non-crop plants (Botias *et al.*, 2016; Mogren and Lundgren, 2016). While foraging pollinators will collect these residues, it is rarely at high enough levels to be immediately lethal; instead the nectar and pollen likely accumulate within the colony (Sandrock *et al.*, 2014; Long and Krupke, 2016; Traynor *et al.*, 2021). The pesticides acquired through foraging can play a role in poor bee health, affecting the more vulnerable larvae developing within the colony (Sanchez-Bayo and Goka, 2014; Doublet *et al.*, 2015). During the lifetime of a honey bee worker, it will consume over 100 mg of pollen (mostly as a larvae) which, if contaminated with pesticides could have lethal or non-lethal effects such as delayed development (Crailsheim *et al.*, 1992; Spurgeon *et al.*, 2016). If a managed colony is placed in an environment with repeated exposure over multiple years that colony could serve as an inferior source of pollination. In this environment the local native pollinators could be reduced or lost, forcing a grower to rely solely on managed pollinators if their services are required.

1.1.5 Watermelon production

Watermelon (*Citrullus lanatus* L.) a member of the family Cucurbitaceae, is commonly grown in the U.S., representing more than $550 million annually in the U.S. alone. Watermelon is a monoecious crop, entirely dependent on insect pollinators to transfer pollen from the male flowers to the stigma of female flowers for fruit set and adequate development (Adlerz, 1966; Walters, 2005). Beginning in the 1990s, seedless watermelon (triploid) became more popular than seeded (diploid) varieties due to consumer preferences and are estimated to make up nearly 85% of U.S. production (AGMRC, 2021). While popular, seedless production requires maintaining polyploidy in the system by crossing female tetraploid and male diploid plants to create the triploid seedless varieties (Zhang *et al.*, 2012). This results in high costs to establish and maintain watermelon fields, leading to efforts to protect plants from pathogens, insects, and disease along with ensuring adequate pollination is achieved (Wijesinghe et al. 2020).

1.1.6 Indiana watermelon production

Indiana is typically a top 5 watermelon producing state, with the state's average annual production value (2016-2020) at nearly $35 million across 6,500 acres (USDA NASS 2020). Much of this production is concentrated in the southwestern region of the state where milder climate provides more favorable conditions. Seedless varieties are most common; growers in the state achieve fruit set by interspacing pollen donating diploid varieties along the row at a 1:2 or 1:3 ratio of seeded: seedless. The high cost of producing seedless watermelon varieties combined with poor in-field germination results in transplanting seedlings to be the most common practice. There are a variety of cultural practices to maintain crop growth, most commonly black plastic mulch and irrigation drip tape are used to control weeds and prevent drought stress to plants during periods where rainfall is insufficient. A single row of plants is transplanted along each row of plastic mulch with 3 to 6 feet separating plants along a row and 6 to 12 feet separating each row.

Watermelon transplanting will usually begin in southern Indiana by mid to late May, avoiding the last freeze dates while still beginning field growth as quickly as possible. One of the major concerns for watermelon growers are pathogens and disease emerging in the field as plants develop. Some of the diseases of concern are anthracnose, fusarium wilt, gummy stem blight, and downy mildew that are controlled through rotation of fungicide products and active ingredients often applied every 1-2 weeks once plant vines begin to extend beyond the plastic mulch (3-4 weeks after transplanting). Bacterial wilt, an insect-vectored disease of cucurbits, does not affect watermelons and is not a concern to the region's growers. Cultural practices to control diseases include crop rotations of non-cucurbit crops for 2-3 years between watermelon crop, use of resistant or resilient varieties, and tools such as MELCAST, the weather-based disease forecast system for Indiana cucurbits (Egel and Latin, 2015). Weed control is primarily achieved using plastic mulch, but preemergence herbicide between rows can improve late-emerging weeds along with tillage between plant rows if vines are turned and moved onto plastic to prevent crop damage. Vine growth leads to crop canopy cover that will also assist in weed suppression.

1.1.7 Watermelon insect pest management

In commercial watermelon production there is a host of arthropod pests that require monitoring to prevent crop damage or loss (Phillips *et al.*, 2021). One of the primary pests of concern is the

striped cucumber beetle *Acalymma vittatum* (F.), representing a risk to cucurbit growers throughout the season. In Indiana and surrounding states there are two generations over each growing season that damage plants across multiple growth stages. Overwintering adults directly feed on new transplants, slowing growth, and even killing plants at high densities. Adults continue to feed on growing plants and, after hatching from eggs deposited in the soil, larvae can feed on plant roots (Foster and Brust, 1995; Haber *et al.*, 2021). When watermelons grow large enough this feeding is no longer a major concern, however adults feed on the rind of maturing fruit which lead to scarring that makes fruit unmarketable.

One advantage of watermelon production is the resistance to *Erwinia tracheiphila*, the bacterium that causes bacterial wilt in many other cucurbits. This disease can devastate fields of cucumbers or cantaloupes and result in complete yield failure if the field becomes infected (Brust, 1997). The pathogen/insect complex leads to very low acceptable pest densities in crops susceptible to bacterial wilt (Phillips *et al.*, 2021), with higher economic thresholds for watermelons before management is recommended (Foster, 2017; Ternest *et al.*, 2020).

Additional pests of concern in the region are squash bugs (*Anasa tristis*), aphids (*Aphis gossypii*), and spider mites (*Tetranychus urticae*). These pests are highly mobile and, if not monitored through frequent scouting, can rapidly grow in population and reach economically damaging levels. To avoid these outbreaks many growers will rely on scheduled spray programs to suppress populations at vulnerable stages (Ternest *et al.*, 2020). Unfortunately, these insecticide applications may have the opposite of the desired effect as these broad-spectrum products are highly disruptive to beneficial insect communities that could otherwise naturally suppress pest populations (Douglas and Tooker, 2016).

Collectively, these insect pests are largely controlled by Indiana watermelon growers using insecticides. The pressure to produce marketable product (USDA AMS, 2016) free of cosmetic damage results in an over-reliance on insecticides to keep developing fruit free of insect pests until harvest. Despite the long-established benefits of an integrated pest management (IPM) approach to management, more conventional practices, such as simply applying insecticides, are easy and cost-effective. Ease of implementation and cost-effectiveness are some of the most influential factors in informing whether a grower will choose to adopt a management practice (Leach *et al.*, 2019). Collected spray records from Indiana watermelon growers found that an average of five insecticide applications were used in each season, with the highest reaching 10 sprays annually

(Ternest *et al.*, 2020). This high-frequency of insecticide use throughout the year can be an effect method of pest control, but in a pollinator-dependent crop such as watermelon there may be non-target effects to pollinators.

1.1.8 Pollination services in watermelon

Pollinator dependent crops make up nearly 10% of worldwide caloric needs (Lorenzo-Felipe *et al.*, 2020) and the importance of these insects is heightened as global population and food demands increase. As discussed earlier, watermelon is entirely dependent on insect pollination for fruit set (McGregor, 1976; Stanghellini *et al.*, 1997), and the widespread selection of seedless varieties make pollination even more essential. Watermelon flowers typically open within one hour after sunrise, close each evening, and will not re-open the next day (McGregor and Waters, 2014). This combination of factors makes it essential that pollinators are consistently present and foraging in the field (Stanghellini *et al.*, 1997; Stanghellini *et al.*, 2002a; Stanghellini *et al.*, 2002b). Female watermelon flowers have a 3-lobbed stigma and pollen from a pollenizer male flower needs to be spread across all lobes (500-1,000 total grains) to ensure market-grade shape and size (Adlerz, 1966; USDA-AMS, 2016; Campbell *et al.*, 2018)). If adequate pollination occurs, a mature fruit will reach market maturity in 40-50 days depending on environment and variety (Phillips *et al.*, 2021).

1.1.9 Honey bee pollination

To ensure adequate pollination is achieved, European honey bees (*Apis mellifera*) are the most popular option as a source of managed pollinators. This is a practice ubiquitous across many major U.S. crops and provide an estimated $15 billion annual value to growers in the U.S. alone (Potts *et al.*, 2010; Calderone, 2012). Honey bee colonies are typically placed in the field several weeks before their service is needed and removed from the field once the need for pollination ends. Honey bees are generalist pollinators and although they will frequently visit non-crop flower resources, the size of their colony (> 30,000 members) makes them an effective source of pollination in many systems (Winston, 1987; Stanghellini *et al.*, 1998). Watermelon is among the crops that employ beekeepers to supply honey bee colonies, Seedless varieties have recommended stocking densities higher (1-5 hives per acre) than other cucurbit crops (Phillips, 2019; Phillips *et al.*, 2021). This

reliance on honey bees amounts to a major cost to watermelon growers with the most recent estimates of regional rental cost averaging $70-80 per hive (USDA NASS, 2017). The transportation and management of honey bee colonies at times means that beekeepers find themselves in conflict with growers over hive loss while in these environments.

The economic importance of honey bees becomes increasingly concerning in recent decades as annual colony losses conspicuously grew to a point where beekeepers found themselves replacing up to half of their hives annually (vanEngelsdorp *et al.*, 2008). This puts an incredible financial stain on beekeepers, leading to economic failure or a dramatic increase in colony rental contract costs (Calderone, 2012). The use of insecticides (such as neonicotinoids) combined with the simplified nature of agriculture landscapes create an inhospitable environment for honey bees and other pollinators (Kremen *et al.*, 2002; Otto *et al.*, 2016; Alaux *et al.*, 2017). There is even evidence that these factors may synergize and create an environment where only intensively managed pollinators (like honeybees) persist in these environments (Bloom *et al.*, 2021).

1.1.10 Managed pollinator alternatives

In addition to honey bees, managed bumble bees have increased in popularity as an additional commercial option for watermelon pollination. The common eastern bumble bee (*Bombus impatiens*) is one of the most common native bumble bee species and produced in functional colonies that allow for easy transportation and installation in fields. While still a social species, bumble bees have an annual life cycle; new queens emerge each spring, build up a colony of several hundred workers who gather resources and eventually produce a new generation of queens who disperse and initiate their own colonies. Commercial colonies of *Bombus impatiens* are reared throughout the year and purchased by growers near peak colony size to optimize their services. Bumble bees are considered effective pollinators; an experiment in North Carolina watermelon fields found bumble bees deposit over three times the pollen compared to honey bees (Stanghellini *et al.*, 2002a). Another advantage of bumble bee pollination is their ability to forage at lower temperatures with less daylight than honey bees (Heinrich, 1979; Corbet *et al.*, 1993). While most frequently deployed in greenhouses or other enclosed environments, watermelon growers in Indiana are using these colonies in field settings with increased frequency. Managed colonies are easy to monitor and evaluate compared to most solitary wild bees, which has made them a popular

choice for research on pollination as well as their flower visitation (Cutler *et al.*, 2014; Gill and Raine, 2014; Brochu *et al.*, 2020; Ingwell *et al.*, 2021).

1.1.11 Wild bee community

Even though watermelon flowers have an open structure that allows for generalist visits, studies have found a high variation in pollen deposited on watermelon flowers across pollinator species (Kremen *et al.*, 2004; Winfree *et al.*, 2018). Many crops rely more heavily on native bees and flies for pollination services than managed bees, although dependence varies across major U.S. crops (Kremen *et al.*, 2004; Klein *et al.*, 2007; Winfree *et al.*, 2008; Garibaldi *et al.*, 2014). An advantage of native pollinators is that they are often more efficient pollinators, more adapted to pollinate in regional environments and provide enhanced pollination services (Winfree *et al.*, 2008; Garibaldi *et al.*, 2014; Campbell *et al.*, 2019). There are thousands of native bee species in the U.S., and while diversity is highest in natural areas, surveys in cucurbit crop fields have found diverse communities of 25-40 different species, depending on pesticide inputs and surrounding landscapes (Smith *et al.*, 2013; Campbell *et al.*, 2019; McGrady *et al.*, 2019). There is also a large dependency on the crop specifically; in an experiment on commercial cucumber, pumpkin, and watermelon fields there was a dramatic difference in the observed community (Bloom *et al.*, 2021). In that study, cucumbers were almost entirely (98%) pollinated by honey bees, pumpkins relied on a small group of honey bees, bumble bees, and specialist squash bee (*Peponapis pruinosa*), while watermelon was a diverse community of 35 species, primarily sweat bees (Halictidae).

Land conversion from natural and seminatural habitats to agriculture has resulted in a loss of flower diversity across the landscape, leading to widespread reductions in wild bee abundance (Dolezal *et al.*, 2019; St. Clair *et al.*, 2020; Quinlan *et al.*, 2021). The Midwestern U.S. has been an area where conversion has been most dramatic (Otto *et al.*, 2016; Dixon *et al.*, 2021; Smart *et al.*, 2021) and likely an area of the country where wild bee losses would be most heavily felt by growers. A comparison of honey bee and wild bee contributions to crop pollination found that wild bees contribute to a majority of pollen transfer in pumpkins, watermelon, tart cherries, and apples (Reilly *et al.*, 2020). These specialty crops often all rely on insecticides to protect yield from insect pests, likely acting as an exposure route to pollinators. Neonicotinoids accumulating in the soil within or adjacent to fields where they are used pose a direct threat to ground-nesting bees that are

often solitary and could not overcome the mortality that a social colony tolerates (Anderson and Harmon-Threatt, 2019; Willis Chan *et al.*, 2019).

1.1.12 Integrated pest management and economic injury concepts

The tactics and ideas behind integrated pest management (IPM) were in use long before the phrase began to be used in applied agricultural settings. To prevent damage to crops from pest insects, pathogens, and weeds those tasked to protect yield combine knowledge of pest biology, cultural knowledge, and modern technologies to create the foundations of IPM today (Kogan, 1998). Early in the development of IPM principles, an emphasis was placed on making environmental and ecological connections between pest and environment (Stern *et al.*, 1959; Geier, 1966). IPM manifests in cropping systems today using economic thresholds and pre-calculated tolerable levels of injury to monitor the natural populations of pests and respond with the appropriate management tactic if crops are threatened. These practices are not new, the original proposal of an economic-injury level (EIL) concept was published over 60 years ago (Stern *et al.*, 1959). Tailored research is required to artificially infest plants at various pest densities to determine what level of damage is sufficient to cause crop loss or can be responded-to with an insecticide application or another control practice. In Indiana cucurbits there has been extensive work previously to help determine these thresholds for striped cucumber beetles and their associated pathogens (Foster and Brust, 1995; Brust *et al.*, 1996; Brust and Foster, 1999).

The effectiveness of IPM to successfully control pests and protect yield is predicated on the compliance of growers to follow recommendations (Mitchell and Hutchison, 2009). To make these programs as effective as possible, we continue to attempt to improve our understanding of what management recommendations growers are more willing to adapt (Zalucki *et al.*, 2009; Peterson *et al.*, 2018). The practice most likely to be adopted and be effective are found to be practices that are easy to implement, easy to evaluate, low-risk, and can save money (Leach *et al.*, 2019). This often puts IPM at a disadvantage compared the current ease and affordability of conventional insecticide use in many cropping systems. Resistance development and non-target effects from insecticides are a growing concern and an emphasis on implementing IPM can ease these worries while protecting yield. A cropping system reliant on insect pollination provides an ideal environment to test these concepts and provide insight to growers on how IPM can provide tangible benefits to their operation.

A recent emphasis in IPM has been adjusting practices to protect pollinator health for both retaining pollination services on farms and environmental conservation. This has even gone so far to be called integrated pest *and pollinator* management or IPPM (Biddinger and Rajotte, 2015). While many of the pollinator-specific aspects of IPPM are built into the foundations of IPM, there is an emphasis on co-managing pest control and pollination goals, often simultaneously through deliberate management practices (Egan *et al.*, 2020; Lundin *et al.*, 2021). While not explicitly referred to as IPPM in this work, the integration of practices to conserve pollinators offers growers a chance to maximize crop yield.

1.1.13 Research goals and justification

There are many challenges to growers in the Midwest, however the unique challenge faced by specialty crop growers is requiring both pest control and pollinators. The goal of this research is to replicate commercial practices and examine how the adoption of IPM can impact pests, pollinators, and crop yield in these systems. Specifically, I began this work to examine how replacing prophylactic insecticide use, such as seed-treatments and calendar sprays, with weekly scouting could provide a more sustainable program for growers without compromising crop yield. These principles are decades old (Stern et al. 1959, Peterson et al. 2018), but there is always risk in any management change, and the higher the risk the less likely that practice is to be widely adopted (Trumble 1998, Peshin 2013). In this work I strove to identify the benefits and risks regional growers face when applying an IPM approach to their system. These practices are applied to both watermelon (regionally important specialty crop) and corn; the latter of which is rotated with watermelon and widely planted in the Midwestern US. The experiments provide a unique ability to observe a multi-crop system across years to gain a better understanding of what changes in the system if IPM is applied.

The largest focus of this work is examining the effect of an IPM system on pest abundance and pollinator visitation and the response of crop yield. Even in corn, a row crop not dependent on pollination, prophylactic insecticide inputs were removed, and pest damage was evaluated at key points in the season along with evaluation of yield in several scenarios to accurately assess any potential benefit from the insect protection. In watermelon fields, dependent on insect visits to produce yield, I monitored pollinator visits including their frequency and what pollinators make up the community. The goal was to observe how the pollinator community responds to pest

management system, and whether any changes in pollination can lead to differences in crop yield. Samples from field soil, leaf tissue, crop pollen, and pollinator colony wax and pollen were collected and accumulated pesticides were quantified and contrasted between the two pest management systems. Using colonies of two of the most popular managed pollinators, I was able to monitor their growth and reproductive capacity in the contrasting environments, informing what role pest management plays for these species. Finally, I included combined pollen quality, pollen pesticide contamination, and landscape composition to explore whether the adoption of IPM can help overcome environmental challenges pollinators face while foraging in simplified agricultural environments. Collectively, this work will improve the understanding on how IPM can improve a grower's operation. These findings can allow later research to apply the experimental design to different cropping systems to lead to more sustainable food production in the Midwest.

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CHAPTER 2. IPM REDUCES INSECTICIDE APPLICATIONS BY 95% WHILE MAINTAINING OR ENHANCING CROP YIELDS THROUGH WILD POLLINATOR CONSERVATION

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Abstract

Pest management practices in modern industrial agriculture have increasingly relied on insurance-based insecticides such as seed treatments that are poorly correlated with pest density or crop damage. This approach, combined with high invertebrate toxicity for newer products like neonicotinoids, makes it challenging to conserve beneficial insects and the services they provide. We used a four-year experiment using commercial-scale fields replicated across multiple sites in the Midwestern U.S. to evaluate the consequences of adopting integrated pest management (IPM) using pest thresholds compared with standard conventional management (CM). To do so, we employed a systems approach that integrated co-production of a regionally dominant row crop (corn) with a pollinator-dependent specialty crop (watermelon). Pest populations, pollination rates, crop yields, and system profitability were measured. Despite higher pest densities and/or damage in both crops, IPM-managed pests rarely reached economic thresholds, resulting in 95% lower insecticide use (97 vs. 4 treatments in CM and IPM, respectively, across all sites, crops, and years). In IPM corn, the absence of a neonicotinoid seed treatment had no impact on yields, whereas IPM watermelon experienced a 129% increase in flower visitation rate by pollinators, resulting in 26% higher yields. The pollinator-enhancement effect under IPM management was mediated entirely by wild bees; foraging by managed honey bees was unaffected by treatments and, overall, did not correlate with crop yield. This proof-of-concept experiment mimicking on-farm practices illustrates that cropping systems in major agricultural commodities can be redesigned via IPM to exploit ecosystem services without compromising, and in some cases increasing, yields.

Significance Statement

Environmental damage from insecticide overuse is a major concern, particularly for conservation of "good" insects such as pollinators that ensure stable production of food crops like fruits and vegetables. However, insecticides are also necessary for farmers to manage "bad" insects (i.e., pests) and thus a more holistic view of crop management needs to account for the proper balance between the beneficial and detrimental aspects of pesticides. Here, we used multi-year field experiments with a paired corn-watermelon cropping system to show that insecticide use can be dramatically reduced (by ca. 95%) while maintaining or even increasing yields through the conservation of wild bees as crop pollinators. These data demonstrate that food production and ecosystem sustainability are not necessarily conflicting goals.

2.1 Introduction

Integrated pest management (IPM) is a central organizing principle to guide pesticide use. At its core, IPM is designed to optimize pesticide inputs, preventing overuse via practices such as scouting with applications dictated by a range of parameters, including economic thresholds, heat unit accumulations, and historical data (i.e., a use-as-needed approach). Although IPM has been a mainstay in agriculture for > 50 years (1), technological and philosophical changes in farming practices over recent decades have made this well-accepted and effective approach to pest management far more difficult to implement in practice (2, 3). A contributing factor to this trend is the introduction and widespread adoption of prophylactic neonicotinoid seed treatments (NST) on staple crops such as corn, soybean, cotton, and wheat (hereafter, 'row crops'). Unlike some transgenic crops (i.e., Bt hybrids), NSTs were not developed in response to new or recurring pest outbreaks; in fact, pest populations remain at historic lows in many U.S. crops (4, 5). As a result, studies have struggled to document a clear agronomic or economic benefit from using NSTs in the U.S. and Canada (6-13), likely due to the sporadic occurrence of the pests they are purported to control. In a recent analysis, < 5% of corn fields in Quebec experienced a measurable benefit from the use of NSTs (14). Yet, > 90% of corn and > 50% of soybean and cotton seed is coated with a neonicotinoid in the U.S. (15, 16). NSTs could, in theory, conform to an IPM framework if proactive, insurance-based pest management is justified by persistent pest pressures (17); however,

the existing data largely do not support this view, especially in northern temperate regions (e.g., the U.S. 'Corn Belt').

The lack of yield benefit from NSTs is also concerning due to accumulating evidence of non-target effects from their overuse (18-20). When evaluated, < 5 % of NSTs were absorbed by the crop (21) with the remaining active ingredient lost to the greater ecosystem (10, 22), where it can persist for years in groundwater (23, 24) and soil (25, 26). The pervasive use of NSTs has led to contamination of waterways near crop fields (27), non-crop wild plants (28-30), pollen and nectar in honey bee colonies (31-33), and even human hair (34) and drinking water (35).

Although a wide diversity of non-target animals are vulnerable to neonicotinoid exposure, pollinating insects have been the most well-studied group, in no small part because of global declines in bee populations (36, 37). The insecticidal toxic load for honey bees has dramatically increased over the past 20-30 years, despite declining application volume (38, 39). This change was most evident in the U.S. Heartland with a 121-fold increase in oral toxicity, an effect attributed almost completely to corn and soybean NSTs. These patterns suggest that neonicotinoid inputs in row crops have the potential to profoundly affect pollinator health across landscapes with potential reverberations in non-corn/soybean habitats.

Most fruits, vegetables, and tree nuts (hereafter, 'specialty crops') are at least partially—and in some cases, entirely—reliant on insect pollinators for yield (40-42). Consequently, NST-mediated impacts have the potential to threaten food production. However, the crops driving neonicotinoid exposure are not the same ones that depend on bees for their services. Corn, soybean, and cotton account for > 80% of neonicotinoid use (15), but both soybean and cotton are primarily considered self-pollinating (despite some recent evidence for yield benefits with bee visitation (43, 44)) and corn is wind-pollinated. Although bees are known to visit these crops for nectar and/or pollen, insect pollinators are not critical to their production. Row crops are cultivated over a large fraction of arable land in the U.S. (9.8% of the continental U.S. is dedicated to corn, soybean, and cotton (45)) and specialty crop fields in this region are often adjacent to at least one of these row crops; therefore, we may expect carryover effects of NSTs on specialty crop pollination. For example, NST-infused dust from corn planting moves hundreds of meters beyond the field border (10, 32, 46), resulting in honey bee mortality (summarized in (47)). Thus, the relatively smaller areas devoted to specialty crops may invariably experience extra-field exposure from nearby row crops. Similarly, specialty and row crops are common rotation partners, resulting in neonicotinoid

soil residues that impact ground-nesting bees (48-50). These spatial and temporal avenues generate several possible exposure routes. A simulation model (46) using field-derived values predicted that NSTs from corn planting in late spring erode honey bee population size enough to reduce capacity for blueberry and cranberry pollination later that summer, resulting in the potential for economic losses to neighboring berry growers. A similar outcome was demonstrated when modeling almond pollination potential for honey bee colonies that reside in the corn-dominated Northern Great Plains for much of the year (51).

In the work described here, we empirically test the hypothesis that IPM implementation, consisting of pest thresholds and removal of NSTs, dramatically reduces insecticide use and improves pollinator function without sacrificing crop yields. To do so, we used a multi-year, multi-site field study, conducted in a dual cropping system representative of agriculture in the Midwestern U.S., and other parts of the world, consisting of a smaller acreage specialty crop paired with (i.e., adjacent to and grown in rotation with) a larger acreage row crop. We compared the effects of IPM vs. conventional insecticide practices across several key metrics: insect pest abundance and damage, pollination, and yield. This design is unique in integrating field measurements of all factors across years, locations, and cropping systems. We paired field corn and seedless watermelon—a functionally dioecious crop that requires bees to move pollen between plants for fruit production. The experiment was conducted over four years (2017-20) across five sites in Indiana, a state that is typically ranked in the top five nationally for both corn and watermelon production (52). In the conventional management (CM) system, we applied industry-standard practices used by growers in the region, characterized by NSTs on corn and preventative, calendar-based insecticides on watermelon. In the IPM system, we used NST-free corn seed with watermelon inputs determined by population thresholds established for arthropod pests. We predicted that the IPM system would have both higher pest densities (while remaining below economic thresholds) and pollinator visitation rates, resulting in equivalent (corn) or higher (watermelon) crop yield and increased farm profitability. This field experiment provides a comprehensive reassessment of IPM principles for both modern row crop and specialty crop pest management in the highly productive and intensively managed agricultural region of the Midwestern U.S.

2.2 Results

2.2.1 IPM systems experienced infrequent pest outbreaks, requiring few insecticide inputs

Neonicotinoid seed treatments target early-season pests; however, early-season corn damage was unaffected by NSTs with corn plant stand similar (P = 0.867) between IPM (11040 ± 145 plants ha^{-1}) and CM (11052 ± 106 plants ha^{-1}) fields (Figure S2.3; see Table S2.6A for full statistical model for this and subsequent pest metrics). Similarly, during the first 3 years of the study, < 1% of sampled plants showed any direct evidence of feeding by western corn rootworm *Diabrotica virgifera virgifera* LeConte—the primary insect pest of corn in this region—across both treatments (overall damage rating: 0.001 ± 0.000 nodes). In the fourth and final year (2020) damage was more prevalent with 33% of IPM corn roots showing evidence of rootworm feeding. This pattern resulted in a significant treatment × year interaction (P = 0.006) with pairwise comparisons showing that IPM fields in 2020 had higher damage ratings than all other treatment × year combinations (Figure S2.4). Despite this statistical increase in pest pressure in the IPM treatment over time, the magnitude of the effect was low (2020 IPM damage rating (on a 0-3 scale): 0.17 ± 0.07 nodes).

Watermelon in the CM treatment received insecticide sprays on a pre-determined schedule that did not depend on scouting. These calendar applications maintained populations of the primary insect pest—striped cucumber beetle (SCB) *Acalymma vittatum* (F.)— well below the published economic threshold of 5 beetles per plant (Figure 2.1A; seasonal mean SCBs per plant = 0.11 ± 0.05). In IPM fields, SCBs also rarely reached their economic threshold (Figure 2.1B; seasonal mean SCBs per plant = 1.18 ± 0.34). Over the three-year experiment, only 4 total IPM insecticide sprays (2018: 1, 2019: 1, 2020: 2) were required across all five sites combined (i.e., 4 applications in 15 site-year growing seasons). In contrast, 77 insecticide applications were made in the CM treatment over the same period across all sites. In the IPM treatment, a single spray per field was sufficient to keep populations below economic thresholds for the remainder of the season; however, in most site-years even a single spray was unnecessary. Appearance of secondary pests—primarily aphids and spider mites—occurred under both management systems (CM = 6, IPM = 4), but, interestingly, these populations only warranted additional pesticide applications (n = 2) in the CM plots (Table S2.5). All other observed secondary pests did not spread to neighboring plants and were likely controlled by abiotic factors (heavy rain) or natural enemies, which were

confirmed by the presence of parasitized aphids or coccinellid larvae/adults on flagged plants known to be previously infested.

2.2.2 Pesticide residues were higher in conventionally managed systems

Neonicotinoids applied to both crops in the CM system were routinely found in sampled plant tissues and soil; 99% (n = 335) of all samples collected had residues of at least one neonicotinoid compared to only 65% (n = 221) of IPM samples.

Neonicotinoids in the pollen of both crops were higher in the CM than IPM treatment. Watermelon pollen had consistently higher concentrations of imidacloprid in CM (median: 6.17 ng/g) compared to IPM (median: < LOD) flowers (Table 2.1); however, residues in CM fields decreased over time with highest values in early-blooming flowers (Table S8). Both clothianidin (CM: 49%, IPM: 5%) and thiamethoxam (CM/IPM median: < LOD) were infrequently detected at low levels in watermelon flowers. Corn pollen, on the other hand, rarely contained imidacloprid residues (CM: 50%, IPM: 10%), but CM corn pollen contained higher levels of both clothianidin (93% detection, median: 1.91 ng/g) and thiamethoxam (100% detection, median: 2.01 ng/g) than IPM corn pollen, which only contained detectable amounts of clothianidin and thiamethoxam in 20% and 10% of all samples, respectively (Table 2.2). This low-level contamination is likely attributable to uptake of carryover NSTs from previous cropping seasons before the experiment began or adjacent fields.

Neonicotinoid residues were also higher in soil and leaf samples within the CM management system, depending on sample date. See Tables S2.7-S2.9 for pesticide summary data across all sample types and years. Non-neonicotinoid pesticides applied to the system—fungicides and the pyrethroid lambda-cyhalothrin—were also detectable, but at varying levels (Table S10). In general, fungicide detection was roughly equivalent across CM and IPM fields, whereas lambda-cyhalothrin was more frequently detected in watermelon leaves and pollen in CM fields (but overall detection rates were relatively low; <20% of samples).

2.2.3 IPM enhanced watermelon pollination

The pollinator community composition was broadly similar across treatments with the most commonly observed taxa being: honey bees, *Apis mellifera* (CM = 35%, IPM = 13%), *Melissodes*

sp. (CM = 22%, IPM = 25%), and *Lasioglossum* + *Halictus* sp. (CM = 26%, IPM = 37%) (see Figure S2.5 and Table S2.11 for a complete description across taxa). Overall abundance of pollinators visiting flowers was 99% greater in IPM (0.64 ± 0.05 pollinators min^{-1}) than CM (0.32 ± 0.02 pollinators min^{-1}) fields (see Table S6B for full statistical model for this and subsequent pollination metrics). Notably, this pattern was driven entirely by wild bees. When treatment effects were tested for managed and wild species as separate groups, there was no impact on honey bee visitation ($P = 0.202$) but wild bee visitation was lower ($P < 0.001$) in CM fields.

Number of flowers visited min^{-1} was 129% greater in IPM (1.25 ± 0.11 visits min^{-1}) than in CM (0.55 ± 0.05 visits min^{-1}) fields (Figure 2.2A). Also, transition visits (observed trips from male to female flower) were 305% higher in IPM (0.18 ± 0.02 transition visits min^{-1}) than CM (0.05 ± 0.01 transition visits min^{-1}) fields (Figure 2.2B).

2.2.4 NSTs did not affect corn yield

There was no statistical difference ($P = 0.097$) in corn yields between management systems, but there was a trend for higher yield in IPM (10602 ± 479 kg/ha) compared to CM (9471 ± 694 kg/ha) fields (Figure 2.3A; see Table S2.6C for full statistical model for this and subsequent yield metrics). Similarly, we conducted a more targeted small-plot trial in 2019 with higher replication and better control of local environmental factors. This follow-up experiment also showed no difference ($F_{1,51} = 0.47$, $P = 0.501$) between +NST (12688 ± 269 kg/ha) and -NST (12511 ± 311 kg/ha) corn yields (Figure S2.6).

2.2.5 IPM watermelons produced higher yields by preserving wild bees

Watermelon yield was 25.7% higher in IPM (9.91 ± 0.84 kg/m^2) than in CM (7.88 ± 0.63 kg/m^2) fields (Figure 2.3B). The significant difference in overall yield between treatments ($P = 0.002$) was driven by the reduced number of watermelons harvested in CM (59.07 ± 4.15) compared to IPM (72.13 ± 5.51) plots. Individual fruit weights were not statistically different (P = 0.071), but IPM melons (6.76 ± 0.18 kg) tended to be larger than those from CM (6.22 ± 0.23 kg) fields. Yield data only included fruit deemed marketable without any rind damage from insect feeding or other deformities. IPM watermelons experienced an increased number of damaged fruits

(55 deemed unmarketable in IPM with only 1 in CM fields); this represented a < 5% loss in potential yield.

There was no relationship between total pollinator visitation and crop yield, likely due to the high stocking of managed honey bee colonies in both pest management systems. To test this possibility, we separately analyzed honey bees, apart from the wild bee community. This subset analysis confirmed that honey bee visitation could not predict watermelon yield (Figure 2.4A; overall slope, $P = 0.097$), whereas higher rates of wild pollinator visitation, driven by lower insecticide use, resulted in correspondingly higher watermelon yield (Figure 2.4B; overall slope, $P = 0.043$, CM slope, $P = 0.218$, IPM slope, $P = 0.728$).

2.2.6 IPM was more profitable than conventional management

The product cost (i.e., no application cost) of Cruiser 5FS on corn was \$31.10 ha^{-1}; however, using industry-provided data (53) the inflation-adjusted cost of a NST at the rate applied in this study was \$57.79 ha^{-1}. Using this cost calculation and the range of field sizes, the use of a NST in CM corn represented a cost of \$330.93 ± 30.93 $field^{-1}$. The cost relative yield (CRY; the minimum percentage in yield gain where the insecticide cost is recuperated) was 3.3%, which was not reached in either the CM/IPM experiment or the within-site NST evaluation, indicating that the cost of NST was not recovered at any of the sites in this experiment.

Watermelon insecticides in the CM system cost \$44.05 ha^{-1} for the soil drench and \$50.28 ha^{-1} for all foliar insecticide applications (\$12.57 per application) for a total cost of \$94.33 ha^{-1} on each field with additional applications required to control secondary pests in some fields increasing this cost. While several insecticide sprays were applied to the IPM watermelons, this was a minority of fields leading to an average cost for IPM insecticides at \$3.35 ± 1.44 ha^{-1} compared to \$100.98 ± 3.49 ha^{-1} across the CM watermelon fields. The insecticide program for CM watermelons had a CRY of 0.70%, however all fields within the CM system failed to reach this threshold and the insecticide applications were never cost-effective. The increased yield from wild pollinator enhancement in the IPM system would result in a financial gain of \$4,512.69 ha^{-1} over the CM system, based on the previous 5-year regional sale price for seedless watermelon (52).

2.3 Discussion

IPM-based approaches, ones that prioritize treating only when insect pests are present at damaging levels, have become increasingly rare across a range of commodities. Instead, a suite of prophylactic approaches to pest management—including insecticidal seed treatments, soil drenches, and calendar sprays—now dominate most U.S. cropping systems, including the corn and watermelon systems studied here. However, our comprehensive field experiment demonstrates that there is no clear rationale supporting this approach from multiple perspectives including insect pest damage and abundance, pollinator visitation and efficiency, environmental pesticide residues, or crop yield and profitability. These varied and integrative perspectives are vital for grower adoption, but surprisingly rare in practice. Hundreds of studies, for example, have tested the negative effects of neonicotinoids and related insecticides on pollinator health in the laboratory and field. The potential threat from these products is incontrovertible. Yet, pollination alone paints an incomplete picture without corresponding data on pest population dynamics and crop production. In previous studies that experimentally reduce insecticide use in crops to determine impact on pollinators, the implications for pests and crops are typically overlooked or omitted, e.g., canola (54), cucurbits (49, 55), apples (56), sunflowers (57). Similarly, in studies where landscape complexity is used as a predictor of pollination services (58, 59) wholesale changes in pest management practices are not explicitly measured or discussed. Farmers are unlikely to change their management practices—no matter how detrimental to bees—if foregoing insecticide treatments leads to excessive crop and economic damage. Conversely, studies on pest/yield relationships (with limited exceptions (60, 61)) involve self- or wind-pollinated crops (7, 11, 62). These experiments often fail to capture the additional losses to yield that nearby or adjacent crops could experience – even though in some cases, the landowner/crop producer is the same individual.

2.3.1 Insecticide use, pest outbreaks, and crop yield

One expected corollary of reducing insecticide inputs over years of the experiment was an increase in pest densities over time. Surprisingly, the only evidence of increasing pest pressure on untreated corn was higher damage from rootworm larval feeding in year four. To isolate the effect of NSTs with minimal confounding factors, corn in our experiment was grown somewhat atypically: without any Bt-traits or crop rotation. Therefore, IPM corn was cultivated under a

'worst case scenario' with no protection for the duration of the study. Despite being entirely defenseless for four consecutive years, only 3 of the 5 fields experienced increased root feeding and only in the final year. These locations were at the northernmost sites, which is the region of the state where rootworm pressure is historically highest (63). This outcome demonstrates that corn rootworm populations in major production areas should not be left unchecked and can increase in a relatively short time, but the industry standard of Bt corn with soybean rotation likely maintains rootworm at sufficiently low levels. It is also important to note that, while we focus on rootworm as the primary corn pest and one for which we observed some evidence of feeding damage, NSTs are largely marketed as targeting secondary pests (e.g., wireworm, seedcorn maggot). These taxa were not present at appreciable densities in any of our experimental fields. Although these cryptic belowground insects are hard to directly sample, indirect evidence of their presence and impact (e.g., poor plant stand in early-season corn) was never observed.

In spite of the rise in rootworm damage over time in NST-free corn, yields were not significantly different across the two systems, reinforcing other published studies that show no yield benefit from NSTs (8, 11, 14). Interestingly, the only factor impacting corn yield had nothing to do with insecticide use. We observed gradual but consistent reductions over time with year 4 yields 28% lower than year 1 yields. This effect was apparent across both IPM/CM treatments. The outcome is not surprising as numerous studies have documented that single-species cultivation has negative feedbacks on crop productivity, including corn (64). These data strongly point to crop rotation as a factor in maintaining high corn yields, and likely far more critical in mitigating rootworm damage than NST use (12). For the purposes of this study, we more narrowly defined IPM in the context of insecticide use, but a 'true' IPM system would employ crop rotation rather than continuous cropping.

Unlike corn, the key insect pest in IPM watermelon colonized in the initial year and was present at moderate densities throughout the entire experimental period, but, similar to the corn system, these elevated densities did not translate to yield reductions, even using the fairly liberal threshold of 5 beetles per plant. These data suggest that watermelon should be routinely scouted to protect against the rare site or year where pests like cucumber beetles exceed their threshold but can mostly be cultivated without insecticide use (65-68). Notably, we only observed outbreaks of secondary pests—aphids and mites—in the CM system where we repeatedly treated the crop with insecticides. Cucurbit growers in our region frequently mention these as pests of concern; however, many of

these same producers also use repeated applications of pyrethroids and neonicotinoids (69), compounds that are highly disruptive to beneficial insect communities that suppress aphid and mite populations (70) Altogether, these observations imply that overly aggressive treatment with broad-spectrum insecticides trigger secondary pest outbreaks in watermelon and that adopting a scouting-based IPM program with fewer inputs prevents the problem.

A major challenge to scouting adoption is that the CRY for watermelon is < 1%, reflecting the reality that insecticides such as pyrethroids are inexpensive relative to other farm inputs (e.g., labor). Moreover, our CRY calculations do not account for the additional cost of scouting in IPM systems, which can be challenging to estimate (69). Some growers scout their own fields for pests, while others hire crop consultants. Similarly, scouting a subset of fields or sporadically observing a few edge plants (vs. walking transects with a specified sample number and location) will undoubtedly reduce costs but also accuracy. In our experiment, insecticide costs were ca. \$101 ha^{-1} in CM compared with \$3 ha^{-1} in IPM. Thus, scouting would need to add at least \$98 ha^{-1} to offset the difference. Other factors that affect the reliability of this estimate include the additional cost (e.g., fuel, equipment, labor) of repeated insecticide applications in CM fields and variation in insecticide price or efficacy. Despite these complexities, Ternest et al. (69) found that the cost of seasonal pest scouting ranges from \$29-\$120 for a field, well within our estimated price point for a commercial watermelon grower to see a positive return from scouting.

The economics of scouting and IPM as a whole also vary widely across cropping systems. We primarily consider watermelon where crop value is relatively high, fields are relatively small, and the pests are mostly aboveground and can be controlled with insecticide sprays. In large acreage row crops such as corn with belowground pests that are both hard to sample and lacking immediate rescue-treatment options, the cost/benefit ratio of scouting may be less favorable. Even among specialty crops, we expect the net value of IPM to be highly variable. Watermelon exhibits a few features that could tip the balance in favor of IPM. Compared with other cucurbits, for example, watermelon has a much higher pest threshold due to its natural resistance to the SCB-transmitted bacterial wilt (*Erwinia tracheiphila*) that kills infected plants (71). Also, seedless watermelon has among the highest reliance on bee pollination (72) and, consequently, the risk of insecticide overuse disrupting fruit production is correspondingly greater in this system. Specialty crops with lower pest tolerances and pollination requirements or those produced in regions with higher pest pressures will experience vastly different trade-offs. These relationships are also dynamic and need

to be reevaluated regularly over time. In our region and many other parts of the world, insect invasions (e.g., brown marmorated stink bug, (73); spotted winged drosophila, (74); spotted lanternfly, (75)) result in a constantly changing landscape of pests and the economics underlying their management.

2.3.2 Routes of insecticide exposure for pollinators

Neonicotinoids were consistently found at higher levels in the pollen of both crops within the CM system compared to IPM. The specific concentrations detected are comparable with related studies. For instance, squash pollen contained 15-19 ng/g of imidacloprid 7 weeks post-application (76) compared to a median value of 6.28 ng/g in this experiment. A trial across the cantaloupe flowering period ranged from 3 to 141 ng/g imidacloprid (77), demonstrating the wide range of potential exposure. Some of this variation is likely explained by bloom time, as we documented much higher levels in early than late flowers. This temporal effect is not trivial. Growers receive price premiums for early melons and these data indicate that the most valued early flush of flowers are the ones that are most heavily contaminated with neonicotinoids.

Bees were also likely exposed via soil residues. Recent studies emphasize the significance of soil-derived neonicotinoid exposure for ground-nesting bees, including imidacloprid in cucurbits (48, 49, 55). This difference in exposure could partly explain why we observed treatment effects on floral visitation for wild bees (most of which are ground-nesters) and not managed honey bees. However, this differential response among pollinators is likely driven in part by other factors inherent to honey bee biology and management (e.g., hives are stocked at high densities with > 20,000 individuals per colony; large individual body size and thus pesticide tolerance compared to many solitary wild species). A recent field experiment on commercial cucurbit farms in the Midwestern U.S. similarly found that insecticide use reduces wild bee visitation with no corresponding effect on honey bees (78). This effect is notable since wild bees in our experiment were both most sensitive to insecticide use and most strongly correlated with crop yield. The latter outcome should be expected – wild bees in general are more efficient than honey bees as crop pollinators (79-81) and in watermelon wild bees are more than twice as effective on a per-capita basis in promoting fruit set and growth (81, 82).

A limitation of our experimental design is that we are unable to differentiate the relative influence of corn and watermelon inputs on crop pollination since the two are confounded (i.e., we

did not independently manipulate insecticide use across the two crops in a factorial design). Because the crops were treated with different neonicotinoids—thiamethoxam in corn and imidacloprid in watermelon—we can infer mobility and exposure across these crop-types by interpreting residues from these active ingredients. Clothianidin, for example, was detected at low levels in 72% of CM watermelon pollen in 2019 compared to 0% in IPM pollen, despite never being applied to watermelon in either treatment. These patterns suggest that watermelon roots scavenge these compounds from a pool of soil residues derived from either ground water movement from the surrounding corn, or carryover effects due to the prior year's NST corn planting. Another likely possibility is that highly mobile bees foraged across crop boundaries, which were well within the flight radius of most taxa. Generalist pollinators like bumble bees tend to avoid cucurbit pollen (83) and readily forage on corn pollen when little else is available (84). Indeed, we observed few bumble bees foraging on watermelon flowers (<10% of visits; Fig. S5), despite stocking fields with managed hives. However, more information is needed on the foraging ranges and behaviors of non-honey bee taxa across crop boundaries; for example, the longhorn bee *Melissodes bimaculatus* is an extremely common, mobile, and effective wild pollinator but its movement within or between crop fields is poorly documented.

A final outcome worth emphasizing is the speed with which the pollinator community responded to IPM implementation. Improvements to bee visitation and yield were observable rapidly, in the first year of the experiment (Fig. 2), even though these farm sites were conventionally managed in previous years and surrounded by conventional agriculture. The response did not require multiple years of insecticide reduction or installation of pollinator habitat. There is a perception that farmland in its current state is devoid of natural life, but these data show that reduced inputs alone, independent of habitat or land use changes, can have demonstrably positive effects in the near-term.

2.3.3 Conclusion

One of the central challenges of global food security in the 21st century is ensuring adequate food supply for a growing population while conserving natural resources. These are often viewed as contradictory endeavors, i.e., a trade-off between agricultural productivity and conservation. Indeed, 'feeding the world' is a common rationale for excessive pesticide use and insurance-based pest management approaches in crop protection. Yet, increasingly, studies find that substantially

lower pesticide inputs result in equivalent yields (85), suggesting that high productivity can be maintained—or even increased, as shown in our study—with less intensive management. This finding dovetails the recent call for ecological intensification of agriculture, for which IPM adoption is a central theme (86-88).

Overall, our study demonstrates that the current, prophylactic approaches offer no consistent benefits to offset the demonstrably negative impacts to both pollinators/pollination and crop yields. The convenience of NST and calendar sprays to manage pests is clearly attractive to some producers. However, this argument rests on the twin assumptions that 1. populations of target pests can be expected to be at economically damaging populations each year, and 2. monitoring-based IPM alternatives expose producers to higher risks and/or upfront costs. Our data do not offer support for these claims in either cropping system, and in fact, show that embracing the use of IPM may offer a readily available "win-win" scenario for crop production and pollinator health across diverse crops.

It is important to note that conducting pest surveys with economic thresholds is not a new phenomenon; thus, our approach was not revolutionary and did not reinvent the wheel. The tools, in principle, have been established for decades, even if they have fallen out of practice. A key step forward is better understanding the thought process underlying when and why farmers decide to use insecticides. There is a myth that farmers only care about profit and refuse to monitor pests because it is too much effort or too time-consuming. Neither of these seem to be universally true. In a recent grower survey of reasons for implementing action thresholds, saving money on insecticide sprays was not among the top three responses and ranked beneath "less harmful to the environment" (89). Similarly, "reducing scouting" and "convenience" were among the bottom several reasons when soybean farmers were surveyed about their pest management decisions in the context of seed treatments, whereas "protecting water quality" and "public safety" were among the top factors (90). These trends are validated by the success of previous extension-based programs in helping growers adopt IPM tactics (89). However, IPM adoption has a long and rocky history that extends far beyond grower education efforts (91-95). This circumstance is particularly complicated for seed treatments where growers may not be making explicit decisions to use neonicotinoids since they are typically the default option offered by seed suppliers (16). In this case, an "extended peer community" that engages farmers, consumers, industry, government, and conservation programs will be vital (96), while ensuring that choice is maintained in crop seed

sales and growers are provided with clear guidelines for how to implement scouting using scientifically-backed pest thresholds.

2.4 Materials and Methods

2.4.1 Site & Experimental Design

The experiment was conducted over four years (2017-20) on five research farms at the Purdue Agricultural Centers (PACs), located across Indiana (Figure S2.1): Northeast (NEPAC; Columbia City, IN), Pinney (PPAC; Wanatah, IN), Throckmorton (TPAC; Lafayette, IN), Southeast (SEPAC; Butlerville, IN), and Southwest (SWPAC; Vincennes, IN). These sites are positioned along a latitudinal gradient across the state with at least 100 km separating one another, ensuring that sites represent a diversity of climatic conditions, soil types, and local pest pressures.

Each site contained of a pair of agricultural fields that were randomly assigned to either a conventional management (CM) or integrated pest management (IPM) program. These treatments were designated in year one of the study (2017) and remained within this management system for the duration of the experiment. CM systems were considered the 'industry standard' and designed to mimic the pest management regime typically found in both row crops and vegetable production, including the routine use of prophylactic insecticides. The IPM system was an experimental treatment that relied on pest scouting to determine the use of insecticides. We only applied insecticides as needed based on published action thresholds as specified in Supplemental Methods. Within a site, paired fields were separated by an average of 5.6 km (range: 4.63-6.63 km), which resulted in similar abiotic conditions (e.g., temperature, precipitation) while providing sufficient buffer for biological independence of CM/IPM treatments, as insect pollinators are unlikely to fly >5 km (97).

2.4.2 Cropping Systems

Fields (area mean: 5.74 ha, range: 4.82-7.73 ha) were planted continuously with corn in all four years of the study. While corn-soybean rotation is common in the Midwestern U.S. (72.3% of all corn acreage in key corn producing states—Iowa, Illinois, and Indiana—from 2015-19), continuous corn is the next most prevalent system, constituting 24.7% of acres (52). Starting in year two of the study (2018) and continuing for three growing seasons, we planted a 0.2 ha

watermelon plot embedded centrally within the corn matrix (Figure S2.2). Corn is the dominant crop grown in Indiana and throughout much of the Midwest (11.74 million ha across Iowa, Illinois, and Indiana). Thus, this design is a microcosm of Midwestern U.S. agriculture, where pollinator-dependent crops such as watermelon are bordered, and often completely surrounded, by corn. The goal of this design was to document the effects of large field crop plantings upon other, adjacent cropping systems. Corn was planted one year in advance of watermelon because neonicotinoid exposure can occur both in-season through a variety of exposure routes, or from the previous year's inputs. This aspect of the experimental design reflects that the vast majority of watermelon acreage on Indiana farmland (77%) is in rotation with either corn or soybean (52). Management practices (e.g., tillage, irrigation, fertilizer, herbicides and fungicides) were standardized across sites such that the only factors differentiating CM/IPM field pairs were insecticide inputs (see Supplemental Methods for management details and field histories).

All corn seed (Spectrum 6334) across both treatments received a fungicide seed treatment (Maxim Quattro: Azoxystrobin 2.5 µg; Fludioxonil 6.5 µg; Mefenoxam 5 µg; Thiabendazole 50 µg of a.i. seed^{-1}); however, CM corn seed was also treated with the neonicotinoid thiamethoxam applied at the maximum rate, marketed for control of corn rootworms and a suite of other secondary pests (Cruiser® 5FS @ 1.25 mg a.i. seed^{-1}). By 2012, > 80% of all U.S. corn seed was coated with at least one neonicotinoid (15), and the CM treatment thus represents the corn seed most commonly used by U.S. farmers. Throughout the experiment and in both treatments, we used a non-transgenic variety that did not express Bt toxins (*Bacillus thuringiensis*), meaning that the untreated 'IPM' seed was unprotected from larvae of the western corn rootworm (*Diabrotica virgifera virgifera* LeConte), the key corn insect pest in the region, and other soil insect pests. This allowed for a 'true' assessment of the efficacy of NST impacts on pest control without the confounding effects of multiple, layered plant protection technologies. However, in practice, all corn seed sold in the U.S. that expresses Bt toxins is also treated with at least one neonicotinoid insecticide (98).

We used a seedless watermelon system, which requires triploid and diploid plants interspersed with one another. All watermelon fields contained the triploid var. 'Fascination' as the seedless crop along with the diploid var. SP-7 as the pollenizer at a 3:1 ratio to ensure adequate pollination. At transplant, CM watermelons were treated with the neonicotinoid imidacloprid (Wrangler® @ 814.09 ml/ha) as a soil drench at the high rate, while IPM watermelons received no insecticides.

Additionally, CM watermelons were sprayed with the high rate of the insecticide lambda-cyhalothrin (Warrior II® pyrethroid @ 140.3 ml/ha) via tractor-drawn air blaster or boom sprayer at 4, 6, 8, and 10 weeks post-transplant, resulting in four foliar applications each season. Application rates for both insecticides (standardized by mL of a.i. per ha; lambda-cyhalothrin = 31.98, imidacloprid = 316.43) are within the range recommended by the label (lambda-cyhalothrin = 21.32-31.98, imidacloprid = 237.94-356.91). Similarly, insecticide rates used in the experiment are slightly higher than but comparable to those applied by watermelon growers in our region, according to on-farm pesticide records reported in (69): lambda-cyhalothrin (n = 18 applications; mean = 26.93, median = 26.66, range = 16.66-33.32), imidacloprid (n = 7 applications; mean = 293.92, median = 297.43, range = 250.22-328.41).

Although watermelon insecticide regimes across growers are more diverse than corn, our prior on-farm survey of insecticide use on 17 Indiana watermelon farms found that producers averaged *ca*. five treatments per field per season and thus the five applications in the CM treatment (1 soil drench + 4 foliar sprays) were intended to reflect this practice (69). The survey further revealed that pyrethroids, including lambda-cyhalothrin, were the three most used active ingredients. Neonicotinoids, including imidacloprid, were also used but at lower frequencies (30% of watermelon growers in (69)). These data guided our pyrethroid-biased regime in the CM treatment. Watermelons in the IPM treatment were left untreated unless insect pests exceeded economic thresholds at a site (see below), in which case the field was also treated with a foliar spray of lambda-cyhalothrin, as above. Additional details on corn and watermelon management (e.g., planting dates, seeding rates) are provided in the Supplemental Methods.

The watermelon-corn matrix was supplemented with managed bees to replicate the pollination practices used by commercial watermelon growers, who typically either rent honey bee hives from beekeepers or purchase bumble bee hives. Increasingly, growers in our region stock with both honey bees and bumble bees in the same field due to their foraging at different times and weather conditions. In each field, two honey bee colonies were placed on opposite corners at the edge of watermelon plots in an arrangement that avoided interference with pesticide application. This stocking rate (1 hive per 0.1 ha) falls within the recommended range for commercial production used by regional growers (99). Additionally, one Quad pollination hive (Koppert Biological Systems, Howell, MI) containing four bumble bee (*Bombus impatiens*) colonies was placed in each field at 4-5 weeks post-transplant to synchronize activity with the watermelon bloom period.

Insect Pest Abundance & Damage. Corn plants were evaluated for both early- and late-season pest damage to assess the efficacy of insecticidal seed treatments. Because foliar insect pests were rarely observed, sampling focused on the more economically damaging guild of soil-dwelling root pests. First, corn stand was evaluated at the V3-V4 stage, along six 5.3 m transects down a row, in which the number of emerged plants was counted. Transect counts were averaged and extrapolated to estimate plants/ha and compare with known planting densities. Poor corn stand, relative to initial planting rates, is often an indication of below-ground seedling damage by insects, including wireworms and seedcorn maggots (100, 101). At corn anthesis, root damage was quantified to determine potential for lodging due to corn rootworm feeding. In every field, 10 random plants were excavated along each of four transects that were > 20 rows from the field edge with > 10 m separating sampled plants within a transect. The root mass was then rinsed and evaluated for damage using the Oleson injury rating scale (102), the established approach for assessing rootworm feeding.

Beginning the week following transplant, watermelon plants were surveyed for pests weekly for a 10-week period extending to harvest. Each survey consisted of five randomly positioned transects, with plants sampled at 10, 20 and 30 m from the plot edge (n=15 plants per plot per week). For each plant, all above-ground tissue was inspected, and the identity and number of insect pests found on the plant or the soil directly below were recorded. If the density of the primary pest, striped cucumber beetle (SCB) *Acalymma vittatum* (F.), exceeded the economic threshold of 5 adult beetles/plant then the plot was treated with a foliar spray of lambda-cyhalothrin within 2 d of the observation (103). See Supplemental Methods for additional details on pest scouting protocol.

2.4.3 Watermelon Pollinators

To assess pest management impacts on pollination we conducted visual observations of watermelon flowers to quantify pollinator visits and community composition. Flower clusters, consisting of at least 5 male and 1 female flowers, were observed for a 3-minute period during which pollinator type, number of flowers visited, and transition of pollen from a male to female flower (i.e., a pollination event) were recorded. Behavioral observations were conducted on the same date at both fields at each site. First observation began 5-6 weeks post-transplanting and

continued for 5 consecutive weeks to encompass most of the blooming period that contributes to harvested yield. See Supplemental Methods for more detail on sampling design.

2.4.4 Crop Yield

Corn maturity was monitored, and the crop was harvested during each of the four years to assess the impact of NSTs on yield. All yield reports were adjusted to account for variation in moisture at harvest and data were standardized to a 15.5% moisture content.

Because corn yields were strongly affected by local factors (e.g., soil type, pH, drainage) determined by random field assignment, we conducted a separate companion study in 2019 using the same two corn seed treatments. This higher-resolution study focused exclusively on yield in smaller, more highly replicated plots with both treatments (neonicotinoid-treated vs. untreated) included in the same field to control for site variation. The trial was repeated at six sites; four of the five original PACs used in the experiment (all but SEPAC) and two additional locations (Davis PAC in Farmland, IN; Agronomy Center in West Lafayette, IN). At each site, we planted 4-9 replicates of two adjacent 5.3m length rows of each corn treatment in a randomized complete block design with the same planting date across all replicates at each site (n=33 total plot replicates for both treated and untreated seed). At harvest, the weight and moisture adjusted yield for each replicate was extrapolated to a per hectare yield.

Beginning at fruit maturity (approx. 80 d), five randomly positioned subplots (5 × 2 m area) of each watermelon field were hand-harvested and used to estimate yield. Mature fruits from each subplot were counted, weighed, and inspected for marketability using USDA grading standards (104) for lack of physical deformities or disease. Subplots were harvested weekly for four consecutive weeks, after which data were summed over time to calculate a total yield per unit area.

2.4.5 Pest Management Profitability

Cost of insecticides applied were either calculated from direct expenditures from purchased product or sourced from external guides (105). The cost of the product (Cruiser 5FS) applied as a NST could be quantified but fails to account for additional costs of seed treatment practices that include labor, infrastructure, specialized equipment, and transportation. A proxy for this calculation can be used based on industry-provided costs for the other commonly used

neonicotinoid in corn pest management, clothianidin (53). We also calculated the cost relative yield (CRY), which is interpreted as the minimum percentage in yield gain required to cover the cost associated with an insecticide treatment and reach a breakeven point where the treatment cost is recuperated (6, 106, 107). CRY was calculated by dividing the insecticide treatment cost by the crop price × crop yield. For both watermelon and corn, price and yield were based on the previous 5-year average (2016-2020) from the state of Indiana (52).

2.4.6 Pesticide Residues

Samples of soil, watermelon leaf tissue, and corn and watermelon pollen were collected during each of the four years and analyzed to detect residues of insecticides and fungicides applied to both corn and watermelon crops using the QuEChERS procedure, followed by LC-MS for pesticide identification and quantification (108). See Supplemental Methods for sample number, preparation, and analytical details.

2.4.7 Statistical Analysis

All statistical analyses were performed using SYSTAT 13 (SYSTAT Software, Inc; Point Richmond, CA) by creating a series of general (continuous data) or generalized (discrete data) linear models. To avoid pseudoreplication, all data points were condensed to a single year/site/treatment to be used in the model by taking the mean for damage evaluations across dates and yield measurements within a field, as well as summing pest counts or pollinator measurements across observation dates for each field. This process resulted in 40 and 30 data points for corn and watermelon, respectively, per response variable; crop differences were due to corn being cultivated for one extra year (2017) than watermelon (see *Cropping Systems* above). Stand counts were natural log transformed while root damage at each site was summed and ×100 to produce integer values and then fit to a zero-inflated distribution. SCB counts and pollinator surveys were summed as total number of beetles or pollinators at each field, to maintain discrete integer values, and fit with a negative binomial distribution. Corn and watermelon yield data were normally distributed and remained untransformed. Models used year (n= 4 corn, n= 3 watermelon), site (n= 5) and management treatment (n= 2) as fixed effects, as well as two-way interactions between treatment and year or site. Post-hoc pairwise comparisons (Fisher's LSD) were used to differentiate any

factors (or interactions) that were significant. Within-field corn yield assessment was analyzed in a separate mixed model with the use of NST and site (n = 6) as fixed effects and spatial block as a random effect. The relationship between crop yield and pollinator visits was explored with regression analysis with a fixed effect of treatment. This relationship was tested against the number of visits from honey bees and the wild pollinator community to contrast the effect from managed vs. wild pollinators. Raw data generated from this study are publicly accessible in the Purdue University Research Repository (109).

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Table 2.1: Neonicotinoids were more frequently detected in watermelon pollen from fields under conventional management. LC-MS/MS was used to quantify imidacloprid, clothianidin, and thiamethoxam from fields (n = 10). Watermelon represents pooled samples (3 g from 50-100 flowers) from each field across 5 consecutive weeks during peak bloom (n = 25 per year). Limit of detection (LOD) was 0.03, 0.01, and 0.025 ng/g for clothianidin, thiamethoxam and imidacloprid, respectively.

Neonicotinoid Residue in **Watermelon** Pollen

Year	Conventional			IPM		
	Percent detection (25)	Median (ng/g)	Range (ng/g)	Percent detection (25)	Median (ng/g)	Range (ng/g)
			Imidacloprid			
2018	96%	4.43	< LOD-82.53	0%	< LOD	< LOD
2019	100%	6.28	1.38-55.86	44%	< LOD	< LOD-1.69
2020	100%	4.84	1.54-22.94	4%	< LOD	<LOD-0.95
			Clothianidin			
2018	24%	< LOD	< LOD-2.12	0%	< LOD	< LOD
2019	72%	0.50	< LOD-1.15	0%	< LOD	< LOD
2020	52%	0.14	<LOD-0.79	0%	< LOD	< LOD
			Thiamethoxam			
2018	24%	< LOD	< LOD-0.21	0%	< LOD	< LOD
2019	16%	< LOD	< LOD-0.87	12%	< LOD	< LOD-0.16
2020	28%	< LOD	< LOD-0.25	8%	< LOD	< LOD-0.15

Table 2.2. Neonicotinoids were more frequently detected in corn pollen from fields under conventional management. LC-MS/MS was used to quantify imidacloprid, clothianidin, and thiamethoxam from fields (n = 10). Corn pollen was taken during anthesis with two replicates per field. Limit of detection (LOD) was 0.03, 0.01, and 0.025 ng/g for clothianidin, thiamethoxam and imidacloprid, respectively.

Neonicotinoid Residue in **Corn** Pollen

Year	Conventional			IPM		
	Percent detection (10)	Median (ng/g)	Range (ng/g)	Percent detection (10)	Median (ng/g)	Range (ng/g)
			Imidacloprid			
2018	10%	< LOD	< LOD-0.11	0%	< LOD	< LOD
2019	30%	< LOD	< LOD-0.73	0%	< LOD	< LOD
2020	100%	0.23	0.11-0.69	30%	<LOD	<LOD-0.71
			Clothianidin			
2018	70%	2.00	< LOD-4.66	10%	< LOD	< LOD-0.85
2019	100%	1.94	0.42-4.54	10%	< LOD	< LOD-0.12
2020	100%	1.91	0.30-2.77	40%	< LOD	< LOD-0.24
			Thiamethoxam			
2018	100%	2.01	0.65-4.18	0%	< LOD	< LOD
2019	100%	2.50	0.94-2.98	0%	< LOD	< LOD
2020	100%	1.81	0.33-2.54	30%	< LOD	< LOD-0.56

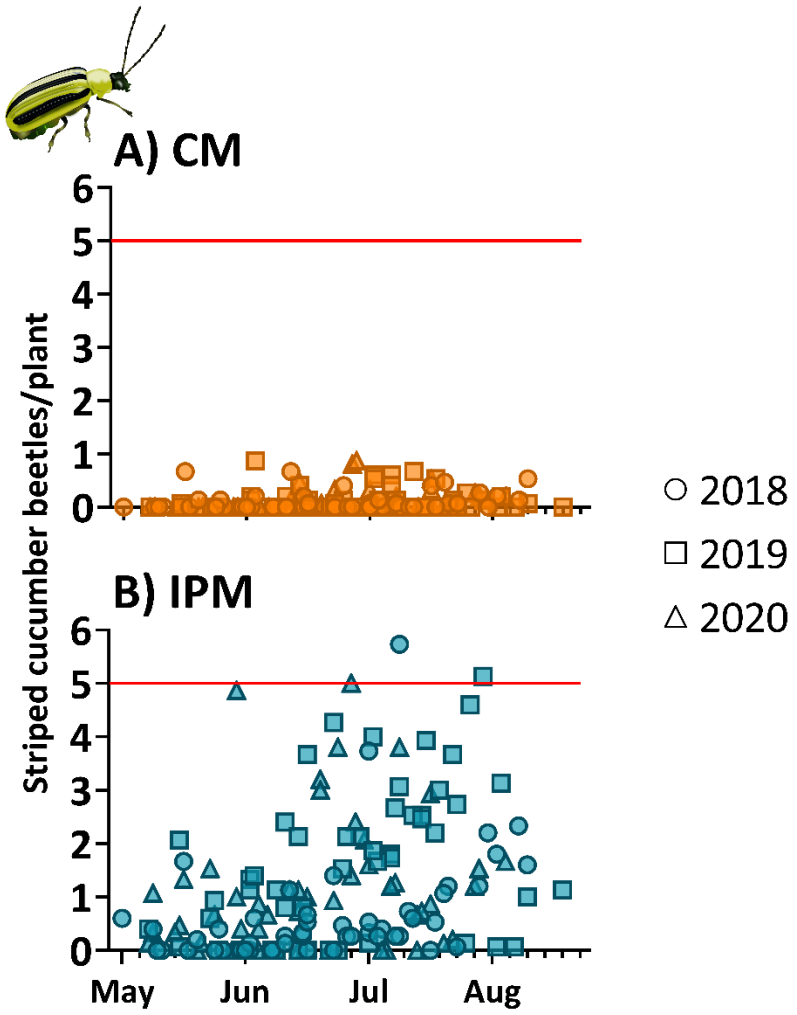

Figure 2.1. Striped cucumber beetles (SCB) were higher in IPM watermelon fields, but infrequently reached levels associated with economic loss. Watermelon fields within both a conventional management (A) and integrated pest management (B) system were scouted weekly and each point represents a 15-plant average of SCBs from seedling transplant until fruit harvest. Red lines in each graph indicate the 5 beetle/ plant economic threshold while circles (2018), squares (2019), and triangles (2020) differentiate experiment years. In IPM fields, each instance where beetle levels reached the economic threshold, insecticide was applied < 2 d following the survey.

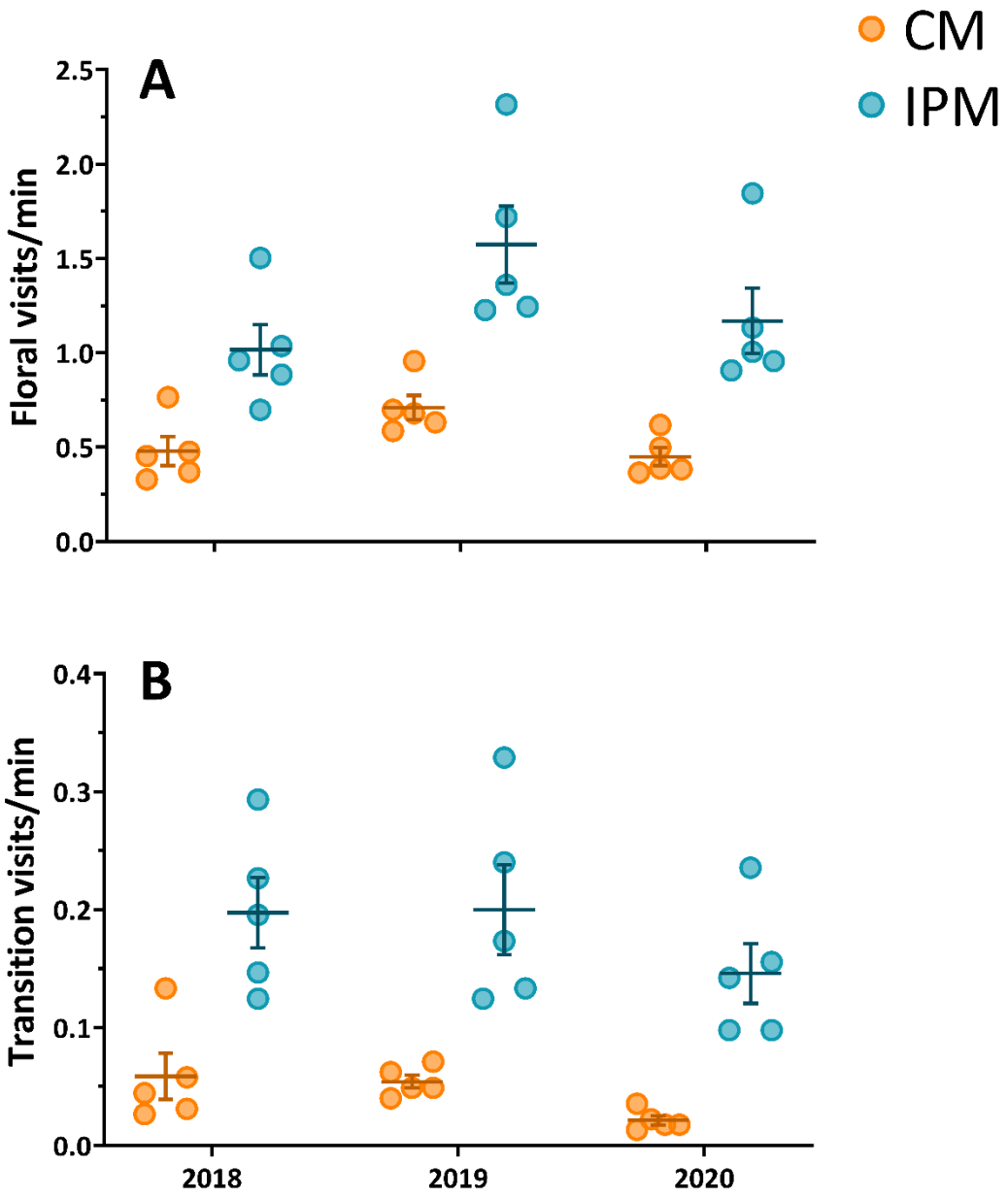

Figure 2.2. The rate of visits to watermelon flowers (A) and transition visits from a male to female flower (B) were both significantly higher in IPM fields. Each point within a cluster (n = 5) represents all observations from a single site during that field season (225 observation minutes). Whiskers within the plot show the mean ± SEM of all sites within each cluster.

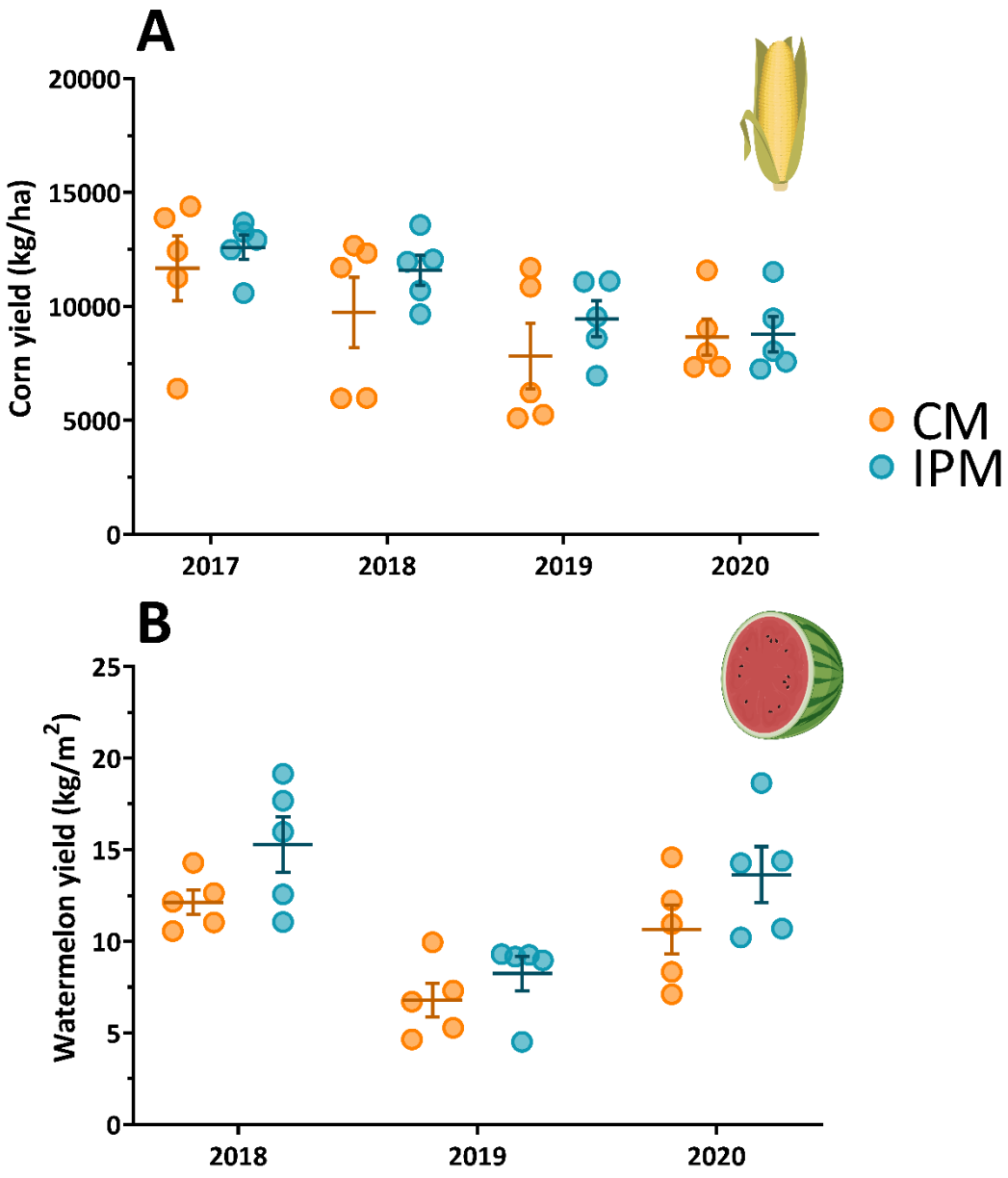

Figure 2.3. Corn yield was unaffected by management system (A), but watermelon yield was significantly higher when grown under an integrated pest management (IPM) system (B). Each point within a cluster (n = 5) represents the yield from a site during that field season. Whiskers within the plot show the mean ± SEM of all sites within each cluster. Corn and watermelon icons from BioRender.

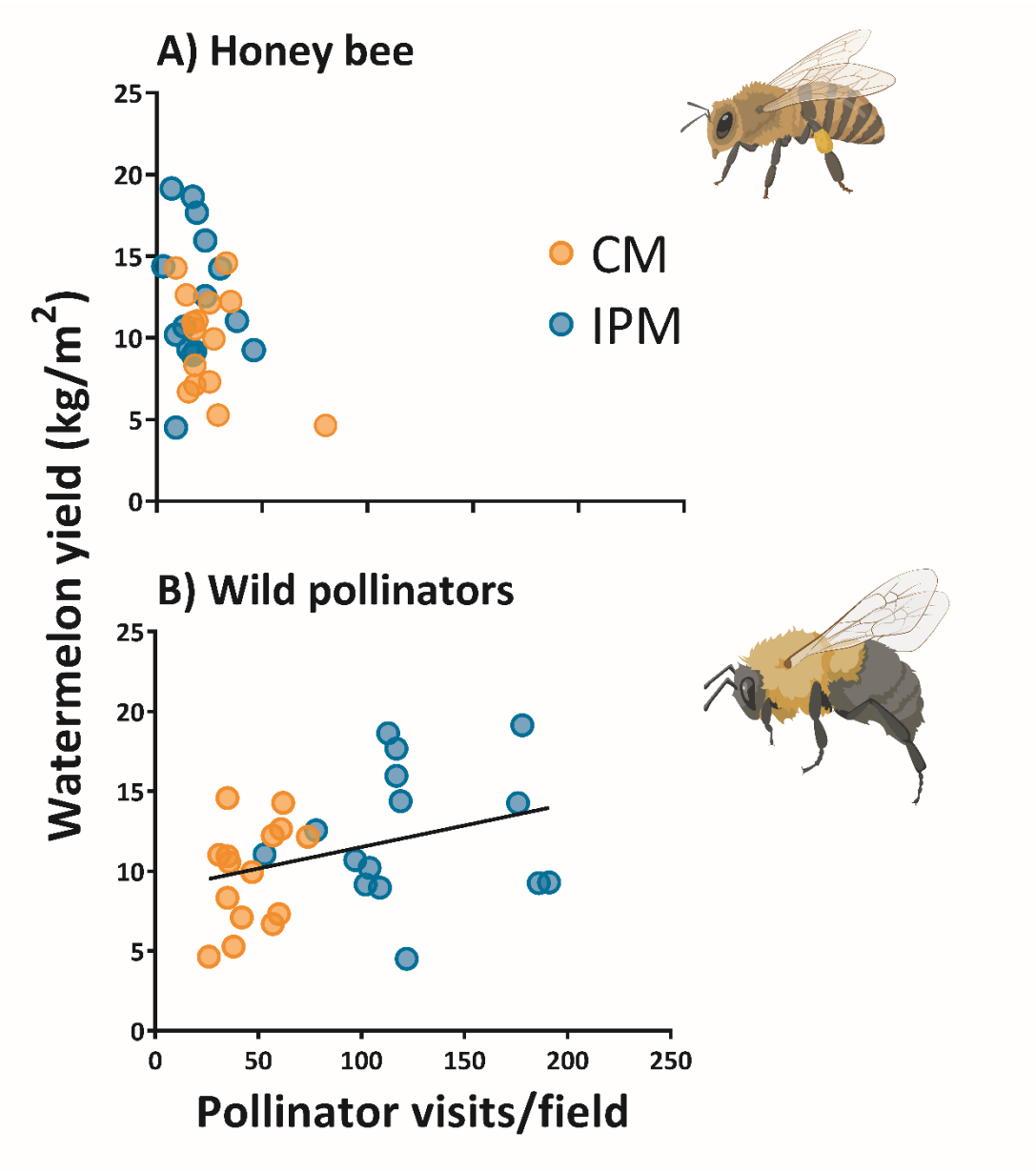

Figure 2.4. Honey bees (A) did not predict watermelon yield but increased wild pollinator visitation (B) in the IPM fields resulted in higher watermelon yield. All plots were stocked with two honey bee colonies at opposite corners of the field. Each point is the total number of observed pollinator visits at a field per site (n = 5 sites with 225 observation minutes) and the corresponding site's average watermelon yield. Best-fit trend line shows relationship using regression model with $P < 0.05$. Bee icons from BioRender

2.6 Supplemental Information

METHODS

Site Selection & Field History. In fall 2016, we met with site supervisors at each PAC to determine field locations. Due to the specific requirements—including a minimum field size of 4.45 ha and a 4 km minimum distance separating field pairs—we had constraints on placement and field history (see Table S2.1 for crop cultivation in each field in 2016, the year before our experiment began). However, land use surrounding focal fields, while highly variable across sites, was nearly the same within sites (i.e., across CM/IPM field pairs, Table S2.2).

Corn Variety, Planting & Fertility. Beginning in 2017, each field was spring chiseled and planted at 76.2 cm row spacing at a planting population of 12,141 plants/ha for all sites, with the exception of SWPAC, which was planted at the lower rate of 11,210 to compensate for the high sand content of soils in this part of the state. All corn seeds in both treatments were coated with Maxim Quattro fungicide blend, which contained the a.i.s fludioxonil (6.5 µg per kernel), mefenoxam (5 µg per kernel), azoxystrobin (2.5 µg per kernel), and thiabendazole (50 µg per kernel). Planting dates were locally determined by site staff based on spring temperatures and precipitation (see Table S2.1 for dates). Corn was side-dressed at the V6 stage with fertilizers at rates of 22-11-00 (SWPAC, SEPAC, PPAC), 19-17-00 (TPAC), and 18-16-00 (NEPAC) N-P-K. These rates were determined by PAC supervisors and varied among sites but were the same across CM/IPM treatment pairs within a given site.

Watermelon Plot Preparation. Prior to each field season from 2018-20 (typically late April through early May), the area within each field dedicated to watermelon production was tilled 2-4 times, depending upon soil type, to facilitate bed-making. Following tillage and drying of soil, 20 black plastic mulch beds (*ca.* 46 m row length; 1.83 m between-row spacing) were installed with irrigation drip tape underneath. After beds were installed, the herbicides Strategy (a.i.s clomazone and ethalfluralin @ rate of 7 L/ha) and Sandea (a.i. Halosulfuron-methyl @ rate of 54.8 mL/ha) were applied to provide early season weed control around the plot and between rows.

Watermelon Propagation & Transplant. Commercial watermelon production typically uses transplants rather than direct-seeding due to inconsistent field germination in spring weather conditions. All watermelons were first planted in greenhouses at the Southwest Purdue Agriculture

Center (Vincennes, IN). The triploid watermelon Fascination (Syngenta) is a regionally popular variety and was used with the diploid "pollen donating" variety SP-7. Watermelon seeds were germinated 4-5 weeks before projected transplant dates in 72-cell trays (#DPS50 HC Companies, Twinsburg, OH, USA) on propagation mats (53.34 cm × 1.52 m plant propagation mats; Gemplers Farm Supply Co., Janesville, WI, USA) at 32°C for the initial 48 hrs and remained at >27°C throughout the germination period. After 3 weeks, transplants were moved to a hardening house, a partially exposed greenhouse, at Throckmorton Purdue Agriculture Center (TPAC), in Lafayette, IN to acclimate to outdoor conditions where they remained until transplant (see Table S2.3 for dates).

At each site, transplant timing was determined using historical weather data to avoid occasionally cold spring temperatures (<7°C) that can kill vulnerable seedlings. Fascination/SP-7 seedlings were planted along each bed at a 3:1 ratio with 1 m inter-plant spacing. Wrangler® insecticide (a.i. imidacloprid) was mixed into transplant water for all CM watermelons and applied at a rate of 814.09 mL/ha. Dead seedlings were removed and replaced for one-week post-transplant. Vines were permitted to grow between plants forming mats along beds with drive alleyways kept open, allowing spray equipment to pass through the interior of the plot without damaging the crop (see Figure S2.2B).

Watermelon Foliar Pesticides. All fungicide sprays were applied each season following recommendations from the MELCAST system (melcast.ceris.purdue.edu), which uses local weather and seasonal data to determine applications. On average, 6-7 fungicide applications were made per watermelon crop each year with spray programs identical across CM/IPM treatments (see Table S2.4 for fungicide use and application dates). CM watermelons were sprayed with the pyrethroid Warrior II® (a.i. lambda-cyhalothrin), a commonly used insecticide to control vegetable pests. Insecticides were applied as described in the main text Methods section (see Table S2.5 for insecticide use and application dates).

Watermelon Pest Scouting. Scouting of insect pests in watermelon plots began at 5-7 days following transplant and continued once each week until harvest began (10-11 weeks post-transplant). Surveys were conducted weekly between 8:00 and 13:00 with temperatures 15-32°C and wind speeds < 16 km/hr. Each survey was conducted by taking 15 total watermelon plants (3

plants along 5 rows) and completely inspecting all above-ground plant material and the region of the soil surface directly below extended vines. All striped cucumber beetles (SCB) were recorded per plant and if populations reached previously established economic thresholds (5 beetles per plant; (1)) insecticide sprays were applied within 1-2 days. This sampling intensity exceeds the current grower-guidelines for number of plants inspected per field and thus can accurately determine pest levels (2). Because CM/IPM fields were sampled sequentially (i.e., one was sampled first before moving to the other), we alternated the sample order each week to avoid sampling bias due to the time of day. When watermelon vines grew together and individual plants could no longer be identified, a 1 m^2 quadrat became the sampling unit. Any insects found on and around plants were averaged to create a per plant plot-level average that would inform whether insecticides were applied. Although SCB is the primary pest in cucurbits, secondary pests—mainly spider mites and aphids—were also noted during these surveys. When observed, infested plants were flagged and populations were closely monitored. If the infestation expanded in subsequent weeks, the area was treated with a foliar insecticide or miticide based on regional recommendations (3).

Sampling Watermelon Pollinators. Weekly sampling of pollinators visiting watermelon flowers was conducted at all sites beginning once female flower production began (5-6 weeks post-transplanting). This weekly collection extended through the peak blooming period of watermelon plants where most of the pollination occurs for the harvested fruit. This typically began six weeks post-transplant and ended at 10 weeks, the onset of harvest. CM/IPM plot pairs at each site were sampled on the same date to account for potential treatment differences in weather conditions and temperature affecting bee activity. As with pest scouting, sampling coincided with optimal conditions for pollinator activity; namely, morning hours (9:00 - 13:00) on days with low wind speeds (< 16 km/h), no precipitation, and relatively little cloud cover. If conditions were unfavorable during a site visit, sampling was postponed until a later date.

Pollinator observations were conducted along five randomly positioned transects (watermelon rows) with three flower clusters selected at 10, 20 and 30 m (n=15 observation points per field). Transects were at least 6 m from the field edge. Observers categorized bees by sight into the following easily-identifiable groups: honey bee (*Apis mellifera*), large bee (*Bombus* sp. and *Xylocopa* sp.), long-horned bee (*Melissodes* sp.), green sweat bee (including *Agapostemon* sp. and

unrelated taxa in the tribe Augochlorine), grey sweat bee (*Halictus* and *Lasioglossum* sp.), squash bee (*Peponapis pruinosa*), and other non-bee insects (Diptera, Lepidoptera, Coleoptera). While cucumber beetles were observed on watermelon flowers, they virtually always remained stationary on the same flower during observation periods and therefore are an unlikely vector for pollen transfer. Each survey resulted in 45 minutes of observation time in each plot and was repeated in each watermelon field across the five-week sampling period.

Pesticide Analysis. Samples were collected in all four years of the study. Soil samples were collected within each watermelon plot in the spring prior to intensive plot tillage and watermelon transplant (May) and again during the fall after the final harvest date (September) but prior to fall tillage and removal of plastic mulch and irrigation. During 2017, soil was only collected in the fall after corn was harvested from the area where the watermelon plot was planned for the following season. For each sample (n=4 for each site/date), we collected 15-20 cores randomly distributed within the collection area from the top 10 cm of soil that was homogenized to form each sample (40 samples in each fall collection 2017-2020 and spring collection 2018-2020; n=280 total). In watermelon fields, we collected 15 randomly selected leaves (@ 1, 2, 4, 6, 9, and 12 weeks post-transplant) from different plants that were homogenized per week to create a single sample at each time point (n=180 total). Additionally, we collected 75 male flowers, removed the pollen/anther complex and combined and homogenized them to create a single analytical sample (3 g) per field. Flower samples were collected weekly over a five-week period from 6-10 weeks post-transplant. Corn pollen was collected by shaking 40-60 plants within a paper bag and later sifting to collect > 6g of pollen, resulting in two samples per field.

Processing methodology for all samples followed a modified QuEChERs protocol for residue quantification (5,6). For all collected soil and pollen a 3 g sample was homogenized with extraction solution (15 ml dH_2O + 15 ml acetonitrile) and 10 µl of internal standard (ISTD) solution (clothianidin-d3, imidacloprid-d4, thiamethoxam-d3, and acetamiprid-d3 at a 10 ng/µl concentration) simultaneously and vortexed. The acetamiprid-d3 was used as the ISTD for analytes that did not have their deuterated analogs in the ISTD mix. Samples were combined with 6 g of magnesium sulfate and 1.5 g of sodium acetate, inverted, vortexed, and centrifuged at 2500 r.p.m. for 10 minutes, after which 10 mL of the top layer of supernatant was transferred to a QuEChERS Dispersive Kit (Agilent Technologies, Santa Clara, CA, #5982-5158) and again inverted, vortexed,

and centrifuged at 4000 r.p.m. for 5 minutes. Supernatant (6 ml) was transferred to a clean 15 ml tube and dried completely in a speed vacuum (Savant SC250EXP, Thermo Scientific, Waltham, MA) Watermelon leaf tissue was homogenized with mortar and pestle to create a l g sample that was placed in a 7ml Precellys tube with 2 g of ceramic beads and 2 ml of dH_2O. All samples were homogenized using a Precellys 24 Tissue Homogenizer (Bertin Instruments, Rockville, MD) on 2 cycles of 180 seconds of homogenization. The tubes were transferred to a 15 ml centrifuge tube and any remaining contents rinsed using 2 ml dH_2O and 4 ml acetonitrile. The internal standards (same as pollen/soil samples) were added to the 15 ml tube and contents were inverted, vortexed, and combine with 1.2 g magnesium sulfate and 0.3 g of sodium acetate. Tubes were again vortexed and centrifuged at 2500 r.p.m. for 10 minutes, after which 1 ml of supernatant was transferred to a 2 ml QuEChERS Dispersive Kit (Agilent # 5982-5321) and inverted, vortexed, and centrifuged at 15,000 r.p.m. for 5 minutes. Resulting supernatant was transferred to a 1.5 ml Eppendorf tube and dried completely in a speed vacuum. All samples were resuspended in 200 µl acetonitrile, vortexed, centrifuged, and all supernatant was transferred to 96-well plates. Just prior to instrument analysis samples were re-suspended with 200 µl 50% acetonitrile dH_2O solution.

We screened samples for the active ingredients of all fungicides and insecticides used during the experiment. Samples were analyzed via liquid chromatography and tandem mass spectrometry at the Bindley BioScience Center at Purdue University, West Lafayette, IN. An Agilent Zorbax SB-Phenyl 2.1×100, 3.5 µm column was used for LC separation and an Agilent 1200 Rapid Resolution LC system coupled to an Agilent 6460 series triple quadrupole mass spectrometer was used to identify pesticide residues based on retention time and co-chromatography with analytical standards of all pesticide targets. Deuterated neonicotinoids were used to quantify the concentration of neonicotinoid presence in samples based on the relative response value. A mix of analytical standards from all other pesticides used in the experiment were subjected to a serial dilution and analyzed on the instrument to create standard curves to quantify their concentration in each sample.

SUPPLEMENTAL REFERENCES

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SUPPLEMENTAL TABLES

Table S2.1. Data on field size, location, prior-year crop (i.e., 2016 before the experiment began), and corn planting/harvest dates.

Site and treatment	Field size (ha)	Location	Previous crop and variety	Corn planting date				Corn harvest date			
				2017	2018	2019	2020	2017	2018	2019	2020
SWPAC CM	5.188	38.7811, -87.4505	Corn P9644AM	28 Apr	30 Apr	19 May	22 Apr	Oct 6	Oct 5	Oct 1	Oct 1
SWPAC IPM	4.985	38.7393, -87.4903	Corn P28T08R	28 Apr	30 Apr	28 May	22 Apr	Oct 2	Sept 29	Oct 23	Oct 3
SEPAC CM	4.828	39.0288, -85.5358	Soybean P39T67R	11 May	9 May	5 Jun	10 Jun	Oct 5	Oct 4	Nov 6	Nov 13
SEPAC IPM	7.732	39.0795, -85.5058	Soybean P35T58R	12 May	8 May	1 Jun	13 May	Oct 2	Oct 5	Nov 1	Oct 14
TPAC CM	4.851	40.2708, -86.8766	Corn Beck 6175	1 May	8 May	2 Jun	7 May	Oct 18	Oct 29	Nov 4	Oct 26
TPAC IPM	5.006	40.3010, -86.9091	Corn P0987AM	5 May	2 May	2 Jun	5 May	Oct 13	Oct 17	Nov 4	Oct 23
PPAC CM	7.859	41.4037, -86.8959	Soybean P28T08R	25 May	1 Jun	27 May	19 May	Oct 24	Oct 15	Nov 1	Oct 26
PPAC IPM	5.702	41.4551, -86.9364	Soybean P278R4	22 May	1 Jun	27 May	19 May	Oct 26	Nov 1	Oct 28	Nov 2
NEPAC CM	6.175	41.1171, -85.4504	Corn P9690AM	27 Apr	4 May	7 Jun	15 May	Oct 11	Oct 9	Oct 29	Oct 12
NEPAC IPM	5.714	41.1957, -85.3962	Corn P0987AM	27 Apr	4 May	7 Jun	15 May	Oct 10	Oct 3	Oct 29	Oct 7

Table S2.2: Composition of the landscape surrounding experimental fields in each treatment at 1 and 3 km radii. Land cover was categorized using the CropScape - Cropland Data Layer (USDA NASS). Values per cell represent averages (± SEM) across the five experimental sites for each land cover category that individually constituted at least 5% of total land area.

Land cover category	*1 km radius (% total area)*		*3 km radius (% total area)*	
	CM	**IPM**	**CM**	**IPM**
Cropland	60.10 ± 7.49	61.18 ± 13.47	60.93 ± 7.07	62.44 ± 10.07
Developed	6.71 ± 1.66	5.73 ± 2.48	12.68 ± 1.13	10.40 ± 2.72
Forest	22.70 ± 8.74	25.13 ± 14.07	19.51 ± 6.66	19.17 ± 10.01
Grassland	8.86 ± 1.56	5.81 ± 3.04	5.35 ± 1.11	5.25 ± 1.31

Table S2.3: Data on watermelon transplant and harvest dates for each year of the study.

Site and treatment	Location	Watermelon transplant dates			Watermelon harvest dates		
		2018	2019	2020	2018	2019	2020
SWPAC CM	38.7811, -87.4505	15 May	17 May	22 May	Jul 25, Aug 1, 8, 14	Aug 1, 9, 16, 23	Jul 21, 28, Aug 4, 11
SWPAC IPM	38.7393, -87.4903	15 May	17 May	22 May	Jul 24, 31, Aug 8, 14	Aug 1, 9, 15, 23	Jul 21, 28, Aug 4, 11
SEPAC CM	39.0288, -85.5358	24 May	3 Jun	22 May	Aug 2, 10, 17, 24	Aug 16, 23, 27, Sept 5	Aug 5, 14, 21, 27
SEPAC IPM	39.0795, -85.5058	24 May	3 Jun	22 May	Aug 2, 10, 17, 24	Aug 16, 23, 27, Sept 5	Aug 5, 14, 21, 27
TPAC CM	40.2708, -86.8766	22 May	28 May	22 May	Aug 3, 9, 16, 23	Aug 6, 13, 21, 29	Aug 6, 12, 19, 26
TPAC IPM	40.3010, -86.9091	22 May	28 May	22 May	Aug 3, 9, 16, 23	Aug 13, 21, 29, Sept 2	Aug 6, 12, 19, 26
PPAC CM	41.4037, -86.8959	11 Jun	11 Jun	2 Jun	Aug 20, 29, Sept 4, 12	Aug 28, Sept 4, 13, 20	Aug 20, 25, Sept 1, 11
PPAC IPM	41.4551, -86.9364	11 Jun	11 Jun	2 Jun	Aug 29, Sept 4, 12, 19	Sept 20, 26, Oct 3, 7	Aug 25, Sept 1, 11, 17
NEPAC CM	41.1171, -85.4504	15 Jun	17 Jun	10 Jun	Aug 31, Sept 7, 14, 21	Aug 30, Sept 6, 11, 18	Aug 28, Sept 3, 10, 16
NEPAC IPM	41.1957, -85.3962	15 Jun	17 Jun	10 Jun	Aug 31, Sept 7, 14, 21	Aug 30, Sept 6, 11, 18	Aug 28, Sept 3, 10, 16

Table S2.4: Watermelon fungicide program. Applications occurred in both CM and IPM fields. Active Ingredients for all products: Initiate: chlorothalonil; Luna Experience: fluopyram, tebuconazole; Cabrio: pyraclostrobin; Inspire Super: difenoconazole, cyprodinil; Aprovia Top: difenoconazole, benzovindiflupyr.

Year	Site	Product/rate	Application dates
2018	SWPAC	Initiate 2.34 L/ha	June 1, 14, 26, 29, July 12, 23, Aug 1
		Luna Experience 1.17 L/ha	June 26
		Cabrio 1.17 L/ha	June 29, July 23
	SEPAC	Initiate 2.34 L/ha	June 8, 18, 28, July 11, 23, Aug 2, 13
		Luna Experience 1.17 L/ha	July 11
		Cabrio 1.17 L/ha	July 23
	TPAC	Initiate 2.34 L/ha	June 14, 28, July 9, 19, 25, Aug 3, 10
		Luna Experience 1.17 L/ha	July 9
		Cabrio 1.17 L/ha	July 19
	PPAC	Initiate 2.34 L/ha	June 28, July 6, 20, Aug 3, 16, 31, Sept 13
		Luna Experience 1.17 L/ha	July 13
		Cabrio 1.17 L/ha	July 27
	NEPAC	Initiate 2.34 L/ha	June 27, July 13, 27, Aug 7, 17, 31, Sept 12
		Luna Experience 1.46 L/ha	July 11
		Cabrio 1.17 L/ha	July 23
2019	SWPAC	Initiate 2.34 L/ha	June 11, 20, July 3, 9, 18, Aug 1
		Inspire Super 1.17 L/ha	July 9
		Cabrio 1.17 L/ha	July 3, Aug 1
	SEPAC	Initiate 2.34 L/ha	June 21, July 5, 19, Aug 2, 16, 30
		Inspire Super 1.17 L/ha	Aug 2
		Cabrio 1.17 L/ha	July 19, Aug 30
	TPAC	Initiate 2.34 L/ha	June 21, July 5, 19, Aug 2, 16, 30
		Inspire Super 1.17 L/ha	July 2
		Cabrio 1.17 L/ha	July 19, Aug 30
	PPAC	Initiate 2.34 L/ha	July 11, 25, Aug 8, 16, 29, Sept 13
		Inspire Super 1.17 L/ha	Aug 16
		Cabrio 1.17 L/ha	Aug 8, Sept 13
	NEPAC	Initiate 2.34 L/ha	July 12, 24, Aug 8, 21, Sept 6, 20
		Inspire Super 1.17 L/ha	Aug 21
		Cabrio 1.17 L/ha	Aug 8, Sept 20
2020	SWPAC	Initiate 2.34 L/ha	June 12, 25, July 2, 14, 24, 31
		Luna Experience 1.17 L/ha	June 25
		Inspire Super 1.17 L/ha	July 2
		Aprovia Top 0.99 L/ha	July 14
	SEPAC	Initiate 2.34 L/ha	June 19, July 1, 10, 24, 31, Aug 12
		Luna Experience 1.17 L/ha	July 1
		Inspire Super 1.17 L/ha	July 10
		Aprovia Top 0.99 L/ha	July 24
	TPAC	Initiate 2.34 L/ha	June 17, July 1, 10, 23, Aug 1, 15
		Luna Experience 1.17 L/ha	July 1
		Inspire Super 1.17 L/ha	July 10
		Aprovia Top 0.99 L/ha	July 23
	PPAC	Initiate 2.34 L/ha	July 6, 16, 31, Aug 11, 26, Sept 3
		Luna Experience 1.17 L/ha	July 16
		Inspire Super 1.17 L/ha	July 31
		Aprovia Top 0.99 L/ha	Aug 11
	NEPAC	Initiate 2.34 L/ha	July 10, 17, 31, Aug 14, 25, Sept 9
		Luna Experience 1.17 L/ha	July 17
		Inspire Super 1.17 L/ha	July 31
		Aprovia Top 0.99 L/ha	Aug 14

Table S2.5: Foliar insecticide program for watermelon. CM treatment always received four applications of Warrior (pyrethroid), but occasionally received additional treatments to control secondary pest outbreaks such as aphids or mites; for example, 2018 @ SWPAC, Portal was also applied. IPM plots only received a Warrior application when cucumber beetles exceeded their economic threshold of five adults per plant. Active Ingredients for all listed products: Warrior II: lambda-cyhalothrin; Portal: fenpyroximate; Assail: acetamiprid.

Year	Site	Treatment	Product/rate	Application Dates
2018	SWPAC	CM	Warrior II 0.14 L/ha	June 14, 26, July 12, 23
			Portal 2.34 L/ha	July 26
		IPM	None	
	SEPAC	CM	Warrior II 0.14 L/ha	June 18, July 11, 23, Aug 3
		IPM	None	
	TPAC	CM	Warrior II 0.14 L/ha	June 14, July 9, 19, Aug 3
		IPM	None	
	PPAC	CM	Warrior II 0.14 L/ha	June 28, July 20, Aug 3, 31
		IPM	Warrior II 0.14 L/ha	July 25
	NEPAC	CM	Warrior II 0.14 L/ha	June 27, July 23, Aug 7, 31
		IPM	None	
2019	SWPAC	CM	Warrior II 0.14 L/ha	June 11, 20, July 3, 18
		IPM	None	
	SEPAC	CM	Warrior II 0.14 L/ha	June 21, July 5, 20, Aug 2
			Assail 0.29 L/ha	Aug 16
		IPM	None	
	TPAC	CM	Warrior II 0.14 L/ha	June 21, July 5, 20, Aug 2
		IPM	None	
	PPAC	CM	Warrior II 0.14 L/ha	July 11, 25, Aug 8, 16
		IPM	Warrior II 0.14 L/ha	July 16
	NEPAC	CM	Warrior II 0.14 L/ha	July 12, 24, Aug 8, 21
		IPM	None	
2020	SWPAC	CM	Warrior II 0.14 L/ha	June 12, 25, July 14, 24
		IPM	None	
	SEPAC	CM	Warrior II 0.14 L/ha	June 19, July 1, 24, 31
		IPM	None	
	TPAC	CM	Warrior II 0.14 L/ha	June 17, July 1, 23, Aug 1
		IPM	None	
	PPAC	CM	Warrior II 0.14 L/ha	July 6, 16, Aug 11, 27
		IPM	Warrior II 0.14 L/ha	July 16
	NEPAC	CM	Warrior II 0.14 L/ha	July 1, 17, Aug 14, 25
		IPM	Warrior II 0.14 L/ha	July 1

Table S2.6: General and generalized linear model output for all response variables. Significant differences are designated by bold text based on a level of $P < 0.05$ for all pest metrics (A), pollinator observations (B), and yield assessments (C).

A.

Response Variable	Explanatory Variable(s)	df	F	*P*
Stand Count	Treatment	1,24	0.03	0.867
Natural log transformed	Year	3,24	1.46	0.136
	Site	4,24	0.49	0.933
	Treatment*Year	3,24	0.34	0.794
	Treatment*Site	4,24	0.48	0.747
Corn Root Damage	Treatment	1,24	5.15	**0.032**
Zero inflated	Year	3,24	5.69	**0.004**
	Site	4,24	1.01	0.420
	Treatment*Year	3,24	5.35	**0.006**
	Treatment*Site	4,24	1.00	0.426
SCB Surveys	Treatment	1,16	72.33	< **0.001**
Neg binomial	Year	3,16	1.42	0.109
	Site	4,16	1.36	0.118
	Treatment*Year	3,16	0.84	0.488
	Treatment*Site	4,16	1.17	0.363

B.

Response Variable	Explanatory Variable(s)	df	F	*P*
Pollinator Abundance	Treatment	1,16	26.21	**0.001**
Neg binomial	Year	2,16	8.13	**0.004**
	Site	4,16	2.75	**0.032**
	Treatment*Year	2,16	0.93	0.319
	Treatment*Site	4,16	0.21	0.864
Floral Visits	Treatment	1,16	180.08	< **0.001**
Neg binomial	Year	2,16	98.15	< **0.001**
	Site	4,16	97.99	< **0.001**
	Treatment*Year	2,16	6.46	**0.034**
	Treatment*Site	4,16	3.85	0.427
Transition Visits	Treatment	1,16	163.21	< **0.001**
Neg binomial	Year	2,16	88.41	< **0.001**
	Site	4,16	116.03	< **0.001**
	Treatment*Year	2,16	2.63	0.411
	Treatment*Site	4,16	0.26	0.849

C.

Response Variable	Explanatory Variable(s)	df	F	*P*
Corn Yield	Treatment	1,24	2.99	0.097
	Year	3,24	6.60	**0.002**
	Site	4,24	2.78	**0.050**
	Treatment*Year	3,24	0.36	0.781
	Treatment*Site	4,24	1.80	0.161
Watermelon Yield	Treatment	1,16	13.72	**0.002**
	Year	2,16	29.50	< **0.001**
	Site	4,16	6.82	**0.002**
	Treatment*Year	2,16	0.62	0.551
	Treatment*Site	4,16	1.39	0.282
Average Melon Weight	Treatment	1,16	3.74	0.071
	Year	2,16	3.52	0.054

Table S2.6 cont.

	Site	4,16	1.92	0.157
	Treatment*Year	2,16	0.27	0.768
	Treatment*Site	4,16	0.06	0.992
Number of Harvested Fruit	Treatment	1,16	9.47	**0.007**
	Year	2,16	18.69	**0.000**
	Site	4,16	3.85	**0.022**
	Treatment*Year	2,16	0.28	0.758
	Treatment*Site	4,16	1.17	0.362

Table S2.7: Results following LC-MS/MS to quantify the concentration of neonicotinoids in watermelon leaves. Limit of detection (LOD) was 0.203, 0.064, and 0.092 ng/g for clothianidin, thiamethoxam and imidacloprid, respectively.

Neonicotinoid Concentration in Watermelon Leaf Tissue

		Imidacloprid					
		Conventional			IPM		
Year	Weeks Post-Transplanting	Percent detection (5)	Mean (ng/g) ± SEM	Median	Percent detection (5)	Mean (ng/g) ± SEM	Median
2018	1	100%	523.63 ± 119.69	636.80	80%	1.04 ± 0.47	0.90
	2	100%	346.64 ± 124.79	349.97	80%	1.40 ± 0.81	0.85
	4	100%	114.56 ± 40.96	103.02	60%	0.58 ± 0.31	0.42
	6	100%	60.49 ± 28.50	28.47	80%	0.35 ± 0.19	0.12
	9	100%	44.09 ± 12.01	41.45	100%	3.63 ± 3.22	0.46
	12	100%	33.74 ± 8.43	37.06	100%	1.34 ± 0.98	0.57
2019	1	100%	456.30 ± 247.49	189.68	80%	0.75 ± 0.28	0.48
	2	100%	164.02 ± 65.93	130.86	80%	0.79 ± 0.29	1.08
	4	100%	52.68 ± 25.73	34.63	80%	0.57 ± 0.22	0.81
	6	100%	10.13 ± 3.53	5.69	80%	0.63 ± 0.29	0.51
	9	100%	8.52 ± 3.73	4.03	80%	0.91 ± 0.42	0.70
2020	1	100%	1191.35 ± 101.17	1182.15	100%	3.64 ± 0.41	3.33
	2	100%	731.45 ± 127.57	414.19	60%	1.71 ± 0.89	0.39
	4	100%	482.29 ± 98.74	384.65	100%	2.27 ± 0.63	2.90
	6	100%	202.08 ± 73.60	138.40	60%	1.68 ± 0.81	0.63
	9	100%	53.05 ± 19.53	47.47	60%	2.94 ± 1.33	1.79
	12	100%	26.36 ± 8.09	21.18	80%	2.86 ± 0.98	4.39
		Clothianidin					
		Conventional			IPM		
Year	Weeks Post-Transplanting	Percent detection (5)	Mean (ng/g) ± SEM	Median	Percent detection (5)	Mean (ng/g) ± SEM	Median
2018	1	100%	3.13 ± 1.57	1.84	60%	0.63 ± 0.29	0.56
	2	100%	1.38 ± 0.49	1.23	60%	0.42 ± 0.21	0.41
	4	100%	1.25 ± 0.24	0.99	80%	0.55 ± 0.20	0.52
	6	100%	0.92 ± 0.17	0.79	40%	0.27 ± 0.17	< LOD
	9	100%	1.11 ± 0.14	1.09	60%	0.34 ± 0.16	0.31
	12	80%	1.20 ± 0.35	1.20	20%	0.15 ± 0.15	< LOD
2019	1	100%	6.60 ± 4.12	2.72	80%	1.70 ± 1.24	< LOD
	2	100%	4.94 ± 2.53	2.85	80%	1.82 ± 1.38	< LOD
	4	100%	2.23 ± 0.73	2.21	80%	2.85 ± 2.23	< LOD
	6	100%	2.72 ± 0.49	2.66	80%	1.51 ± 1.17	< LOD
	9	100%	1.77 ± 0.51	1.78	80%	0.50 ± 0.31	< LOD
2020	1	60%	3.26 ± 1.36	2.82	0%	0.00 ± 0.00	< LOD
	2	60%	2.16 ± 0.84	2.46	0%	0.00 ± 0.00	< LOD
	4	80%	2.48 ± 0.62	2.67	0%	0.00 ± 0.00	< LOD
	6	80%	2.07 ± 0.62	2.23	0%	0.00 ± 0.00	< LOD
	9	80%	1.52 ± 0.41	1.72	0%	0.00 ± 0.00	< LOD
	12	80%	4.40 ± 2.36	2.98	0%	0.00 ± 0.00	< LOD
		Thiamethoxam					
		Conventional			IPM		
Year	Weeks Post-Transplanting	Percent detection (5)	Mean (ng/g) ± SEM	Median	Percent detection (5)	Mean (ng/g) ± SEM	Median
2018	1	100%	1.33 ± 0.28	0.99	0%	0.00 ± 0.00	< LOD
	2	100%	0.60 ± 0.16	0.52	0%	0.00 ± 0.00	< LOD
	4	100%	0.45 ± 0.15	0.27	0%	0.00 ± 0.00	< LOD
	6	80%	0.54 ± 0.19	0.58	0%	0.00 ± 0.00	< LOD
	9	60%	0.24 ± 0.12	0.27	20%	0.11 ± 0.11	< LOD

Table S2.7 cont

	12	20%	0.13 ± 0.13	< LOD	0%	0.00 ± 0.00	< LOD
2019	1	80%	5.78 ± 5.11	0.43	60%	0.29 ± 0.12	0.38
	2	80%	4.99 ± 2.94	3.19	40%	0.56 ± 0.53	< LOD
	4	80%	1.76 ± 1.41	0.32	20%	0.37 ± 0.37	< LOD
	6	60%	0.21 ± 0.14	0.09	0%	0.00 ± 0.00	< LOD
	9	40%	1.40 ± 1.16	< LOD	0%	0.00 ± 0.00	< LOD
	12	60%	0.70 ± 0.51	0.28	0%	0.00 ± 0.00	< LOD
2020	1	80%	3.54 ± 1.36	1.61	20%	0.15 ± 0.13	< LOD
	2	100%	2.66 ± 0.84	1.73	60%	0.92 ± 0.43	1.53
	4	100%	1.44 ± 0.62	1.15	20%	0.07 ± 0.06	< LOD
	6	100%	0.90 ± 0.62	0.31	0%	0.00 ± 0.00	< LOD
	9	20%	0.11 ± 0.41	< LOD	0%	0.00 ± 0.00	< LOD
	12	0%	0.00 ± 0.00	< LOD	0%	0.00 ± 0.00	< LOD

Table S2.8: Results following LC-MS/MS to quantify neonicotinoids in watermelon pollen. Limit of detection (LOD) was 0.03, 0.01, and 0.025 ng/g for clothianidin, thiamethoxam and imidacloprid, respectively.

Neonicotinoid Concentration in Watermelon Pollen

		Imidacloprid					
		Conventional			IPM		
Year	Weeks Post-Transplanting	Percent detection (5)	Mean (ng/g) ± SEM	Median	Percent detection (5)	Mean (ng/g) ± SEM	Median
2018	5	100%	36.91 ± 34.07	24.76	0%	0.00 ± 0.00	< LOD
	6	100%	14.63 ± 10.32	18.55	0%	0.00 ± 0.00	< LOD
	7	100%	4.75 ± 3.56	4.35	0%	0.00 ± 0.00	< LOD
	8	80%	2.97 ± 3.23	3.12	0%	0.00 ± 0.00	< LOD
	9	80%	2.34 ± 2.08	2.14	0%	0.00 ± 0.00	< LOD
2019	5	100%	23.47 ± 21.19	17.43	60%	0.40 ± 0.73	0.3
	6	100%	13.55 ± 10.96	11.34	40%	0.30 ± 0.42	< LOD
	7	100%	6.60 ± 3.36	6.28	20%	0.09 ± 0.09	< LOD
	8	100%	3.83 ± 1.36	3.49	40%	0.15 ± 0.22	< LOD
	9	100%	5.65 ± 3.07	4.90	80%	0.52 ± 0.49	0.38
2020	5	100%	19.04 ± 1.21	18.38	0%	0.00 ± 0.00	< LOD
	6	100%	9.26 ± 1.54	8.58	0%	0.00 ± 0.00	< LOD
	7	100%	5.37 ± 0.77	4.71	20%	0.19 ± 0.19	< LOD
	8	100%	3.77 ± 0.57	3.65	0%	0.00 ± 0.00	< LOD
	9	100%	3.60 ± 0.75	3.13	0%	0.00 ± 0.00	< LOD
		Clothianidin					
		Conventional			IPM		
Year	Weeks Post-Transplanting	Percent detection (5)	Mean (ng/g) ± SEM	Median	Percent detection (5)	Mean (ng/g) ± SEM	Median
2018	5	0%	0.00 ± 0.00	0.59	0%	0.00 ± 0.00	< LOD
	6	60%	0.69 ± 0.87	0.52	0%	0.00 ± 0.00	< LOD
	7	60%	0.47 ± 0.45	< LOD	0%	0.00 ± 0.00	< LOD
	8	0%	0.00 ± 0.00	< LOD	0%	0.00 ± 0.00	< LOD
	9	0%	0.00 ± 0.00	< LOD	0%	0.00 ± 0.00	< LOD
2019	5	60%	0.35 ± 21.19	0.78	0%	0.00 ± 0.00	< LOD
	6	80%	0.63 ± 10.96	0.70	0%	0.00 ± 0.00	< LOD
	7	100%	0.70 ± 3.36	0.44	0%	0.00 ± 0.00	< LOD
	8	80%	0.42 ± 1.36	< LOD	0%	0.00 ± 0.00	< LOD
	9	40%	0.25 ± 3.07	< LOD	0%	0.00 ± 0.00	< LOD
2020	5	80%	0.18 ± 0.05	0.16	0%	0.00 ± 0.00	< LOD
	6	40%	0.21 ± 0.13	< LOD	0%	0.00 ± 0.00	< LOD
	7	60%	0.16 ± 0.06	0.22	0%	0.00 ± 0.00	< LOD
	8	40%	0.13 ± 0.08	< LOD	0%	0.00 ± 0.00	< LOD
	9	40%	0.26 ± 0.15	< LOD	0%	0.00 ± 0.00	< LOD
		Thiamethoxam					
		Conventional			IPM		
Year	Weeks Post-Transplanting	Percent detection (5)	Mean (ng/g) ± SEM	Median	Percent detection (5)	Mean (ng/g) ± SEM	Median
2018	5	80%	0.9 ± 0.7	0.75	0%	0.00 ± 0.00	< LOD
	6	60%	0.06 ± 0.06	0.74	0%	0.00 ± 0.00	< LOD
	7	40%	0.03 ± 0.04	< LOD	0%	0.00 ± 0.00	< LOD
	8	20%	0.03 ± 0.03	< LOD	0%	0.00 ± 0.00	< LOD
	9	0%	0.00 ± 0.00	< LOD	0%	0.00 ± 0.00	< LOD
2019	5	40%	0.19 ± 0.38	< LOD	0%	0.00 ± 0.00	< LOD
	6	40%	0.05 ± 0.07	< LOD	40%	0.05 ± 0.06	< LOD
	7	20%	0.03 ± 0.03	< LOD	0%	0.00 ± 0.00	< LOD
	8	0%	0.00 ± 0.00	< LOD	0%	0.00 ± 0.00	< LOD
	9	0%	0.00 ± 0.00	< LOD	20%	0.03 ± 0.03	< LOD
2020	5	20%	0.03 ± 0.03	< LOD	0%	0.00 ± 0.00	< LOD

Table S2.8 cont.

6	40%	0.08 ± 0.05	< LOD	0%	0.00 ± 0.00	< LOD
7	40%	0.05 ± 0.03	< LOD	20%	0.03 ± 0.03	< LOD
8	20%	0.03 ± 0.02	< LOD	0%	0.00 ± 0.00	< LOD
9	20%	0.02 ± 0.02	< LOD	20%	0.02 ± 0.02	< LOD

Table S2.9: Results following LC-MS/MS to quantify the concentration of neonicotinoids in soil from within the watermelon field. Limit of detection (LOD) was 0.03, 0.01, and 0.025 ng/g for clothianidin, thiamethoxam and imidacloprid, respectively.

Neonicotinoid Concentration in Soil Samples

	Imidacloprid					
	Conventional			IPM		
Sampling Period	Percent detection (20)	Mean (ng/g) ± SEM	Median	Percent detection (20)	Mean (ng/g) ± SEM	Median
Fall 2017	45%	0.31 ± 0.14	< LOD	35%	0.19 ± 0.08	< LOD
Spring 2018	100%	2.86 ± 0.10	1.77	75%	0.41 ± 0.08	0.49
Fall 2018	100%	116.1 ± 0.10	101.05	50%	1.06 ± 0.38	0.17
Spring 2019	100%	9.39 ± 0.01	5.17	100%	1.58 ± 0.24	1.27
Fall 2019	95%	21.53 ± 0.10	10.95	60%	4.46 ± 2.77	0.19
Spring 2020	100%	3.51 ± 0.92	2.66	100%	0.88 ± 0.14	0.76
Fall 2020	100%	106.28 ± 17.41	86.83	100%	1.02 ± 0.17	0.81

	Clothianidin					
	Conventional			IPM		
Sampling Period	Percent detection (20)	Mean (ng/g) ± SEM	Median	Percent detection (20)	Mean (ng/g) ± SEM	Median
Fall 2017	90%	9.23 ± 1.97	7.18	80%	3.88 ± 1.06	2.75
Spring 2018	90%	23.26 ± 6.58	14.93	65%	5.88 ± 1.69	3.90
Fall 2018	90%	8.92 ± 1.83	6.34	75%	3.35 ± 0.86	2.75
Spring 2019	100%	2.98 ± 0.44	2.65	10%	0.31 ± 0.23	< LOD
Fall 2019	100%	2.16 ± 0.37	1.57	85%	1.19 ± 0.16	1.22
Spring 2020	100%	5.35 ± 0.67	6.79	100%	1.22 ± 0.26	0.82
Fall 2020	100%	3.98 ± 0.38	5.05	100%	1.17 ± 0.20	0.74

	Thiamethoxam					
	Conventional			IPM		
Sampling Period	Percent detection (20)	Mean (ng/g) ± SEM	Mean	Percent detection (20)	Mean (ng/g) ± SEM	Mean
Fall 2017	100%	0.19 ± 0.04	0.15	25%	0.04 ± 0.01	< LOD
Spring 2018	100%	0.82 ± 0.18	0.72	55%	0.12 ± 0.02	0.11
Fall 2018	100%	0.30 ± 0.05	0.26	45%	0.08 ± 0.02	< LOD
Spring 2019	100%	0.66 ± 0.15	0.45	20%	0.03 ± 0.01	< LOD
Fall 2019	100%	0.45 ± 0.07	0.41	0%	0.00 ± 0.00	< LOD
Spring 2020	80%	2.60 ± 0.60	0.85	30%	0.25 ± 0.17	< LOD
Fall 2020	100%	0.63 ± 0.09	0.57	0%	0.00 ± 0.00	< LOD

Table S2.10: Non-neonicotinoid pesticides used during the experiment. Because the methodology was tailored to neonicotinoids, the exact concentrations could not be reliably quantified and thus we more conservatively report percent of samples with detectable levels of pesticides. LOD (ng/g) for leaf tissue: Chlorothalonil: 4.604, Fluopyram: 0.0603, Pyraclostrobin: 0.0205, Difenoconazole: 0.0041, Cyprodinil: 0.042, Lambda-cyhalothrin: 1.838. LOD (ng/g) for soil/pollen samples: Chlorothalonil: 0.602, Fluopyram: 0.0252, Pyraclostrobin: 0.0044, Difenoconazole: 0.0012, Cyprodinil: 0.0026, Lambda-cyhalothrin: 1.216.

	Soil (n = 140)		Leaf (n = 90)		Watermelon Pollen (n = 75)		Corn Pollen (n = 30)	
Product	CM	IPM	CM	IPM	CM	IPM	CM	IPM
Chlorothalonil	37%	41%	84%	80%	92%	95%	0%	7%
Fluopyram	16%	11%	21%	32%	11%	15%	0%	0%
Pyraclostrobin	11%	6%	12%	10%	12%	8%	0%	3%
Difenoconazole	6%	16%	7%	9%	7%	12%	3%	0%
Cyprodinil	1%	0%	0%	0%	0%	0%	0%	0%
Lambda-cyhalothrin	4%	1%	16%	2%	13%	1%	0%	0%

Table S2.11: Averages (mean ±SEM) for all pollinator observations per minute. Each pollinator category is listed along with overall pollinator abundance, visitation, and transition visits.

	2018		2019		2020	
	CM	IPM	CM	IPM	CM	IPM
Honey bee	0.08 ± 0.01	0.10 ± 0.05	0.16 ± 0.05	0.09 ± 0.029	0.11 ± 0.016	0.06 ± 0.02
Melissodes sp.	0.05 ± 0.02	0.11 ± 0.03	0.11 ± 0.03	0.21 ± 0.026	0.06 ± 0.015	0.15 ± 0.02
Large bees	0.03 ± 0.01	0.05 ± 0.02	0.01 ± 0.01	0.08 ± 0.01	0.01 ± 0.004	0.04 ± 0.01
Green sweat bees	0.04 ± 0.02	0.11 ± 0.06	0.00 ± 0.00	0.04 ± 0.01	0.00 ± 0.00	0.06 ± 0.02
Grey sweat bees	0.10 ± 0.04	0.18 ± 0.07	0.07 ± 0.03	0.28 ± 0.1	0.09 ± 0.03	0.26 ± 0.04
Squash bee	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01
Non-bee insects	0.02 ± 0.01	0.04 ±0.01	0.01 ± 0.00	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.00
Total Abundance	0.31 ± 0.04	0.58 ± 0.07	0.36 ± 0.03	0.72 ± 0.10	0.30 ± 0.03	0.61 ± 0.07
Floral Visits	0.49 ± 0.08	1.02 ± 0.13	0.71 ± 0.06	1.57 ± 0.21	0.45 ± 0.04	1.17 ± 0.16
Transition Visits	0.06 ± 0.02	0.20 ± 0.03	0.05 ± 0.01	0.20 ± 0.04	0.02 ± 0.00	0.15 ± 0.02

SUPPLEMENTAL FIGURES

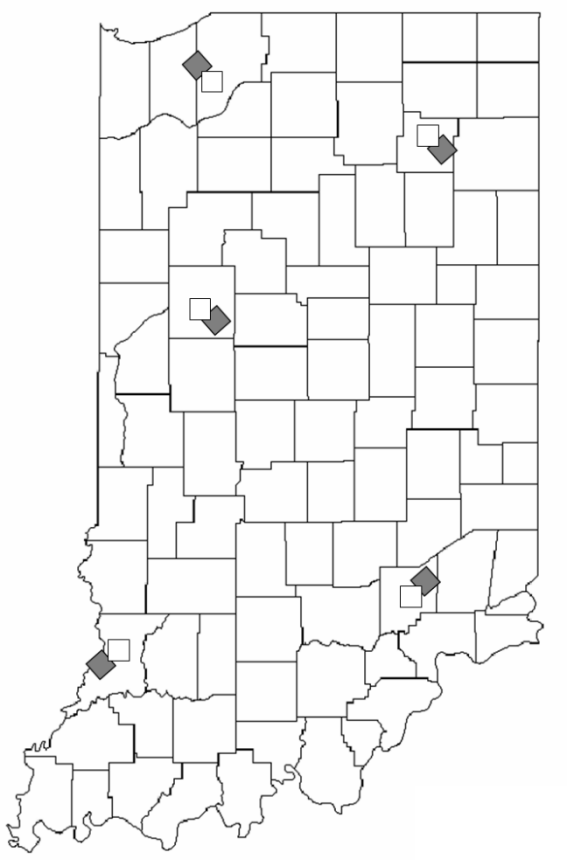

Figure S2.1. Map of Indiana with location of field sites used in 2017-2020. Grey diamonds and white squares represent the integrated pest management (IPM) and conventional management (CM) sites, respectively.

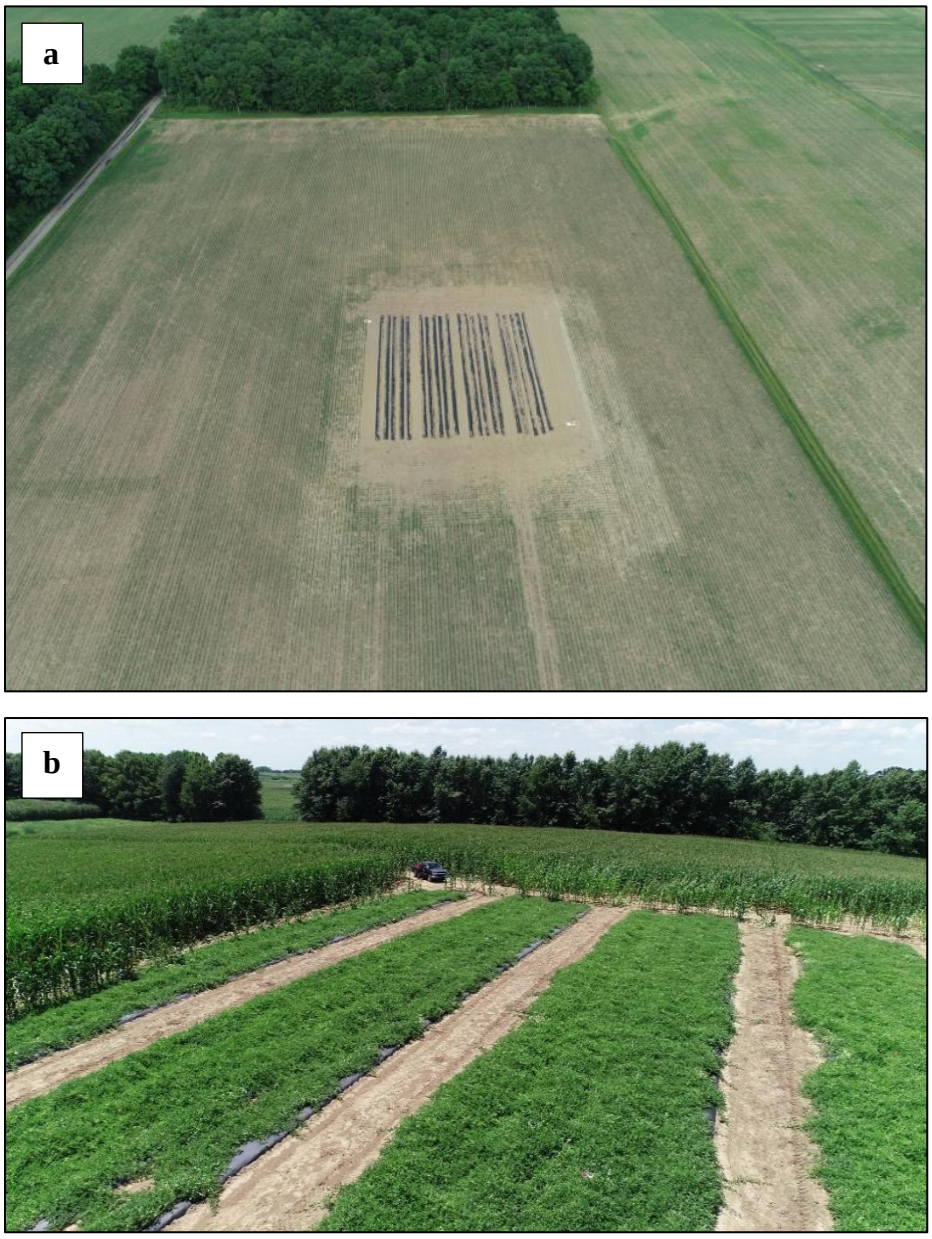

Fig. S2.2. Aerial view of mixed corn-watermelon cropping system, showing a site a) early in the season (May) following watermelon transplant, and b) later in the summer (July) during crop growth. Uncultivated alleys between rows allowed access for equipment to apply foliar pesticides.

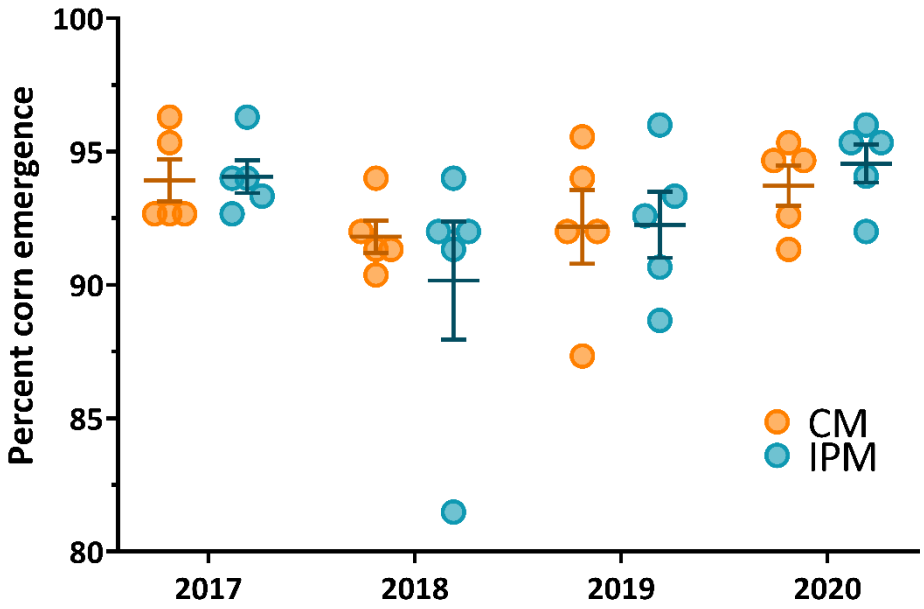

Fig. S2.3. Corn emergence did not differ between management systems. Each point within a cluster (n = 5) represents all observations from a single site during that season. Transects of V3-V4 corn plants were counted and compared to planted densities within each field. Whisker within the plot show the mean ± SEM of all sites within cluster. Results based on general linear model using $P < 0.05$.

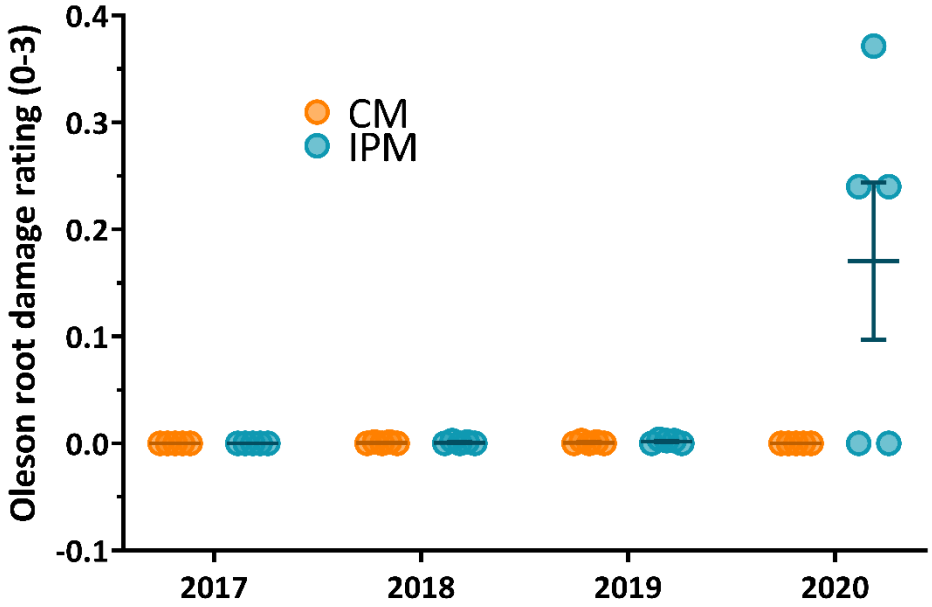

Fig. S2.4. Corn root damage was only different between +/- NST treatments after the 4th year. Each point within each cluster (n = 5) represents all observations from a single site during that season. Corn roots at each site (n = 40) were excavated, washed, and scored using the Oleson root scale (0-3). Whiskers within the plot show the mean ± SEM of average root damage within each cluster of points. Results based on generalized linear model with post hoc pairwise comparisons using $P < 0.05$.

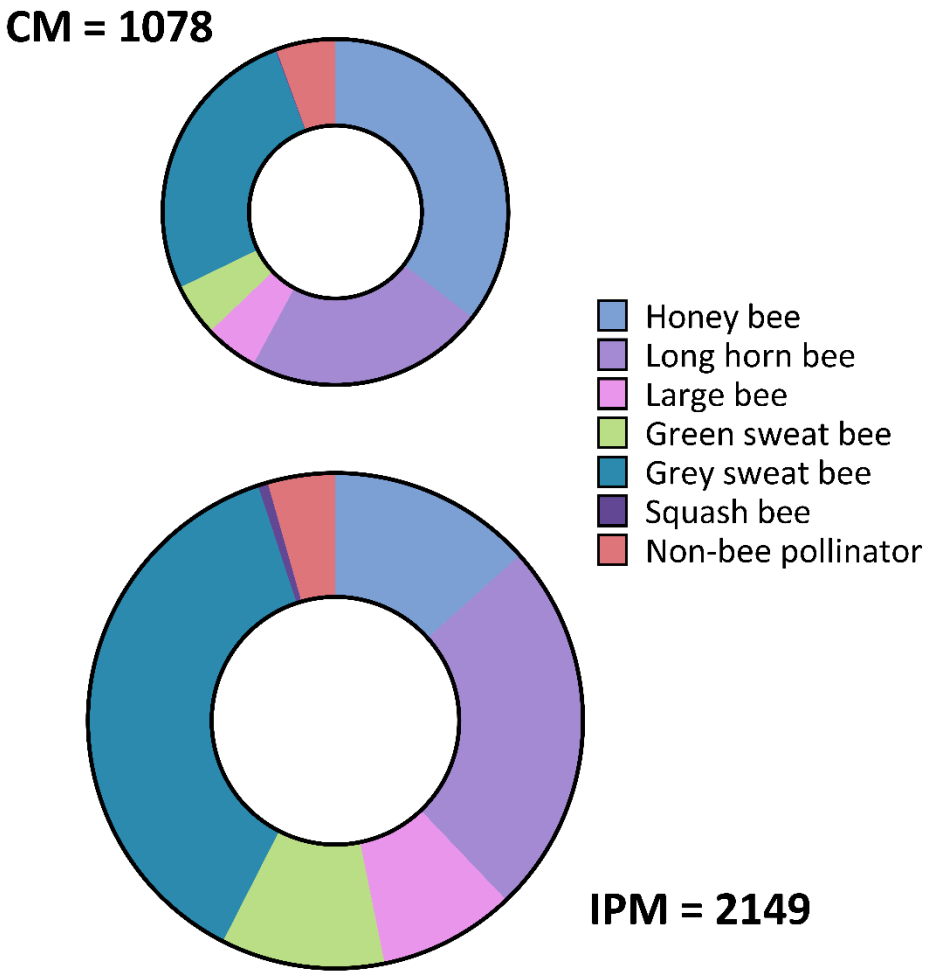

Fig. S2.5. Composition of the pollinator community was similar between pest management systems, but abundance was lower in CM fields (top) compared to IPM fields (bottom). Each doughnut chart represents the observed watermelon flower visitors across 3,375 minutes of observation from 2018-2020. Graphs are scaled to the proportion of pollinators observed at the CM fields (1,078) compared to IPM fields (2,149). Colors of each graph are representative of the categories of pollinators that could accurately be visually identified as they visited flowers.

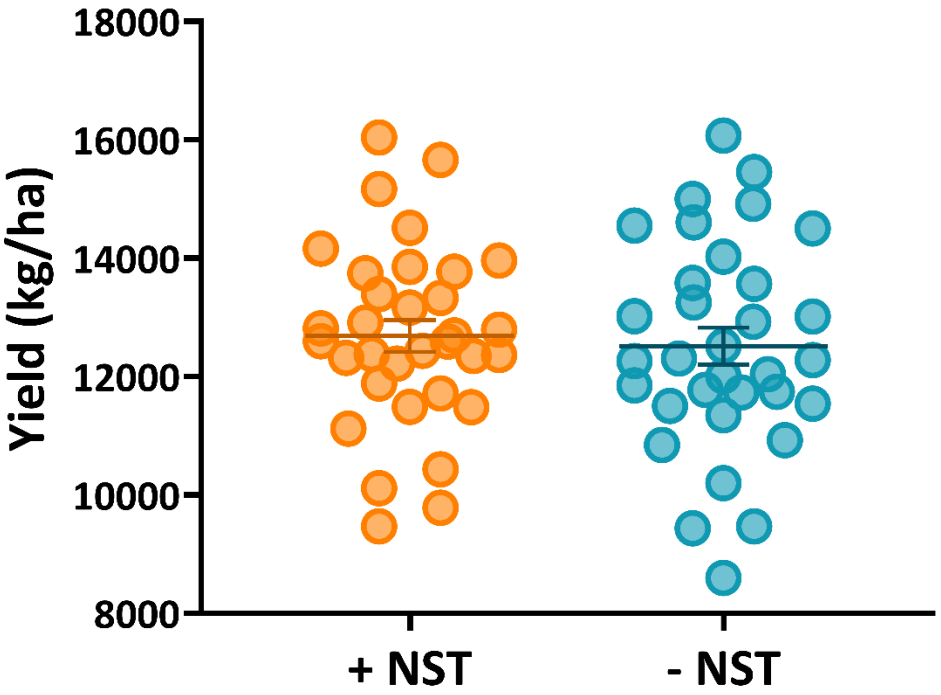

Fig. S2.6. Within-field plots showed that corn yield was unaffected by NST use. Corn rows with and without NST were replicated within the same field at different sites (n = 6) to test the effect on yield. Each point was the average yield within a plot and whiskers represent overall treatment mean ± SEM. Analysis used linear mixed models with post hoc comparisons between NST based on $P < 0.05$.

CHAPTER 3. IMPLEMENTING IPM IN CROP MANAGEMENT SIMULTANEOUSLY IMPRVOVES THE HEALTH OF MANAGED BEES AND ENHANCES THE DIVERSITY OF WILD POLLINATOR COMMUNITIES

Abstract

With worldwide food production demands increasing, there is mounting pressure on growers to balance protecting crops from pests while maintaining pollination services. Honey bees are the most commonly used commercial pollinators, but the service provided by both managed and wild pollinators can be reduced by exposure to pesticides used in crop fields. To examine the effect of pest management on crop pollinators, we created commercial-scale fields of pollinator dependent watermelon surrounded by corn, regionally important crops in the Midwestern U.S. These fields were paired at each location with the only difference being pest management regimes: a standard set of conventional management (CM) practices vs. an integrated pest management (IPM) system that uses scouting and pest thresholds to determine if/when insecticides are used. Between these two systems we measured the health, growth, and abundance of managed and wild pollinators from 2018-2020. IPM led to increased growth and development of two popular managed pollinators (honey bee and common eastern bumble bee) and increased abundance, richness, and diversity of pollinator species compared to CM fields. These improvements to pollinator health through adoption of IPM demonstrate that simple changes to pest management can alter pollination services. By replicating realistic changes to management, this experiment provides one of the first demonstrations where tangible improvements to pollinator conservation result from IPM implementation in agriculture.

3.1 Introduction

The global dependence on insect pollination to economically important crops (estimated at $215 billion globally[1]) has prompted many growers to rely on managed species, primarily the European honey bee (*Apis mellifera* L.), for supplemental pollination. However, honey bees are increasingly threatened by agricultural intensification[2-4]. Declines in the health of honey bee colonies are well-documented [5-7], with common drivers of colony losses identified as lack of high-quality forage[8-10], parasites (e.g., Varroa mite) and their associated diseases[11,12], and increased

toxicity of insecticides[13,14]. The effect of insecticides on honey bee colony health is especially important to consider given the dramatic increase in agricultural landscape toxicity for invertebrates in recent decades[15-17].

The primary contributor to insecticide hazard in the US has been the rapid and widespread use of neonicotinoids, a group of systemic and highly insect-specific products that have grown into the most widely used insecticide class[18,19]. Neonicotinoids are extremely toxic to pollinators; honey bee oral LD_{50} has been observed as low as 3.7 ng for clothianidin [20-22]. Estimates of pollen consumption throughout a honey bee worker's life (*ca*. 100mg [23]), mean that even short periods of exposure in floral resources could lead to mortality of larvae or adults. With most major US row crops receiving a neonicotinoid seed treatment (NST), honey bees living within agricultural landscapes are likely exposed to these products[13,24]. Indeed, analyses of hive materials commonly report neonicotinoid residues at biologically relevant levels[25-28]. Although the combination of laboratory toxicity tests and field exposure in bee diets is often used to infer negative health outcomes, this approach has been criticized for potentially overestimating risk[29].

Field studies examining the effect of NSTs on honey bee colonies have been conducted in different cropping systems—mostly corn and canola—with mixed results. Honey bee colonies placed adjacent to fields using NSTs experienced higher worker mortality[30,31], impaired immunity[31,32], increased pathogen loads[32] and reduced overwintering success[33]. However, others have found no consistent colony-level effects using similar field designs[34-37]. Even within the same study, NST-mediated impacts on honey bees can vary dramatically across landscape contexts[33]. Clearly, additional large-scale field experiments simulating realistic exposure are needed to clarify the contribution of NSTs to honey bee health in agricultural areas.

While honey bees are the most well-studied species, other managed pollinators (e.g., bumble bees, mason bees) and native wild bees are similarly exposed to these products, potentially eliminating key contributors to crop pollination (van der Sluijs et al. 2013, Reilly et al. 2020). Non-honey bee species are important to acknowledge due to their advantages as a source of pollination: bumble bees are more efficient cucurbit pollinators[38,39] and forage under more adverse weather conditions[40,41] than honey bees. When multiple species were directly compared in the same experimental set-up, a few studies show that insecticides have no discernible effect on honey bees, while the same applications reduce wild bee visitation and performance[36,42]. Reproductive success and population growth of both solitary bees and social bumble bees are reduced by neonicotinoid

exposure[21,33,43] but see[44]. Bee species respond to pesticides differently[45] with smaller body size generally increasing vulnerability, which means that risk is greater for many of the native solitary bees[46].

Current research approaches on how NSTs and other insecticides impact pollinators suffer from a few limitations. First, studies tend to focus on a single crop, even though many pollinators, especially generalists like honey bees and many bumble bees, forage widely across neighboring habitats and cropping systems[47-49]. Moreover, the most field-tested system for NST-bee interactions, corn, is wind-pollinated and thus beekeepers do not intentionally place honey bees in or near these fields. Corn represents a broader land use, however, that intersects with bee foraging ranges, particularly in the Midwestern US (94% of foragers in Indiana are at exposure risk[50]), and honey bees readily collect corn pollen in the absence of alternatives[28]. The extra-field exposure of corn NSTs for bees in adjacent crops that require pollinators (e.g., fruits and vegetables) is poorly investigated. A second problem is that virtually all experiments employ an all-or-nothing strategy that compares the presence vs. absence of insecticides. The value of an experimental control completely free of insecticide use is debatable, depending on historical pest pressure, crop economics, and farmer behaviors. In corn[50-52] and soybean[53-55], for example, NSTs seem to contribute little or nothing to yield and thus an NST-free control may be appropriate. Yet, in higher-value specialty crops, foregoing insecticides altogether is unrealistic. In these systems, an insecticide-free control is theoretically useful for estimating the overall impact of insecticides on pollinators, but in practice would rarely be implemented on commercial farms. An alternative approach could employ an integrated pest management (IPM) system with economic injury levels guiding a reduced-insecticide "control" compared to a prophylactic or calendar-based spray regime. Under this scenario, the control field could be insecticide-free or have one to several applications if pest populations exceed their action threshold. Recent reviews emphasize that pollinators should be more explicitly accounted for in pest management decisions—from IPM to IPPM (integrated pest *and pollinator* management)[56-58]—yet we have few empirical cases documenting how IPM implementation affects pollinator health.

We conducted a multi-year, multi-site experiment across Indiana using a dual cropping system to contrast a conventional insecticide program with an IPM system, evaluating the health of both managed and wild pollinators. This experimental design placed seedless watermelon, a pollinator-dependent crop that almost always receives managed bees, within a larger corn field to

simulate conditions typical for our region [59]. Specialty crops such as watermelon are often surrounded by and rotated with row crops such as corn. We hypothesized that the reduced insecticide applications within IPM cropping system would result in healthier managed bee colonies and higher watermelon floral visitation and diversity from the wild pollinator community. Secondarily, we expected that the magnitude of response to insecticide use by managed bees would be weaker than for wild bees. This study provides some of the first data linking the adoption of IPM with a more abundant and species-rich pollinator community, a critical step to providing growers with evidence-based solutions to sustainable crop management and on-farm bee conservation.

3.2 Materials and Methods

3.2.1 Experimental Design

This four-year experiment took place from 2017-2020 on five of the Purdue Agricultural Center (PAC) research farms across Indiana, USA. At each site, a pair of fields (separated by 4.63-6.63 km; average 5.6 km) were randomly assigned to either a conventional management (CM) or integrated pest management (IPM) program. Regardless of treatment, all fields had the same crop arrangement: the entire area (4.8-7.7 ha) was planted with corn, except for 0.2 ha of watermelons embedded within the corn matrix, surrounded on all four sides. The two treatments differed only in insecticide inputs; all other management practices (e.g., tillage, fertilizer, herbicides/fungicides) were standardized for each pair of sites. For additional detail on site history, land use, and crop management, see [60]. Corn was planted in all four years of the study (beginning in 2017), whereas watermelon started one year later (2018). The purpose of this staggered start date was to allow the first year for corn to impose initial treatment differences in insecticide use that carryover to subsequent years. Thus, during the initial year of watermelon-pollinator surveys, ground-nesting bees were potentially exposed to soil residues from the prior year's corn crop.

CM fields mimicked the insecticide inputs typical of Indiana row crop and vegetable production. Corn seed (var. Spectrum 6334) was coated with thiamethoxam (Cruiser 5FS @ 1.25 mg a.i. per seed), one of the most widely used neonicotinoid products by US farmers. Transplanted watermelons (var. 'Fascination') received imidacloprid (Wrangler® @ 814.09 ml/ha) as a soil drench. While not as ubiquitous as NST in corn, neonicotinoids applications as a tray drench as

seedlings or soil drench at transplanting are common practices[61]. Additionally, CM watermelons were sprayed with the insecticide lambda-cyhalothrin (Warrior II® pyrethroid @ 140.3 ml/ha) via tractor-drawn air blaster or boom sprayer at 4, 6, 8, and 10 weeks post-transplant, resulting in four foliar applications each season. These sprays were made as late in the day as possible to avoid peak bee foraging times, with a majority of applications taking place later than 17:00. The CM insecticide program is based on prior on-farm surveys of Indiana watermelon growers and thus replicates a typical spray regime, consisting of *ca*. five applications per season with neonicotinoids and/or pyrethroids [61].

In the IPM system, corn seed was left untreated, except for fungicides, which were coated on seeds in both treatments (Maxim Quattro: Azoxystrobin 2.5 µg; Fludioxonil 6.5 µg; Mefenoxam 5 µg; Thiabendazole 50 µg of a.i. per seed). Similarly, IPM watermelons received no insecticides, except if the primary pest—striped cucumber beetle (*Acalymma vittatum*)—exceeded its economic threshold of 5 beetles per plant during weekly scouting[62]. When pests crossed their threshold, we applied a foliar spray of lambda-cyhalothrin, as described above. However, this only occurred four-times across the 15 site-years; once each in 2018 and 2019 and twice in 2020. None of the IPM fields were treated more than once in a growing season.

3.2.2 Honey bee colony establishment

Colonies of honey bees (*A. mellifera*) were regionally sourced from Bastin Honey Bee Farm LLC (Knightstown, IN, USA). In 2018 and 2019, 2.7 kg packages with mated queens were used, while poor weather conditions in 2020 forced the use of nucleus (nuc) hives that were modified to have reduced food stores/capped brood and an increased number of bees to mimic the packages used in earlier years. Bees were housed in pre-weighed 8-frame Langstroth hives with plastic foundation frames (#HK-560 Hackensack, MN, USA). Hives were only used once per year of the experiment and later replaced, i.e., we did not place the same hive out in multiple years so that each unique year-site was not confounded by conditions experienced in prior years of the study.

Each field received two hives, placed at opposite corners in the space at the transition between the watermelon and corn crops, in an arrangement to avoid farm management (e.g., driving lanes, irrigation). This design also prevented hives from being directly sprayed with insecticide and the stocking rate was within the recommended range of 1-5 colonies per acre[63].

Once purchased, all colonies were installed and placed within a three-day period: 9-11 May 2018, 2-4 May 2019, and 19-20 May 2020. In 2018 and 2020, all corn was planted prior to hive placement; however, in 2019 weather conditions delayed corn planting and thus all colonies were already established, and the hive entrances were blocked during the field planting (Table S1). Establishment was confirmed by observing new eggs or larvae in frames. Only one package did not have a viable queen (2019) and after replacement 2 days later, successful eggs were observed. Colonies remained in the field until late-September or early-October (spanning the full management periods for both crops, except for corn harvest), after which they were overwintered in an apiary yard within Martell Forest located outside of West Lafayette, IN. In the apiary, all colonies were provided with supplemental sugar solution (1:1 ratio sucrose: water) prior to temperatures dropping consistently below 0°C. In the following spring, hives were checked for successful overwintering and recorded as either alive or dead. Surviving colonies were removed from the hive boxes and all frames were replaced prior to the next field season when a new set of colonies were used.

3.2.3 Honey bee colony growth

Colony size is one of the strongest predictors of overwintering success[64], brood production[65], and weight of accumulated foraging resources[64]. After placement into each pre-weighed hive box, colonies were weighed (Doran 7400, Doran Scales Inc., St. Charles, IL, USA) to calculate initial weight and then reweighed approx. every two weeks until hive removal from the field (10-11 measurements per hive per year). When colony inspections showed brood production in more than half of upper box frames (typically late June), two honey supers were pre-weighed and added to all colonies to allow additional resource storage throughout the season. The pre-weight measurements of hive boxes and supers were subtracted from colony weight measurements to accurately quantify colony population and resource gathering.

Successful development of new brood represents a greater number of bees within the hive to transition to a foraging role for pollen/nectar gathering and capped brood (pupation) allows for a standardized timepoint to measure the production of new brood in a colony[9,30]. As an additional measure of colony growth, photographs of frames were taken to quantify capped brood[66]. Photographs were taken from each colony monthly from July-September in 2018 and June-September in 2019 and 2020. The frames inside the second hive body were used and organized by

labeling all frames 1-8 with the first being the northmost frame. During picture sessions, each side of frames 2, 4, 6, and 8 were brushed free of bees, photographed, and returned, maintaining the same frame order and orientation within the hive body. All hives had pictures taken within one week of one another for each month's sample. Individual images (n = 1,600) were opened in Microsoft Paint (Microsoft Corporation, Redmond, WA) and all cells in the frame dedicated to capped brood were filled in with the same color. Colorized frame photos were analyzed using ImageJ (US NIH, USA) to quantify the area of each frame dedicated to capped brood within each colony.

3.2.4 Honey bee mortality

While most of the honey bee mortality (80-98%) occurs away from the hive (Johansen and Mayer 1990, Porrini et al. 2002), the cleanliness behavior within the hive allows for at-hive mortality to serve as a proxy or "mortality index" for comparisons across hives [30,34]. Plywood boards (1m × 1m with 5 cm raised edges on all sides) were treated with a white paint/stain and placed directly in front of each colony to collect dead bees from within the hive that were removed by other members of the colony. The number of dead or dying (categorized by spasms or twitching behavior when prodded) individuals on the board was measured each Monday, Wednesday and Friday and summed for a weekly total mortality. Multiple within-week measurements were used to reduce the number of dead bees lost due to scavenging from small mammals or birds. For each count, the board was removed from in front of the hive to a safe distance to count all bees. Dead individuals were removed along with any detritus and the board was replaced in front of the hive. Mortality was measured for the duration of colony placement in the field, ending the week prior to overwintering.

3.2.5 Varroa mite counts

Although each colony was newly established and less at-risk to severe mite infestation (Traver et al. 2018), we counted varroa mites to track any first-year accumulations. Counts were conducted three times each year, mid-July, early August, and mid-September, using a Varroa Easy Check (Véto-pharma, Palaiseau, France) container to evaluate colony mite levels. The bottom collection portion of the container was filled with ethanol to the point where it was nearly touching

the inner collection cup. Then, using a bee brush (#M00751, Dadant, Hamilton, IL, USA) approx. 300 bees (1/2 cup) from a frame containing brood were placed into the inner collection cup and the lid was secured immediately to prevent escaping bees. The container was shaken for 60 seconds to kill all bees and any mites on them, which fall through holes in the inner collection vessel. The transparent outer bowl allowed for mite counting, and then the entire container was emptied and washed once with water to remove all remaining bees or mites prior to the next mite count. The number of counted mites was divided by three to calculate the percent infestation of the hive.

3.2.6 Bumble bee colonies

Colonies of bumble bees are becoming a popular alternative to honey bees to provide managed pollination services in watermelon due to their success in enclosed environments and efficiency as pollinators in diverse crops. The common eastern bumble bee (*Bombus impatiens* Cresson) is a native species within the study region and the most common managed bumble bee species available for the eastern United States. A Quad colony (Koppert Biological Systems, Howell, MI, USA) containing 4 separate *B. impatiens* colonies was placed in the field when watermelon bloom began (4-5 weeks following transplant) to synchronize colony growth with the crop bloom period. At placement, each individual colony was labeled and weighed (Tayler Precision Products TE22FT, Capacity 10kg × 1g) and left in the space between corn and watermelon crops under a tarp for protection from rain and sun. Each week, the entrance to the colonies was temporarily altered such that foraging bees could return but new foragers could not leave. After *ca*. 1 hr in this condition, colony weight was measured and the entrance was reopened. This process was repeated for 6 weeks, after which colonies were placed in a -20°C freezer to kill all remaining bees and preserve the colony for later inspection.

After at least 5 days in the freezer, colonies were dissected in the lab. We recorded the number and weight of all workers, males, and queens that were still alive at the time of freezing, along with counts of already dead bees (i.e., died prior to freezing). The two groups were distinguishable based on appearance and location – dead bees showed clear signs of decay and were often found on the edges of the colony away from the nest material. Additionally, we recorded the number of constructed cells with nectar resources, eggs, larvae, and pupae of both worker and queen types (distinguished by size). Counts of these metrics within the colony, coupled with seasonal weight change, informed the condition and health of the colony in either treatment group.

3.2.7 Wild pollinator communities

Weekly collections of insect visitors to watermelon flowers were conducted at all sites to measure the community of wild bees and other taxa contributing to crop pollination. Collections began around 6 weeks post-transplant of watermelon seedlings and ended after completion of 5 consecutive weekly surveys; this was typically from late June to early August. Collections took place on the same date at each pair of fields per site to account for daily or weekly differences in weather conditions affecting pollinator foraging activity. Sampling occurred between 9:00 and 13:00 with low cloud cover, wind speeds < 16 km/h, and temperatures between 15 and 32°C. This weekly collection extended through the peak blooming period of watermelon.

Pollinators were collected with a hand-held insect vacuum (Bioquip #2820GA) with a collection chamber to capture insects visiting watermelon flowers. Sampling occurred along a transect extending from the field edge and walking between plant rows for a 15 min period, collecting all insects actively visiting watermelon flowers. This sampling time typically allowed for the entire field to be surveyed. Upon completion, the collection chamber was removed and placed in a cooler until returning to lab where it was stored at -20°C until later identification. The process resulted in 75 mins total collection time per field per year (=15 min weekly transect × 5-week duration). All pollinators were pinned and identified to the lowest taxonomic level. Most specimens were identified to species, except for hoverflies (Syrphidae) and several *Lasioglossum sp*. (Halictidae) that were identified to morphospecies. Bee specimens were identified using taxonomic keys[67-69] and reference specimen from Purdue Entomology Research Collection (PERC, West Lafayette, IN).

3.2.8 Pesticide Residues

Samples from within both the honey bee and bumble bee colonies were taken in each experimental year. Beebread samples from honey bee colonies was chosen because it represents a nutrient-rich food source consumed by both larvae and adults within the colony[70,71]. A sample of approximately 10 g wax/beebread was taken from one of the inner frames from the top hive body and differentiated from other stored food resources by the presence of packed cells with a shiny outer appearance indicative of beebread. A section of wax/beebread was removed from each honey bee colony was collected during the first watermelon harvest, at which point all insecticides in the

crop had been applied and hives are often removed in commercial operations. Samples were immediately placed in a cooler and stored in a -20°C freezer until processing. During the bumble bee colony dissections, approximately 5 g of nest material (open and nectar-containing cells) was collected into small freezer bags and placed in a -20°C freezer. When processing began, each wax or nest material sample was finely ground in liquid nitrogen using a mortar and pestle until the sample was reduced to a powder and a 0.5 g aliquot was used for the residue analysis. All materials were sanitized with ethanol between each sample to avoid contamination.

Processing methodology for samples followed a modified QuEChERs protocol for residue quantification optimized for the high-lipid matrix of the wax/nest material[26,72]. For bee bread/nest material, 0.5 g of sample was mixed with extraction solution (15 ml dH_2O + 15 ml acetonitrile) and 10 µl of internal standard solution (clothianidin-d3, imidacloprid-d4, thiamethoxam-d3, and acetamiprid-d3 at a 10 ng/µl concentration) simultaneously and vortexed. Samples were combined with 6 g of magnesium sulfate and 1.5 g of sodium acetate, inverted, vortexed, and centrifuged at 2500 r.p.m. for 10 minutes, after which 10 mL of the top layer of supernatant was transferred to a QuEChERS Dispersive Kit (Agilent Technologies, Santa Clara, CA, #5982-5456) and again inverted, vortexed, and centrifuged at 4000 r.p.m. for 5 minutes. Supernatant (6 ml) was transferred to a clean 15 ml tube and dried completely in a speed vacuum (Savant SC250EXP, Thermo Scientific, Waltham, MA). All samples were resuspended in 200 µl acetonitrile, vortexed, centrifuged, and the supernatant was transferred to 96-well plates. Immediately prior to instrument analysis, samples were re-suspended with 200 µl 50% acetonitrile dH_2O solution.

We screened samples for the active ingredients of all fungicides and insecticides used in both our corn and watermelon plots during the experiment. Samples were analyzed via liquid chromatography and tandem mass spectrometry at the Bindley BioScience Center at Purdue University, West Lafayette, IN. An Agilent Zorbax SB-Phenyl 2.1×100, 3.5 µm column was used for LC separation and an Agilent 1200 Rapid Resolution LC system coupled to an Agilent 6460 series triple quadrupole mass spectrometer was used to identify pesticide residues based on retention time and co-chromatography with analytical standards of all pesticide targets. Deuterated neonicotinoids were used to quantify the concentration of neonicotinoids in samples based on the relative response value. A mix of analytical standards from all other pesticides used in the experiment were subjected to a serial dilution and analyzed on the instrument to create standard curves to quantify their concentration in each sample. This protocol prioritized the detection and

quantification of neonicotinoids, which made quantification of some of the other pesticides impossible. This optimized protocol limited the detection clarity of the non-neonicotinoid pesticides, therefore non-neonicotinoid products applied to the watermelon fields were not quantified and instead reported only as a presence/absence for each sample.

3.2.9 Statistical Analysis

Statistical analyses were performed using SYSTAT 13 (SYSTAT Software, Inc; Point Richmond, CA) by creating a series of general linear models for pollinator abundance and performance response variables. Pseudoreplication was avoided by averaging colony parameters for multiple honey bee and bumble bee hives at each field to use field as the replicate for each treatment/year[73]. Similarly, surveys of the wild pollinator community were summed across collection dates for a single community total for each site/treatment/year. This approach resulted in 30 data points for each response variable with treatment ($n = 2$), year ($n = 3$), and site ($n = 5$) treated as fixed effects in the model, as well as the two-way interactions between treatment and year or site. Honey bee hive mortality model included an additional treatment "post-insecticide" that included all CM and IPM hive mortality counts during the two observation periods immediately following a pyrethroid spray to that field. Post-hoc pairwise comparisons (Fisher's LSD) were used to differentiate any factors (or interactions) that were significant ($p = 0.05$). Additional repeated-measures analyses were conducted with general linear models on seasonal changes to honey bee colony growth ($n = 10$), capped brood area ($n = 4$), varroa mite load ($n = 3$), and bumble bee colony growth ($n = 6$) across each season with treatment ($n = 2$) as a fixed effect. Data were transformed (square-root or log) as necessary to meet assumptions of normality (summarized for each response variable in Table S2). Because insecticide data generally contain many zeroes and thus cannot be transformed to achieve a normal distribution, we analyzed pesticides using a generalized linear model with a binomial distribution. To do so, we converted concentration data to presence/absence based on whether any sample contained quantifiable residues over the limit of detection.

3.3 Results

3.3.1 Honey bee colonies placed in IPM crops were more productive and heavier than in CM system

Across all three experimental years, honey bee colony weight gain was 80% higher ($P < 0.001$) in IPM (30.09 ± 1.96 kg) than CM (16.70 ± 1.90 kg) colonies (Figure 1A, Table S2A for statistical model for all honey bee metrics). Similarly, the peak measurement of growth was significantly higher ($P < 0.001$) in IPM (36.22 ± 1.97 kg) than CM (24.81 ± 1.91 kg) colonies. Immature bee populations, measured through area devoted to capped brood, was 132% higher ($P < 0.001$) in IPM colonies (1377.4 ± 61.09 cm^2) than CM (592.84 ± 62.57 cm^2) (Figure 2). Repeated measures analysis showed that metrics of colony growth varied significantly over the year ($F_{9,252} = 59.44$, $P < 0.001$; $F_{3,54} = 7.88$, $P < 0.001$ for weight and capped brood, respectively). There was also an interaction between treatment and seasonality for colony weight ($P = 0.013$), but no such relationship for immature bee populations ($P = 0.493$).

3.3.2 Varroa mite levels were unaffected by pest management system, but IPM hives had lower mortality and greater overwintering success

The varroa mite infestation rate throughout the season did not differ ($P = 0.166$) between CM (3.77 ± 1.02 mites per hive per season) and IPM (1.26 ± 0.34 mite per hive per season). There was an increase in mites across the season with 0.1%, 0.27%, and 0.64% infestation rates in late July, August, and September, respectively. Hive mortality was significantly different among CM, IPM, and post-insecticide treatments ($P < 0.001$). IPM (5.02 ± 2.62 bees/hive), CM (10.02 ± 4.81 bees/hive), and post-insecticide (20.6 ± 7.44 bees/hive) were all different from one another based on post-hoc comparisons (Figure 3). Successful overwintering occurred in only 10% of colonies from CM fields compared to 57% survival from IPM hives.

3.3.3 Bumble bee colonies grew larger and were more reproductively successful in IPM fields

The final weight change of *B. impatiens* was significantly higher ($P < 0.001$) in IPM (63.38 ± 6.98 g) than in CM (-45.13 ± 7.69 g) colonies, which averaged a decline in weight over the 6 weeks in the field (Table 1, Figure 1B; Table S2B for statistical models for all bumble bee metrics). This weight change was reflected in worker bee count (CM: 31.00 ± 4.18,

IPM: 55.93 ± 5.61) and the total worker weight (CM: 3.44 ± 0.49 g, IPM: 6.65 ± 0.89g), which were significantly higher in IPM colonies at $P < 0.001$ and $P = 0.001$, respectively. Trends of more robust colonies in IPM fields remained consistent with higher queen weight ($P < 0.001$), live queen counts ($P = 0.001$), larval counts ($P = 0.014$), egg counts ($P = 0.002$), worker honeypot counts ($P = 0.028$), and total cell counts ($P = 0.003$) compared to CM colonies (Table 1). CM colonies also had more than twice as many dead workers (57.52 ± 7.46) than the IPM (27.35 ± 2.95) colonies.

3.3.4 IPM fields contained a larger and more diverse pollinator community

A total of 4,909 pollinators from 41 morphospecies were collected from experimental fields over the three-year period (Figure 4; see Table S2C for statistical summary of wild pollinators and Table S3 for raw data across all species). The most abundant species were honey bees (n = 1,381), *Melissodes bimaculatus* (n = 997), *Lasioglossum pilosum* (n = 469), and *Augochlora pura* (n = 389). The pollinator community was dominated by wild species (n = 3,235 observations) compared to managed species (n = 1,674 observations). This difference was more pronounced in IPM fields; managed pollinators represented 44% and 26% of the collection from the CM and IPM fields, respectively. The average abundance of pollinators collected was 147% higher ($P < 0.001$) in IPM (161.53 ± 18.70) than in CM (65.33 ± 8.49) fields (Figure 5A). Species richness was similarly 128% higher ($P < 0.001$) in IPM (15.67 ± 1.38) than in CM (6.87 ± 0.65) fields (Figure 5B). While there was no effect of management system on species evenness (J') ($P = 0.958$), pollinator communities were more diverse (Shannon H') ($P < 0.001$) in IPM (2.03 ± 0.09) than in CM (1.37 ± 0.11) fields (Figure 5C-D).

3.3.5 Neonicotinoid residues were detected more frequently in managed colonies from CM fields

Honey bee comb from IPM fields contained at least one neonicotinoid in 63% of samples, while all CM hives contained residues of at least one neonicotinoid product at high enough levels to be quantified (Table 2A). Imidacloprid was significantly higher ($P = 0.005$) in CM (1.78 ± 0.33 ng/g) than in IPM hives (0.21 ± 0.06 ng/g) (see Table S2D for statistical models for all residue variables). Similarly, both clothianidin ($P < 0.011$) and thiamethoxam ($P < 0.001$) were significantly higher in CM than IPM hives. Bumble bee nest material contained at least one neonicotinoid in 97% and 13% of samples from CM and IPM fields, respectively (Table 2B).

There were significantly higher residues of imidacloprid ($P < 0.001$) in CM (0.46 ± 0.01 ng/g) than in IPM (0.02 ± 0.01 ng/g) hives. While the average concentration of clothianidin ($P = 0.007$) was higher in CM hives, there was no difference in thiamethoxam from IPM hives. Detection of all non-neonicotinoid pesticides was similar between treatments (Table S4).

3.4 Discussion

Implementation of an IPM-based approach to pest management can improve the health of both managed and wild pollinators. There was no lag-period in an IPM system's improvement and even in the experiment's first year there was a significant benefit to the growth and reproductive capability of two species of managed pollinators. In a pollinator-dependent crop the health of pollinators supplying pollination may be as essential to consider as the health of the plants themselves. At typical stocking rates of 1-2 honey bee hives per acre for cucurbit crops, the rental cost for a single 20 acre watermelon field ranges from $1,755-2,510 (average-sized field[61], adjusted for inflation from most recent 2017 data)[74]. With such a high investment cost there is a clear benefit to maximizing honey bee colony growth and flower visits while they are in the field and maintaining positive relationships with the beekeeper supplying them. Outside of this annual cost of commercial colonies (both honey bee and bumble bees), the conservation of wild bees in these crops may be equally, or more, important for ensuring adequate pollination is reached.

In all experimental years, honey bee colonies in the IPM fields were more productive at accumulating food resources and producing new brood than CM fields. End of the season weight gain was nearly twice as high in IPM fields, indicating that IPM colonies collected floral resources (i.e. pollen and nectar) at a much higher rate. Growth patterns of IPM colonies were similar to previous examples in agricultural landscapes (Iowa soybean fields[9]); high growth during crop bloom and a leveling off or decline in weight at the end of summer. The overall colony growth was likely facilitated by the larger areas of capped brood in IPM hives. Although the images analyzed are only a fraction (~25%) of the hives total available space for egg laying/larval development, this snapshot of reproductive growth over time demonstrates a significantly higher amount of capped brood even after less than two months in the different environments. IPM hives with higher weight, worker numbers, and increased resources represent a colony that is more likely to successfully overwinter or provide a beekeeper with a capable hive for the following season[64].

The lack of a fully factorial design in this experiment, to replicate grower practices in both crops, did not allow us to differentiate the relative effects of the neonicotinoid insecticides used in either corn or watermelon or the foliar pyrethroid applications to the watermelon crop. The quantification of neonicotinoids provides some idea of what products are accumulating in the colonies, but we cannot be certain what portion of this exposure came from outside of the experimental arena but still within the range of foraging. The at-hive mortality index measurement demonstrates some of the effect the pyrethroid sprays have on honey bee colonies. The counts of dead bees following an insecticide spray were 99% and 310% higher than CM and IPM counts, respectively. This increase in mortality after an insecticide spray, even in CM fields with higher baseline mortality counts, reveal that in-season foliar insecticide applications directly impact pollinator health, even when applications are timed to minimize the likelihood of honey bee exposure. A previous experiment[75] similarly found that applications of the pyrethroid insecticide lambda cyhalothrin in combination with exposure to neonicotinoids increased honey bee worker mortality. Synergism between pesticides has been reported at colony and landscape scales[42,76,77], and could contribute to managed bee colonies in the CM fields experiencing significant reductions to growth and health. Instead of looking at the effect of the removal of a single product or active ingredient, the IPM system we used here represents the simultaneous removal or reduction of several different products across both the corn and watermelon crop. New honey bee colonies were used each year and the lack of initial food resources compounded with the negative impacts of foraging on cucurbits[78] may be additional stressors to colonies that lead to the strong negative effects in CM colonies. Conversely, the use of new colonies and frames each experimental year ensured a more consistent starting point across all colonies and minimized previously acquired pesticide residues. While there are example of honey bee colonies being unaffected by field-relevant neonicotinoids[36,79], the possibility of pesticide synergy may have led to pronounced negative effects in CM colonies.

Bumble bees experienced similar negative effects to colony growth when placed in a CM compared to an IPM system. Popularity of bumble bees has increased with colonies sourced from commercial insectaries easily placed in the field to briefly augment pollination during crop bloom[80]. Bumble bees can also serve as a proxy for wild pollinators; *B. impatiens* is native to the eastern U.S. and can help identify the impacts of agricultural intensification and pesticide use on native species. The decrease bumble bee in-field abundance and colony fitness in the CM fields is a clear

indication of the hazards of insecticide use to bumble bees. The negative effects of neonicotinoid exposure are well documented in bumble bees[43,72,81], but these studies are often conducted in a controlled laboratory or enclosed greenhouse environment that eliminate or restrict the ability to forage. A field study found that a related bumblebee, *B. terrestris*, in crops treated with neonicotinoid and pyrethroid seed treatments had reduced growth and weight loss compared to colonies in untreated fields[36]. Colony dissection allows for an examination of the effect insecticide exposure may have on resource gathering and reproductive development[72,82]. A lower number of eggs, larvae, workers, and queens in CM compared to IPM colonies demonstrates that higher frequency insecticide use created an environment hazardous enough to lead to deleterious effects to colony growth.

Despite our observation of fewer honey bee and bumble bee foragers in CM fields, samples from both colonies consistently contained neonicotinoid residues from both crops. Neonicotinoid residues have been found in the soil and pollen in row crop[32,83] and cucurbit[60,84,85] fields. These residues accumulating in managed colonies have been previously documented[26], but this study identifies that IPM adoption was sufficient in reducing the concentration and detection frequency of insecticide residues within the colony of both pollinators. In many cases the range of values in CM colonies were well above previously found oral LD50 values for pollinators[86,87]. An important context for these values is that it requires direct feeding by bees within the colony. Beebread from honey bee colonies is a source of food for larvae and adults, but enough would have to be eaten to result in lethal or sublethal effects[88,89]. A previous survey of honey bee wax detected several fungicides and pesticides at high levels (~ 1 µg/g), these detections are far higher than any in this experiment, and likely represent an accumulation of residues over multiple years of exposure.

It is impossible to determine whether any collected *B. impatiens* were from a wild or commercial colony, therefore collected specimen were considered “managed”, meaning the proportion of wild species in the watermelon pollinator community is likely even larger. This difference in managed/wild pollinator communities demonstrates that a conventional approach to pest management more strongly affects wild pollinators and increases the reliance on managed bees to provide pollination. The average species richness in IPM sites was 128% higher than CM sites, demonstrating a more favorable environment for wild pollinators that are often solitary compared to the managed species with 100-50,000+ members. Wild pollinators are more effective pollinators than honey bees; in an experiment using watermelons in Florida *Melissodes, Bombus,*

and *Agapostemon,* were all found to deposit similar or even greater amounts of pollen to watermelon flowers compared to honey bees[39]. These three genera were among the most abundant, and all were more common in IPM than CM fields. In commercial pumpkins (a related cucurbit crop) in Pennsylvania there was a similarly diverse community of 37 species foraging on flowers during peak yield with most visits (>78%) coming from honey bees, *B. impatiens,* and the cucurbit specialist squash bee *Peponapis pruinosa* [90]. Organic cucumber fields in Indiana found 28 different species but honey bees similarly the most abundant species with 66% of visits[91]. A comparison of pollinator communities in Midwest pumpkin, cucumber, and watermelon fields similarly found that the watermelon pollinator community was more diverse and less reliant on managed species[42]. In the comparison study cucumbers were nearly entirely visited by honey bees (98%) with pumpkins and watermelon at 41% and 42% respectively. The wild bee community found in this experiment was primarily Halictidae spp. and *Melissodes bimaculatus*, similar to what Bloom et al. found in commercial watermelon fields. A consistent trend across these experiments was the strong negative effect pesticides have on wild bee abundance and diversity in agriculture fields.

3.4.1 Conclusion

Indiana is routinely among the top five watermelon producing states, with neighboring states similarly leading the nation in other cucurbit crops[92]. These systems are often rotated with and surrounded by row crops that nearly always use NST regardless of regional pest pressure[93]. The use of NSTs combined with the foliar insecticides in cucurbits[42] leads to high environmental stress on pollinators within these agroecosystems. Frequent within- and extra-field insecticides in a crop reliant on pollinators can lead to concerns that risks to pollinator health may compromise yield. This experiment examines how simple changes to insect pest management—namely, the adoption of a previously developed, scouting-based IPM program—can impact the health of managed colonies and entire pollinator community. These changes to pest management are well-established and have existed as proven practices for decades. Some of the most influential factors in grower decision making, cost and ease of implementation[94] can be addressed through this work and provide growers a compelling piece of evidence that IPM can be successfully put into practice. The findings demonstrate that IPM is a viable and readily accessible approach for effective pest management and increasing pollination services for commercial watermelon producers.

Wild pollinators have been found to be more sensitive to environments with high insecticide use[95] and their higher abundance, richness, and diversity in IPM compared to CM fields in this system support earlier observations. The recruitment and retention of wild pollinators represents a potential increase in pollination services that are "free" to growers, and lead to increased fruit quality and weight. Removing prophylactic insecticides such as seed treatments that provide negligible yield improvements[51,52,96], or unnecessary calendar-based foliar sprays can be an important step in improving conditions for pollinators and maximizing the opportunity to realize the yield benefits they provide.

Collectively, the findings from this experiment provide evidence that commitment to IPM can provide an environment more suitable for pollinators. Maintaining food security and minimizing environmental degradation are often overlapping concerns and sometimes conflict with one another. However, these experiments demonstrate that those goals are not mutually exclusive, and less intensive insect control via IPM provides an opportunity to conserve essential pollinators while still providing growers with the tools to manage key insect pests.

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Table 3.1: Performance variables measured from dissection of *Bombus impatiens* colonies after 6-week placement in experimental fields. Each column represents experimental years (2018-2020) and whether the colonies were placed within a conventional management (CM) or an integrated pest management (IPM) system. Each value is the average (± SEM) from four colonies placed inside each of the ten experimental fields.

B. impatiens hive variables	2018		2019		2020	
	CM	IPM	CM	IPM	CM	IPM
Colony weight change (g)	-16.05 ± 3.05	35.9 ± 2.81	-56.6 ± 8.18	69.4 ± 4.65	-62.75 ± 6.27	84.85 ± 5.66
Queen weight (mg)	292.81 ± 36.97	687.89 ± 87.61	158.21 ± 39.34	554.76 ± 48.82	308.45 ± 65.58	522.39 ± 41.18
Worker weight (mg)	1762.99 ± 199.16	4334.01 ± 366.07	4652.15 ± 330.47	10297.13 ± 954.38	3909.56 ± 399.19	5305.3 ± 317.3
Worker count (no. hive^{-1})	18.8 ± 1.7	51.95 ± 3.87	33.7 ± 14.1	77.3 ± 7.02	42.75 ± 4.36	48.55 ± 2.09
Total cell count (no. hive^{-1})	188.6 ± 9.68	257.6 ± 8.97	194.2 ± 9.07	296.45 ± 13.59	259.4 ± 16.51	326.35 ± 20.04
Worker honeypots (no. hive^{-1})	66.3 ± 7.13	87.6 ± 9.59	59.2 ± 5.55	133.35 ± 10.29	82.6 ± 7.29	89.3 ± 7.03
Worker larval cells (no. hive^{-1})	18.6 ± 1.87	23.7 ± 3.37	20.55 ± 2.3	30.25 ± 3.51	24.95 ± 3.13	46.25 ± 4.8
Live eggs (no. hive^{-1})	6.1 ± 2.19	23.61 ± 3.41	13.7 ± 2.51	63.05 ± 13.27	5.75 ± 1.85	31.2 ± 3.95
Dead worker count (no. hive^{-1})	58.9 ± 6.11	34.6 ± 2.22	60.75 ± 5.52	20.3 ± 2.28	52.9 ± 9.28	27.15 ± 2.99

Table 3.2: Neonicotinoid insecticide residues in both honey bee comb (A) and bumble bee nest material (B). Any sample below the limit of detection (0.0275, 0.0235, and 0.0056 ng/g for clothianidin, imidacloprid, and thiamethoxam, respectively) was considered the minimum value.

A. Neonicotinoid Residue from Honey Bee Colonies

Year	Conventional			IPM		
	Percent detection (10)	Median (ng/g)	Range (ng/g)	Percent detection (10)	Median (ng/g)	Range (ng/g)
			Imidacloprid			
2018	100%	1.07	0.325-3.28	40%	< LOD	< LOD-0.64
2019	90%	1.06	< LOD-3.73	40%	< LOD	< LOD-0.44
2020	100%	2.39	0.58-9.08	50%	0.12	<LOD-1.36
			Clothianidin			
2018	100%	1.12	0.48-2.24	20%	< LOD	< LOD-0.64
2019	100%	1.63	0.36-2.52	40%	< LOD	< LOD-1.06
2020	80%	1.41	< LOD-2.83	30%	< LOD	< LOD-0.86
			Thiamethoxam			
2018	40%	< LOD	< LOD-3.01	0%	< LOD	< LOD
2019	20%	< LOD	< LOD-2.42	0%	< LOD	< LOD
2020	90%	0.26	< LOD-0.90	20%	< LOD	< LOD-0.23

B. Neonicotinoid Residue from Bumble Bee Colonies

Year	Conventional			IPM		
	Percent detection (10)	Median (ng/g)	Range (ng/g)	Percent detection (10)	Median (ng/g)	Range (ng/g)
			Imidacloprid			
2018	70%	0.21	< LOD-0.46	0%	< LOD	< LOD
2019	70%	0.25	< LOD-0.66	20%	< LOD	< LOD-0.36
2020	100%	0.89	0.32-2.16	0%	< LOD	<LOD
			Clothianidin			
2018	40%	< LOD	< LOD -6.22	0%	< LOD	< LOD
2019	100%	1.7	0.32-4.34	30%	< LOD	< LOD-1.06
2020	40%	< LOD	< LOD-1.74	0%	< LOD	< LOD
			Thiamethoxam			
2018	0%	< LOD	< LOD	0%	< LOD	< LOD
2019	0%	< LOD	< LOD	0%	< LOD	< LOD
2020	30%	0.26	< LOD-0.90	0%	< LOD	< LOD

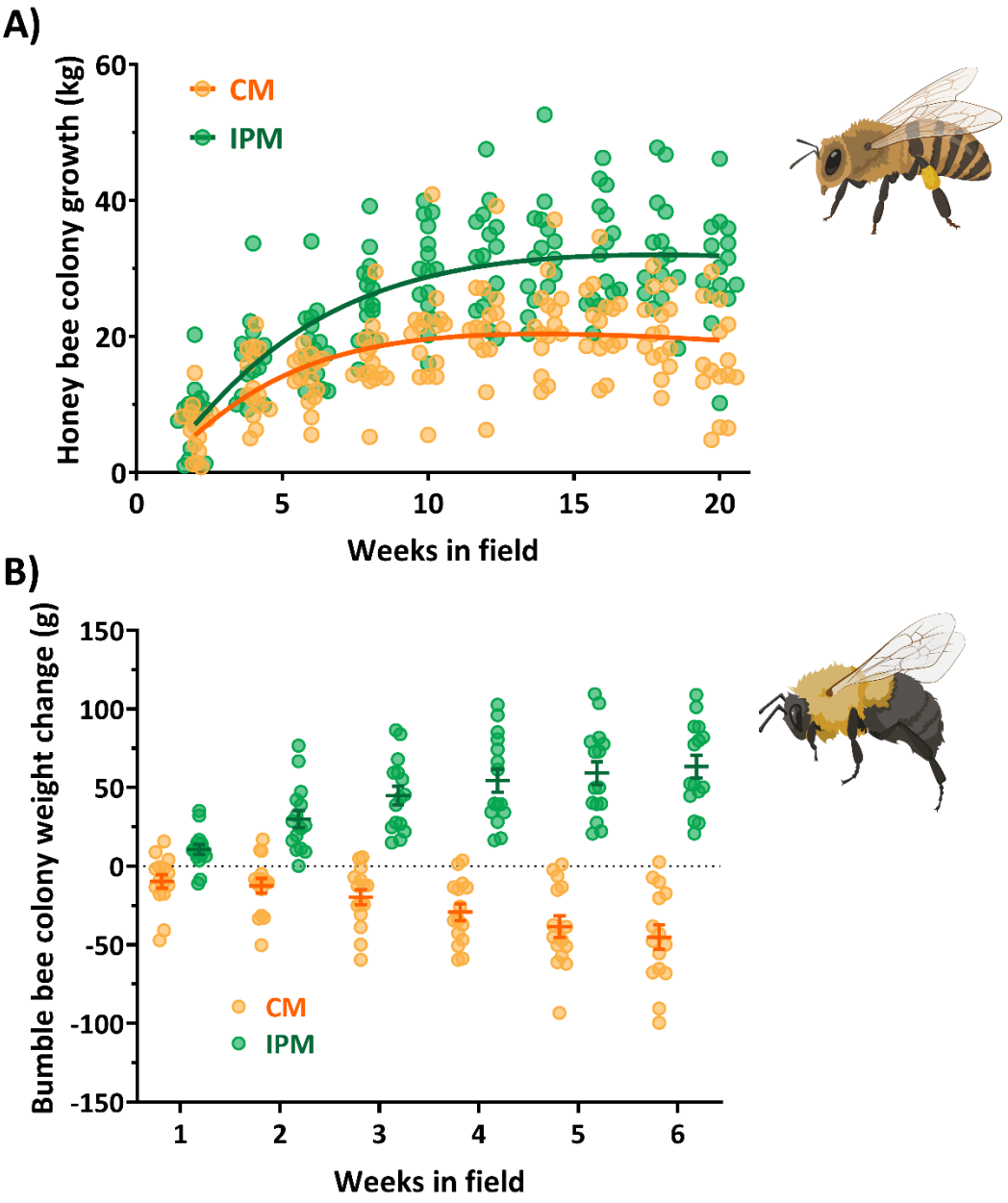

Figure 3.1: The growth of both honey bee (A) and bumble bee (B) colonies was affected by the management system of the field where they were placed. Each point is the average of all colonies—HB (n = 2) or BB (n = 4)—at each site from 2018-2020. Curve fit lines for honey bee colonies follow a lognormal path (R^2 for CM = 0.362 and IPM = 0.559). Points above or below the dotted line on 1B indicate weight gain or loss respectively from bumble bee colonies at each site based off initial weight measured at placement in the field. Whiskers within the bumble bee plot show the average (± SEM) of all sites across the experiment's three years. Bee icons from BioRender.

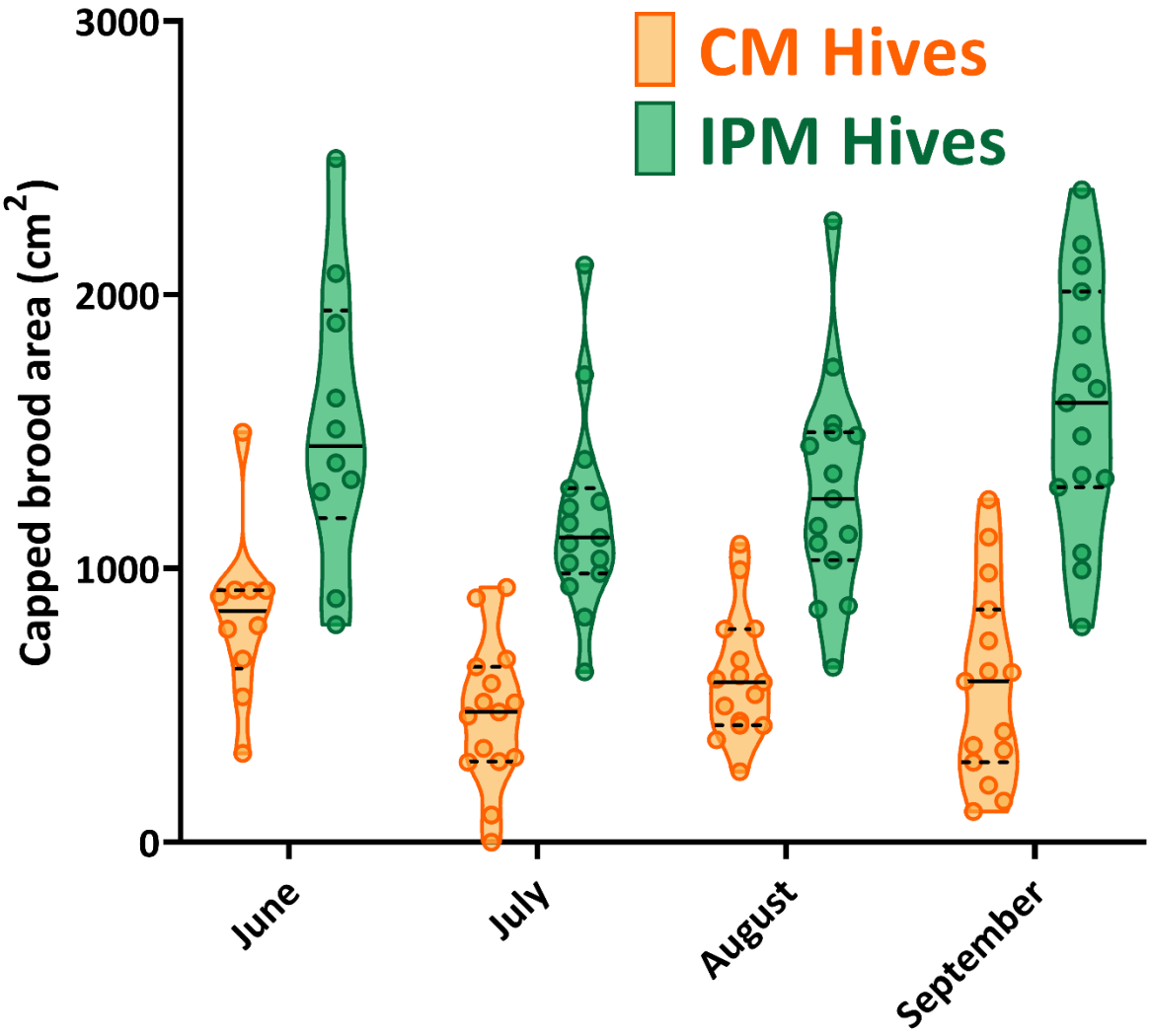

Figure 3.2: The area devoted to capped brood was higher in honey bee colonies within IPM fields. Points in the violin plots were the average area of capped brood from the front and back of 4 frames within each hive body. Each point is the mean value of total brood area from two colonies at each site (n = 5) from 2018-2020 from July, August, and September, and only 2019-2020 in June. Solid lines within violin plot represent 50th percentile (median), with lower and upper dashed lines indicating 25th and 75th percentiles, respectively.

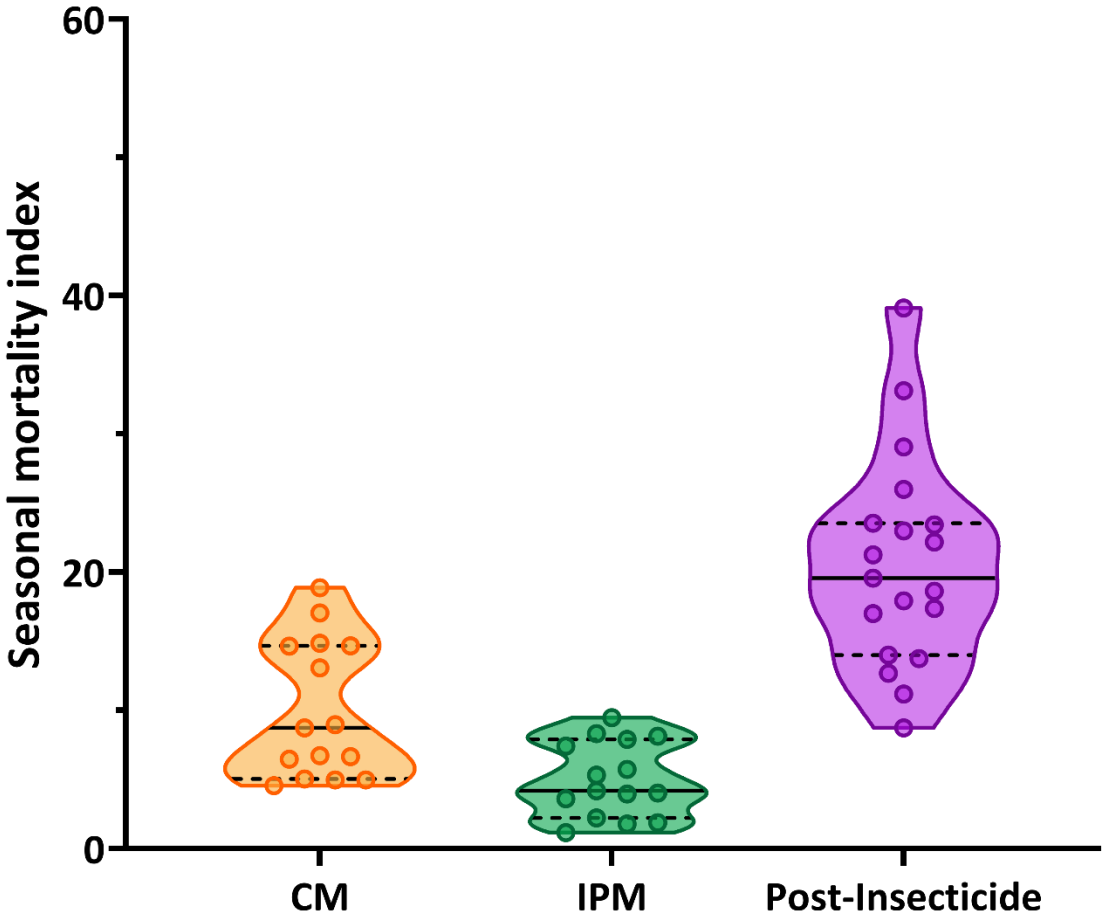

Figure 3.3: Seasonal average of at-hive mortality was highest following insecticide sprays to watermelon. Points in each violin plot were the seasonal mean of at-hive mortality measured using a collection board in front of each hive. Each point is the average from all hives (n = 2) at each of the five experimental sites from each treatment from 2018-2020. The purple post-insecticide treatment represents the two mortality counts that followed any pyrethroid foliar application from both the CM (n =15) and IPM (n = 4) treatments. Solid lines within plot represent 50th percentile (median), with lower and upper dashed lines indicating 25th and 75th percentiles, respectively.

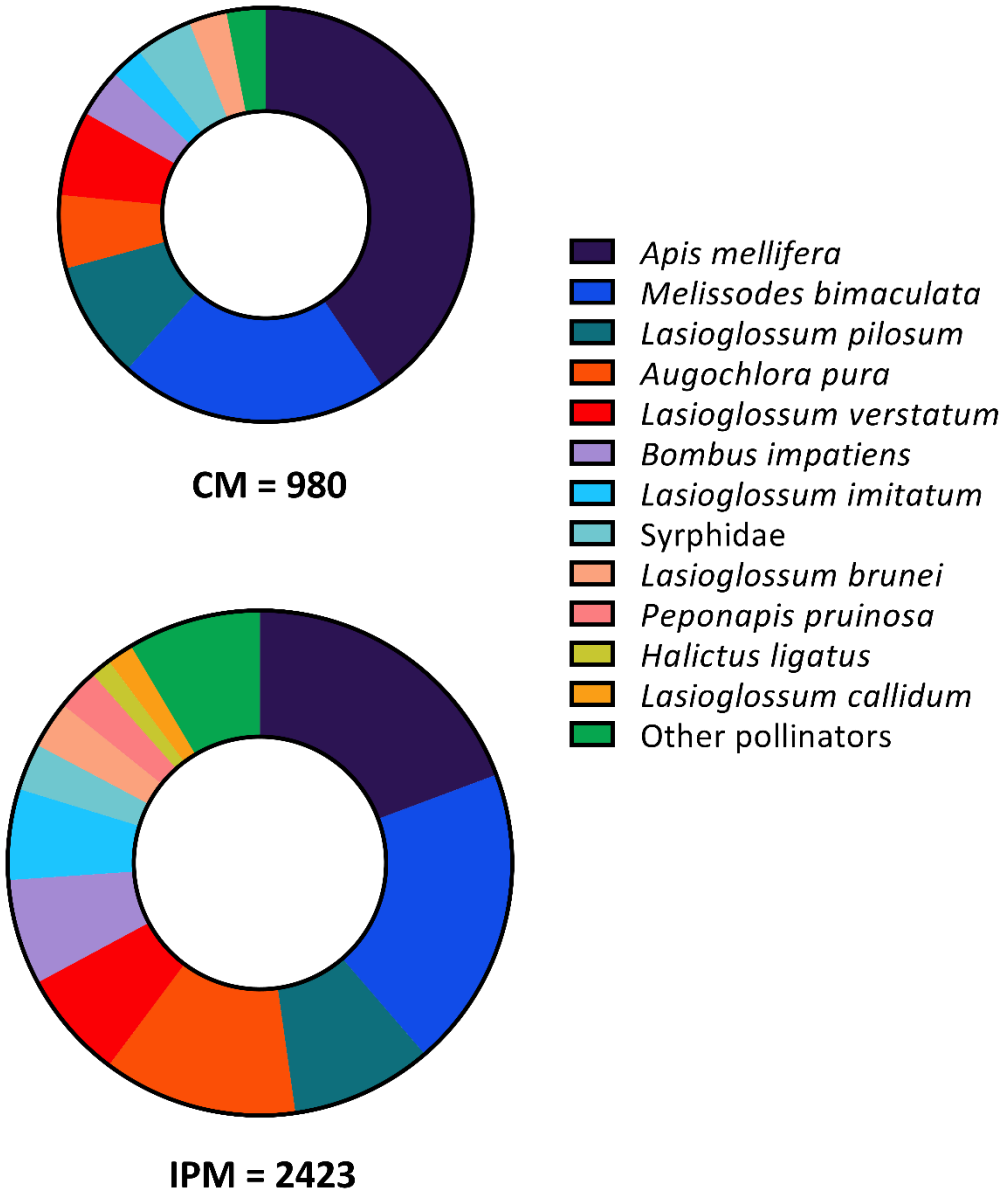

Figure 3.4: Pollinator community was less abundant and species rich in CM fields (above) compared to IPM fields (below). Each doughnut chart shows collected pollinators from surveys of watermelon flowers during bloom from 2018-2020 (1,125 total sampling minutes for each graph). Graphs are scaled to the proportion of pollinators observed between the two fields and colors represent pollinators identified to the lowest taxonomic unit possible. Any species that represented ≤ 1% of either system's community was grouped into the "other pollinators" category.

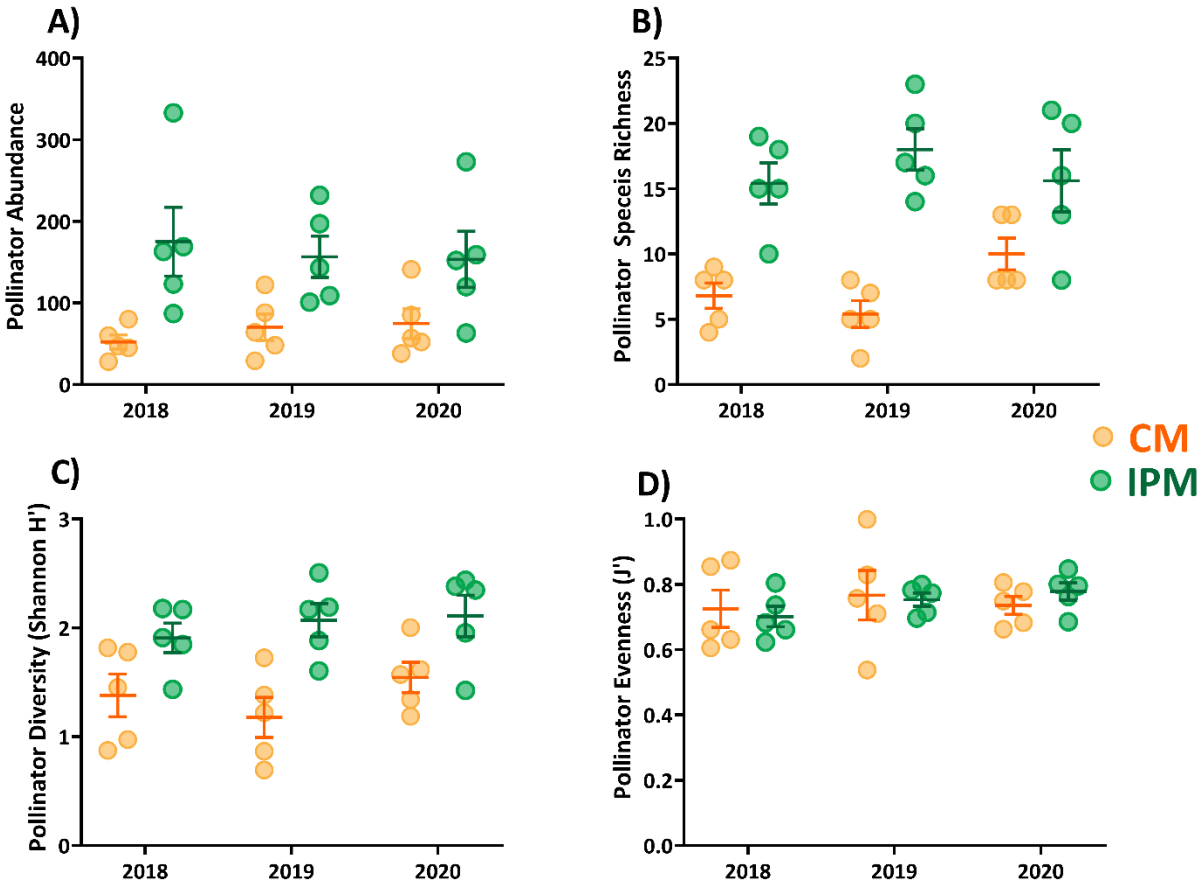

Figure 3.5: Pollinator communities were different between the two pest management systems. The abundance (A), species richness (B), diversity (C), and evenness (D) of watermelon pollinators were measured by collecting individuals visiting flowers from 2018-2020. Each point within a cluster (n = 5) represents 5 weekly collections during that field season (75 total minutes). Whiskers within the plot show the average ± SEM of all sites within each cluster.

3.6 Supplemental Information

SUPPLEMENTAL TABLES

Table S3.1: Information on the location and timing of honey bee colony placement and removal for all experimental years and sites. All hives within a year were placed at all sites within a 48 hour period after purchase from a local supplier.

Year	Location	Treatment	Colony ID	Field placement	Field removal
2018	TPAC Lafayette, IN	IPM	1-2	9 May	30 Sept
		CM	3-4		
	SEPAC Butlerville, IN	IPM	5-6	9 May	2 Oct
		CM	7-8		
	PPAC Wanatah, IN	IPM	9-10	10 May	1 Oct
		CM	11-12		
	NEPAC Columbia City, IN	IPM	13-14	10 May	1 Oct
		CM	15-16		
	SWPAC Vincennes, IN	IPM	17-18	11 May	3 Oct
		CM	19-20		
2019	TPAC Lafayette, IN	IPM	21-22	3 May	2 Oct
		CM	23-24		
	SEPAC Butlerville, IN	IPM	25-26	2 May	1 Oct
		CM	27-28		
	PPAC Wanatah, IN	IPM	29-30	3 May	29 Sept
		CM	31-32		
	NEPAC Columbia City, IN	IPM	33-34	3 May	29 Sept
		CM	35-36		
	SWPAC Vincennes, IN	IPM	37-38	4 May	30 Sept
		CM	39-40		
2018	TPAC Lafayette, IN	IPM	41-42	20 May	7 Oct
		CM	43-44		
	SEPAC Butlerville, IN	IPM	45-46	19 May	7 Oct
		CM	47-48		
	PPAC Wanatah, IN	IPM	49-50	20 May	6 Oct
		CM	51-52		
	NEPAC Columbia City, IN	IPM	53-54	20 May	6 Oct
		CM	55-56		
	SWPAC Vincennes, IN	IPM	57-58	19 May	5 Oct
		CM	59-60		

Table S3.2: General linear model output for all response variables. Significant differences are designated by bold text based on a level of $P < 0.05$ for all honey bee colonies (A), bumble bee colonies (B), wild pollinator surveys (C), and neonicotinoid residues (D). Any transformations to normalize data or separate repeated measures analyses are stated underneath response variables.

A. Honey bee colony parameters

Response Variable	Explanatory Variable(s)	df	F	*P*
Final Weight Change	Treatment	1,16	66.55	< **0.001**
	Year	2,16	18.21	< **0.001**
	Site	4,16	4.34	**0.015**
	Treatment*Year	2,16	0.47	0.633
	Treatment*Site	4,16	1.80	0.179
Average Brood Area	Treatment	1,16	123.11	< **0.001**
	Year	2,16	3.99	**0.039**
	Site	4,16	0.66	0.632
	Treatment*Year	2,16	0.01	0.987
	Treatment*Site	4,16	0.23	0.917
Varroa Seasonal Average	Treatment	1,16	2.11	0.166
Log(x+1) transformed	Year	2,16	0.31	0.739
	Site	4,16	2.97	0.052
	Treatment*Year	2,16	0.31	0.736
	Treatment*Site	4,16	0.84	0.517
At-Hive Mortality	Treatment	2,28	51.12	< **0.001**
	Year	2,28	11.79	< **0.001**
	Site	4,28	1.43	0.25
	Treatment*Year	4,28	0.60	0.669
	Treatment*Site	8,28	0.58	0.788
Seasonal Brood Area	Time	3,16	9.05	**0.001**
Repeated measures	Time*Treatment	3,16	0.84	0.493
Varroa Mite Counts	Time	2,27	9.05	**0.001**
Repeated measures	Time*Treatment	2,27	0.84	0.493

B. Bumble bee colony parameters

Response Variable	Explanatory Variable(s)	df	F	*P*
Colony Weight Change	Treatment	1,16	193.22	< **0.001**
	Year	2,16	0.13	0.880
	Site	4,16	1.58	0.228
	Treatment*Year	2,16	13.76	< **0.001**
	Treatment*Site	4,16	0.57	0.688
Worker Weight	Treatment	1,16	25.98	< **0.001**
Square root transformed	Year	2,16	15.44	< **0.001**
	Site	4,16	2.77	0.063
	Treatment*Year	2,16	1.65	0.224
	Treatment*Site	4,16	1.09	0.393
Queen Weight	Treatment	1,16	72.33	< **0.001**
	Year	2,16	1.04	0.377
	Site	4,16	1.48	0.254
	Treatment*Year	2,16	0.64	0.542
	Treatment*Site	4,16	0.81	0.535
Queen Count	Treatment	1,16	15.57	**0.001**
	Year	2,16	2.72	0.096
	Site	4,16	1.27	0.323
	Treatment*Year	2,16	1.13	0.348
	Treatment*Site	4,16	0.62	0.654

Table S3.2 cont.

Live Worker Count	Treatment	1,16	18.25	**0.001**
Square root transformed	Year	2,16	3.94	**0.041**
	Site	4,16	0.69	0.608
	Treatment*Year	2,16	3.14	0.071
	Treatment*Site	4,16	1.30	0.313
Dead Worker Count	Treatment	1,16	10.64	**0.005**
Square root transformed	Year	2,16	0.63	0.548
	Site	4,16	0.40	0.805
	Treatment*Year	2,16	0.51	0.614
	Treatment*Site	4,16	0.32	0.863
Worker Larvae Count	Treatment	1,16	7.56	**0.014**
Square root transformed	Year	2,16	3.84	**0.044**
	Site	4,16	1.43	0.269
	Treatment*Year	2,16	1.21	0.323
	Treatment*Site	4,16	0.93	0.473
Egg Count	Treatment	1,16	13.34	**0.002**
Log(x+1) transformed	Year	2,16	0.79	0.473
	Site	4,16	2.38	0.095
	Treatment*Year	2,16	0.63	0.543
	Treatment*Site	4,16	0.85	0.513
Worker Honeypots	Treatment	1,16	5.83	**0.028**
	Year	2,16	0.63	0.547
	Site	4,16	0.52	0.725
	Treatment*Year	2,16	2.11	0.154
	Treatment*Site	4,16	0.78	0.556
Total Cells	Treatment	1,16	11.95	**0.003**
	Year	2,16	3.21	0.067
	Site	4,16	0.67	0.624
	Treatment*Year	2,16	0.25	0.783
	Treatment*Site	4,16	0.70	0.602

C. Pollinator Survey

Response Variable	Explanatory Variable(s)	df	F	*P*
Pollinator Abundance	Treatment	1,16	45.48	< **0.001**
Square root transformed	Year	2,16	0.15	0.861
	Site	4,16	8.40	**0.001**
	Treatment*Year	2,16	1.25	0.313
	Treatment*Site	4,16	0.50	0.702
Species Richness	Treatment	1,16	61.73	< **0.001**
	Year	2,16	0.47	0.633
	Site	4,16	4.22	**0.016**
	Treatment*Year	2,16	0.78	0.477
	Treatment*Site	4,16	0.27	0.892
Shannon (H') Diversity	Treatment	1,16	41.47	< **0.001**
	Year	2,16	1.58	0.236
	Site	4,16	6.43	**0.003**
	Treatment*Year	2,16	1.28	0.306
	Treatment*Site	4,16	0.26	0.899
J' Evenness	Treatment	1,16	0.01	0.958
	Year	2,16	0.91	0.425
	Site	4,16	2.66	0.071
	Treatment*Year	2,16	0.42	0.662
	Treatment*Site	4,16	1.21	0.343

Table S3.2 cont.

D. Neonicotinoid residues

Response Variable	Explanatory Variable(s)	df	F	*P*
Honey bee: Imidacloprid	Treatment	1,16	10.89	**0.005**
Binomial distribution	Year	2,16	1.56	0.241
	Site	4,16	0.33	0.851
	Treatment*Year	2,16	2.12	0.169
	Treatment*Site	4,16	0.88	0.452
Honey bee: Clothianidin	Treatment	1,16	8.17	**0.011**
Binomial distribution	Year	2,16	0.17	0.848
	Site	4,16	0.25	0.905
	Treatment*Year	2,16	0.49	0.621
	Treatment*Site	4,16	1.06	0.118
Honey bee: Thiamethoxam	Treatment	1,16	33.34	< **0.001**
Binomial distribution	Year	2,16	6.63	**0.009**
	Site	4,16	1.17	0.362
	Treatment*Year	2,16	2.12	0.129
	Treatment*Site	4,16	0.83	0.524
Bumble bee: Imidacloprid	Treatment	1,16	39.59	< **0.001**
Binomial distribution	Year	2,16	1.32	0.390
	Site	4,16	1.01	0.436
	Treatment*Year	2,16	1.36	0.321
	Treatment*Site	4,16	1.40	0.392
Bumble bee: Clothianidin	Treatment	1,16	17.52	**0.007**
Binomial distribution	Year	2,16	6.29	0.073
	Site	4,16	2.36	0.152
	Treatment*Year	2,16	0.32	0.301
	Treatment*Site	4,16	1.21	0.380
Bumble bee: Thiamethoxam	Treatment	1,16	3.12	0.119
Binomial distribution	Year	2,16	0.46	0.774
	Site	4,16	1.27	0.295
	Treatment*Year	2,16	0.74	0.605
	Treatment*Site	4,16	1.16	0.342

Table S3.3: All collected and identified species of pollinators in watermelon fields from 2018-2020. Yearly columns from both the conventional management (CM) and integrated pest management (IPM) systems were summed in the total column. Species order was based on the total frequency they were observed. All pollinators were identified to the lowest taxonomic level, frequently at a species level except for several *Lasioglossum* species and hover flies (Syrphidae).

Species/morphospecies	2018		2019		2020		Total
	CM	IPM	CM	IPM	CM	IPM	
Apis mellifera	75	146	159	194	163	128	1381
Melissodes bimaculatus	66	156	93	180	48	133	997
Lasioglossum pilosum	4	63	27	73	59	84	469
Augochlora pura	49	232	3	24	4	46	389
Lasioglossum verstatum	12	24	27	52	26	92	338
Bombus impatiens	13	56	19	68	5	40	293
Lasioglossum imitatum	0	58	14	48	11	35	239
Syrphidae *spp.*	19	25	2	23	23	25	165
Lasioglossum brunei	9	31	2	27	18	15	149
Peponapis pruinosa	0	0	0	9	1	56	76
Halictus ligatus	2	1	3	20	2	11	64
Lasioglossum callidum	2	19	0	4	4	18	55
Halictus confusus	0	14	0	9	1	1	35
Lasioglossum luecocomum	1	4	0	6	0	17	34
Augochlorella aurata	0	0	0	8	1	11	29
Lasioglossum sp. 1	0	7	1	5	0	8	27
Halictus rubicundus	0	6	0	6	1	2	22
Lasioglossum oceanicum	0	0	0	5	0	8	18
Agapostemon splendens	1	3	0	3	0	6	16
Lasioglossum illioense	0	3	0	4	0	2	13
Triepeoious remigatus	0	1	1	2	2	1	12
Chaulioganthus pensylvanicus	2	2	0	2	1	1	11
Lasioglossum zephyrum	0	4	0	2	0	3	11
Halictus parallelus	0	1	0	1	1	4	9
Lasioglossum sp. 2	0	1	0	1	1	4	9
Calliopsis andyreniformis	0	3	0	0	0	4	7
Xylocopa virginica	0	1	0	2	0	1	6
Lasioglossum albipenne	0	2	0	0	0	4	6
Difourea marginata	0	2	0	1	0	0	4
Halictus sp.1	0	0	0	2	0	0	4
Lasioglossum sp. 3	0	1	0	0	0	3	4
Megachile brevis	1	1	0	0	1	0	4
Agapostemon virescens	0	1	0	1	0	0	3
Agapostemon sericeus	0	2	0	0	0	0	2

Table S3.3 cont.

Holcopasite calliopsidis	0	1	0	0	0	1	2
Agapostemon texanus	0	0	0	0	0	1	1
Andrena asteris	0	0	0	0	0	1	1
Augochloropsis metalica	0	0	0	0	0	1	1
Ceratina calcarata	0	1	0	0	0	0	1
Hylaeus sp.	0	1	0	0	0	0	1
Nomada tyrrellensis	0	1	0	0	0	0	1

Table S3.4: List of non-neonicotinoid pesticides applied to the watermelon field during the experiment. Quantification procedures were specifically tailored to quantify neonicotinoid residues, resulting in an inability to detect the fungicide active ingredient chlorothalonil, or quantify non-neonicotinoid products. Percent detection of all honey bee wax and bumble bee nest material is reported from both conventional management (CM) and integrated pest management (IPM) systems.

	Percent detection in honey bee wax (n = 30)		**Percent detection in bumble bee nest material** (n = 30)	
Product	**CM**	**IPM**	**CM**	**IPM**
Chlorothalonil	na	na	na	na
Fluopyram	20%	33%	37%	23%
Pyraclostrobin	50%	30%	30%	43%
Difenoconazole	20%	3%	23%	27%
Cyprodinil	0%	0%	0%	0%
Lambda cyhalothrin	23%	0%	5%	0%

CHAPTER 4. UNTANGLING POLLEN CONTENT: CAN IPM ADOPTION IMPROVE THE COLLECTION OF NUTRITIOUS POLLEN AND REDUCE INSECTICIDE CONTAMINATION

Abstract

Landscape simplification and increased pesticide use across agricultural landscapes presents challenges to pollinators and their contribution to crop yield. Pollen is an essential food source for honey bee colony growth and the quality of this pollen, along with its contamination with insecticides, will likely impact colony health and development. To create more sustainable environments an effective first step may be applying integrated pest management (IPM) practices to reduces pesticides and allow for unimpaired foraging. Using paired plots with similar surrounding landscapes we examined the effect of local IPM adoption on the protein content and neonicotinoid contamination of honey bee-collected pollen. These sites were along a gradient of landscape composition and as a result presented another opportunity to test whether IPM adoption can reduce insecticide applications to crop fields and improve the conditions of a simplified cropland-dominated landscape. From 2018-2020 pollen was collected between June and September with higher protein and lower insecticide residues in pollen from IPM colonies compared to those with conventional insecticide inputs. We found that honey bee pollen collected from landscapes with larger amounts of cropland had lower protein content but failed to find any relationship with landscape categories and neonicotinoid exposure. These findings reinforce previous findings that non-crop pollen resources are likely a source of insecticide exposure throughout the season. The chronic exposure to neonicotinoid insecticides via pollen may lead to less efficient foraging by honey bees and less nutritious pollen. IPM practices improved the efficiency of foragers indicated that there are changes to foraging behavior that could improve their ability to provide pollination.

4.1 Introduction

Pollinator health is an issue of worldwide concern for both conservation purposes and the role of pollinators in agricultural systems (Winfree *et al.*, 2011; Cardinale *et al.*, 2012). The most economically important pollinator is the honey bee (*Apis mellifera*), which provides >$15 billion

in value to crop pollination each year in the U.S. alone (Potts *et al.*, 2010). Honey bee colonies have faced lower survival in the last two decades, which has come at a cost to beekeepers that is passed on to growers renting hives (Smart *et al.*, 2018b). Colonies placed in landscapes frequently surrounded by cropland experience decreased nutrient intake (Dolezal *et al.*, 2016; Alaux *et al.*, 2017), honey production (Sande *et al.*, 2009) and experience higher colony mortality than colonies surrounded by higher amounts of natural areas(Otto *et al.*, 2018). Some studies have found that agricultural crops, if foraging resources are available throughout the season, can maintain productive colonies despite the trade-off with increased risk of pesticide exposure (Alburaki *et al.*, 2017; St. Clair *et al.*, 2020). Due to the economic importance of honey bees to agriculture, it is critical that beekeepers can identify the hazardous aspects of placing their colonies within agricultural landscapes and what tactics or practices can be implemented to reduce or mediate these risks to honey bees.

Several co-occurring anthropogenic factors could, individually or collectively, make crop fields a difficult environment for pollinators to persist. Managed and wild pollinators are sensitive to losses of floral diversity and simplified landscapes can lead to biodiversity loss and poor colony health (Winfree *et al.*, 2009; Vanbergen *et al.*, 2013; Goulson *et al.*, 2015). Honey bee colonies require a consistent source of pollen and nectar to supply food to both existing larvae/adults within the colony, and to build up stores if the colonies are to successfully overwinter (Doke *et al.*, 2019). When these colonies are placed in crop fields to provide pollination the non-crop plants are an essential source of forage outside of crop bloom (Requier *et al.*, 2015; Smart *et al.*, 2016). A limited floral diversity in and around crop fields presents nutritional challenges to colonies providing pollination services (Dolezal *et al.*, 2019; Smart *et al.*, 2019). Pollen availability within the landscape is especially important as it constitutes the sole source of protein in pollinator diets (Wright *et al.*, 2018). High-intensity agriculture landscapes (simplified landscapes with frequent insecticide applications) have been linked to a lower protein content in pollen collected by honey bees (Donkersley *et al.*, 2017; Smart *et al.*, 2018b). Multiple studies have found seasonal variation in the protein content (Smart *et al.*, 2018a; Quinlan *et al.*, 2021) and plant source (Wood *et al.*, 2019; Mogren *et al.*, 2020; Zawislak *et al.*, 2021) collected by honey bees. Intensively managed crop fields could lead to a reduction of non-crop floral resources in the colonies foraging range and reduce pollen quality (Pernal and Currie, 2001; Quinlan *et al.*, 2021).

The risk to pollinators in intensive agricultural landscapes extends beyond simplified foraging resources. In the last 20 years, the toxicity load for bees in agricultural environments has increased, driven by the near-universal use of neonicotinoid seed coatings on large acreage crops such as corn and soybean (DiBartolomeis *et al.*, 2019; Douglas *et al.*, 2020). Neonicotinoids have systemic activity that allows expression throughout the plant tissues; however, this characteristic also allows the active ingredient to move outside of the crop field and be detected in non-crop plant tissues (Pecenka and Lundgren, 2015; Botias *et al.*, 2016). In the Midwestern U.S., where row crops are planted on a large portion of the landscape (USDA NASS 2020) and routinely treated with neonicotinoid seed treatments this represents a sizeable risk of exposure to foraging honey bees in this region (Krupke *et al.*, 2017). A further risk to consider is that pollinators are unable to detect neonicotinoids in floral tissues and fail to selectively avoid foraging on resources with high neonicotinoid contamination (Muth *et al.*, 2020). Even if neonicotinoid exposure is not high enough to be lethal there is the possibility that foraging may be impaired as a sublethal effect (Van der Sluijs *et al.*, 2013; Dively *et al.*, 2015). Sublethal chronic exposure of neonicotinoids to foraging bumble bees resulted in suboptimal foraging decision, resulting in less efficient foraging trips, lower quantities of pollen, and foragers more frequently failed to successfully return from foraging trip (Gill and Raine, 2014; Siviter *et al.*, 2021). Sublethal effects can manifest in foraging pollinators in several ways; olfactory learning and communication can be impaired (Muth *et al.*, 2020), ineffective flight (Kenna *et al.*, 2019), and decreased locomotion and motor function synchrony (Henry *et al.*, 2012; Williamson *et al.*, 2014). The ubiquitous use of neonicotinoids and other insecticide products may act synergistically in environments with simplified pollen diets or those that are protein deficient (Tosi *et al.*, 2017a; Barraud *et al.*, 2020; Pecenka *et al.*, 2021). Therefore, it is difficult to determine whether efforts to create more "pollinator friendly" environments at a field-scale can ameliorate the effects of landscape insecticide use.

We conducted a multi-year experiment across agricultural landscapes in Indiana to evaluate how insecticide applications around the hive affect the pollen that honey bees collect throughout the season. We used an experimental design of watermelon (a pollinator dependent crop) surrounded by corn (widespread wind-pollinated row crop) to contrast conventional management (CM) insecticide use in both crops with an integrated pest management (IPM) system where insecticide applications are reduced or eliminated based on scouting. Paired sites across the state varying in surrounding landscape composition allowed us to test environments that provide honey

bees with high-quality forage. This unique experimental design also allowed use to observe the effects of insecticide use on honey bee foraging. We specifically investigate i) whether the protein content of collected pollen is related to the surrounding landscape, ii) whether higher cropland composition within the honey bees' foraging range will lead to increased neonicotinoid concentration in pollen, and iii) whether the implementation of IPM can affect pollen pesticide concentration, protein content, or any interaction of these factors and the landscape beyond our research plots. We hypothesized that landscapes with higher amounts of cropland will contain lower protein content and greater levels of insecticide residues in pollen samples from honey bees. We additionally hypothesize that stress of the pesticide-contaminated pollen from CM fields will limit the ability of honey bee colonies to forage efficiently and limit the seasonal protein content of collected pollen, a stressor less effecting IPM colonies. Collectively, we aim to connect trends in pollen quality and insecticide contamination and find out whether local changes to pest management can improve forager efficiency. The results of this study can help provide insight on whether IPM at a local level can improve the quality of pollen and minimize the exposure risks of insecticides to pollinators.

4.2 Materials and Methods

4.2.1 Experimental Design

This experiment took place from 2017-2020 on five of the Purdue Agricultural Center (PAC) research farms across Indiana, USA. At each site, a pair of fields were randomly assigned to either a conventional management (CM) or integrated pest management (IPM) program. The pair of sites were separated by an average of 5.6 km (range: 4.63-6.63 km) but regardless of distance and location all fields had the same arrangement of a corn field with a smaller field of watermelons embedded within the corn matrix. The size of the corn field varied by site (4.8-7.7 ha) but the watermelon plot was always 0.2 ha and surrounded on all four sides by the corn field with at least 30 m from any corn field edge. The only difference between the two treatments at each site was the insecticide inputs; tillage, fertilizer, fungicides, and all other management practices were consistent based on conventional practices for the region and standardized for each pair of sites each year.

Corn was planted beginning in 2017 while the first watermelon plot started the following year after which both crops were planted through the duration of the experiment (2020). This first corn year was to impose an initial treatment effect of insecticide use in corn that would by typical in commercial fields that rotate the crops in subsequent years. CM corn (var. Spectrum 6334) was coated with the neonicotinoid thiamethoxam (Cruiser 5FS @ 1.25 mg a.i. per seed) along with Maxim Quattro fungicide seed treatment. These were chosen to replicate typical grower practices and are some of the most widely used products by US farmers.

Watermelons in Indiana are nearly always transplanted in the field and seedless (var. 'Fascination') and seeded (SP7) watermelons were transplanted at a 3:1 ratio. The transplanting date varied across sites based on frost-free dates, but both fields at each site pair was always transplanted within 48 hours. Similar to the corn fields, the only difference between the two watermelon treatments is the insecticides; at transplant the CM watermelons received imidacloprid (Wrangler @ 814.09 mml/ha) as a soil drench in addition to foliar sprays with lambda-cyhalothrin (Warrior II @ 140.3 ml/ha) approximately 4, 6, 8, and 10 weeks post-transplant. The insecticide applications used were based on previous surveys of Indiana growers and replicate the products and frequency commonly used (Ternest *et al.*, 2020). IPM watermelons received insecticides only based on weekly scouting that began after transplant using the economic thresholds and recommendations for pests; primarily striped cucumber beetles, but secondary pests such as aphids and spider mites were also monitored throughout the season (Brust and Foster, 1999; Phillips *et al.*, 2021). If striped cucumber beetle densities reached threshold (5 beetles per plant), lambda-cyhalothrin was applied at the same rate as the CM field.

4.2.2 Landscape Quantification

Land-use data from all four experimental years were accessed using the Cropland Data Layer (CDL, USDA National Agricultural Statistics Service) and imported into ArcMAP 10.5.1 (ESRI, Redlands, CA). For each site, we extracted 1-km and 3-km buffers around the center of each watermelon field, approximating a short and long honey bee foraging range (Cariveau et al. 2013, Danner et al. 2014). CDL listed 31 land-use types within the extracted areas, but we excluded any classification that made up < 0.1% of the surrounding landscape, leaving 18 land-use types (Table S1). These categories were again consolidated into the categories of cropland, managed

forage crops, developed, forest, and semi-natural. These categories account for > 98% of the landscapes across all study sites and represent a majority of the regional land use.

4.2.3 Honey Bee Colony Source and Management

Honey bee colonies were sourced from a local apiary (Bastin Honey Bee Farm, Knightstown, IN) for all experimental years. In 2018 and 2019 new 2.7 kg packages with mated queens were used to start colonies, while unfavorable conditions in 2020 forced the use of nucleus hives that were modified with reduced food stores to more closely mimic the condition of the packages used in earlier years. All colonies were transported to the field sites within a three-day period each year (9-11 May in 2018, 2-4 May in 2019, and 19-20 May in 2020) and placed into 8 frame Langstroth hives with plastic foundation frames (#HK-560 Hackensack, MN). Each site received two hives that were placed at opposite corners of the open space between the corn and watermelon field, within the recommended range of honey bee stocking density for watermelons (Ullmann et al. 2017). Colonies were only used during a single experimental year and all bees and frames were replaced each spring to prevent a confounding effect due to conditions experienced across years.

4.2.4 Pollen Collection

At each site, pollen was collected using a Superior Pollen Trap (Bastin Honey Bee Farm, Knightstown, IN, USA) that was installed on one hive at all sites in mid-June to allow bees to acclimate to the modified entrance. These traps can be engaged to force returning foragers to enter through a constricted space that removed pollen from the corbicula to a collection drawer. For each sampling period, the pollen trap entrance was engaged for approx. 48-hours collection period after which the pollen was removed, and the pollen trap disengaged to allow for unobstructed pollen collection by the colony. Each drawer of collected pollen was emptied into a plastic bag and returned to the hive. Each bag of pollen was transferred to an ice-filled cooler until return to the lab and stored in a -20° C freezer. Pollen collection began in late June (22-June to 1-July) each year and continued biweekly to early September (31-Aug to 9-Sept) for 6 samples from each field from 2018-2020 (n = 180).

4.2.5 Pollen Nutrition Analysis

To quantify nutritional quality of pollen samples we used the crude protein content as a proxy following the Bradford protocol (Bradford, 1976; Mogren and Lundgren, 2016; Vaudo *et al.*, 2020). Crude protein is defined as proxy for quality because even high-protein pollens will be nutritionally inadequate for colony health without high amounts of essential amino acids (McCaughey *et al.* 1980). These essential amino acids are in highest demand during periods of reproductive growth within the colony to feed developing larvae and young nurse adults (Crailsheim 1990, Paoli *et al.* 2014). Briefly, a 3g portion of each pollen sample was filtered to remove any non-pollen debris from the sample and ground using a mortar and pestle. Using a microbalance, a 1 mg sample was placed in a 2 mL centrifuge tube. Each tube was combined with 1.5 mL NaOH, vortexed for 10 minutes, and set overnight. Bradford reagent and protein standards (Bio-rad assay kit IV, product # 500-0204) were removed from the refrigerator several hours prior to following steps to allow all components to reach room temperature. After an acclimation period, 100 µL of NaOH and 50 µL from each sample were added to each plate sample well followed by 150 µL of Bradford reagent that was mixed using the pipette. Protein standards were 0, 25, 50, 100, 250, 500 µg and plated in triplicate to create the standard curves. Plates were read on spectrophotometer (Eon #130131E, BioTek Instruments Inc, Winooski VT) at 595 nm and quantified using standard curves to calculate a µg/mg protein value.

4.2.6 Pesticide Residue Analysis

An additional 0.5 g from the filtered portion of each pollen sample described earlier was placed in a 50 mL tube for pesticide residue analysis following modified QuEChERs protocol (Long and Krupke, 2016; Ingwell *et al.*, 2021). Each sample was vortexed with a mixture of an extraction solution (15 ml dH_2O + 15 ml acetonitrile) and 10 µl of internal standard solution (clothianidin-d3, imidacloprid-d4, thiamethoxam-d3, and acetamiprid-d3 at a 10 ng/µl concentration). Samples were combined with 6 g of magnesium sulfate and 1.5 g of sodium acetate, inverted, vortexed, and centrifuged at 2500 r.p.m. for 10 minutes, after which 10 mL of the top layer of supernatant was transferred to a QuEChERS Dispersive Kit (Agilent Technologies, Santa Clara, CA, #5982-5456) and again inverted, vortexed, and centrifuged at 4000 r.p.m. for 5 minutes. Supernatant (6 ml) was transferred to a clean 15 ml tube and dried completely in a speed vacuum

(Savant SC250EXP, Thermo Scientific, Waltham, MA). All samples were resuspended in 200 µl acetonitrile, vortexed, centrifuged, and the supernatant was transferred to 96-well plates. Immediately prior to analysis, samples were re-suspended with 200 µl 50% acetonitrile:dH_2O solution.

All fungicides and insecticides used during the experiment were screened using liquid chromatography and tandem mass spectrometry at Bindley BioScience Center at Purdue University, West Lafayette, IN. An Agilent Zorbax SB-Phenyl 2.1×100, 3.5 µm column was used for LC separation and an Agilent 1200 Rapid Resolution LC system coupled to an Agilent 6460 series triple quadrupole mass spectrometer was used to identify pesticide residues based on retention time and co-chromatography with analytical standards of all pesticide targets. Deuterated neonicotinoids were used to quantify the concentration of neonicotinoids in samples based on the relative response value. A mix of analytical standards from all other pesticides used in the experiment were subjected to a serial dilution and analyzed on the instrument to create standard curves to quantify their concentration in each sample. This protocol prioritized the detection and quantification of neonicotinoids, this led to confidence in the detection of these products but residue amounts were variable when compared to the standard curves conducted for pesticide quantification. This variations resulted in a high limit of quantification (LOQ) values for the non-neonicotinoid pesticides and led to the decision to present all non-neonicotinoid pesticides only as a presence/absence for each sample.

4.2.7 Pollen Weight and Color Analysis

As an additional method of pollen evaluation, in 2019 and 2020 we collected individual pollen loads from foraging bees returning to the hive. This was achieved by restricting the entrance to the hive and capturing returning foragers (identified by visible pollen loads on their corbicula) with soft forceps and removing the pollen by gently brushing the pollen against the edge of a 1.5 ml centrifuge tube to collect the entire pollen load from both legs. This was repeated for at least 10 foragers at each field during one collection period in July, August, and September. Each collection under favorable foraging conditions; during morning hours (9:00 – 13:00) on day with wind < 16 km/h, little cloud cover, and temperatures > 18 °C. All tubes from a site were placed in a plastic bag together and immediately placed on ice until returning to the lab and were stored in a -20° C freezer. For analysis, all pollen loads were weighed using a microscale (0.01 mg resolution)

and photographed on top of a white background with consistent lighting from above. Each picture was opened in the application Digital Color Meter (Apple Inc.) and a large section of the pollen (without including any of the white background) was converted to a hexadecimal RGB triplet and converted to a Pantone TPX (https://connect.pantone.com/) name and number. This is a universal color standard commonly used in graphic design but allows for a standard value for each unique color. In the event of several options provided, the closest Pantone equivalent was selected. In all individual pollen collections, the two pollen loads collected from each bee were the same in all but one instance. Using image software reduces potential bias from visual comparisons of pollen color categories to a Pantone color (Stoner *et al.*, 2019). Pollen load measurements give context to individual forager efficiency and pollen collection, while the color analysis was completed to see whether it could serve as a proxy to quantify the diversity of the pollen resources available in the landscape. Previous analysis of individual pollen pellets found that nearly all pellets were composed of >92% of a single pollen source, making this proxy an possible alternative method for quantifying foraging diversity for beekeepers without the funding or skills to identify pollen grains (Stoner *et al.*, 2019).

4.2.8 Statistical Analysis

The landscape categories were compared between treatments using two-sample T-tests to confirm that the landscape composition was similar for each site pair. Imidacloprid, clothianidin, and thiamethoxam were transformed (ln(x+1)) to improve normality and analyzed using generalized linear mixed models. These models used treatment (n = 2), sample date (n = 6), and their interaction as fixed effects with year as a random effect. The relationship between landscape categories surrounding the colonies and pollen nutrition/insecticide residues was explored with a mixed regression analysis with each of the landscape categories and treatment as the fixed effects. These tests contrasted all landscape categories mixed with treatment against pollen protein content and the concentration of the three neonicotinoids. The foliar insecticides and fungicides applied to the experimental watermelon fields likely contributed to environmental hazard in the honey bees' foraging radius, however a previous assessment of the environmental toxicity across agricultural landscapes overwhelmingly associated high risk areas in regions where neonicotinoid seed treatments are used (Douglas *et al.*, 2020). To avoid pseudoreplication, each point in the regression analysis was an averaged value across a single year/site/treatment (n = 30) for each regression.

While this condensation of samples removes any variation seen across the season, each sample making up the regression are made up of the same number of collection times. To explore the relationship of pollen toxicity and nutrition all pollen these values for all samples (n = 180) run in a regression comparing protein content to a pollen toxicity score (Σ (neonicotinoid concentration * (1/oral LD50)) to incorporate both concentration and relative toxicity of each neonicotinoid active ingredient). A final analysis was conducted on the weight and richness of Pantone colors collected from individual honey bee foragers. Generalized linear mixed models used month collected (n = 4), treatment (n = 2), and site (n = 5) as fixed effects with year as a random effect. All statistics were performed using SYSTAT 13 (SYSTAT Software Inc).

4.3 Results

4.3.1 Landscape composition varied across sites but was similar between each site pair

There was a difference across the 5 sites in all the landscape categories surrounding colonies (Table 4.1). Landscape composition was similar at both 1 km and 3 km radii, so all analysis and figures were conducted only on the 3 km data. This distance was chosen because it is a large enough radius to include a majority of the range of honey bee foraging trips (Danner *et al.* 2014). The landscape categories were not different between CM and IPM sites for any of the categories (Table 4.1B, Table S4.2). Landscape surrounding hives was predominantly cropland (63.8%) with corn (26.3%) and soybeans (33.7%) comprising nearly this entire value. While there was variation in the amount of cropland surrounding the colonies (37.3-84.5%), only one pair of sites had < 50% of the surrounding 3 km radius composed of cropland. Woodland and semi-natural areas were the next largest landscape components, comprising 20.5% and 7.3% respectively. Semi-natural area showed relatively small variation across sites (4.8-9.2%), but woodland was highly variable, ranging from 4.1% to 48.8%. The remainder of the habitat was comprised of alfalfa/hay (1.0%) and developed area (6.9%).

4.3.2 Pollen collected by honey bee colonies in IPM fields had higher seasonal protein and lower neonicotinoid residues than CM colonies

Average protein content from pollen was higher ($p < 0.001$) in IPM colonies (86.38 ± 6.29 µg/mg) compared to CM colonies (68.39 ± 4.99 µg/mg; Figure 4.1). Across the sampling periods,

the lowest average protein content was during the first sampling period (late-June) at 63.26 ± 4.02 µg/mg and peaked in the late August collection 82.50 ± 6.54 µg/mg. However, there was no difference in pollen protein content across the season ($p = 0.063$).

All pollen samples collected from CM colonies contained detectable levels of at least 1 neonicotinoid compared to only 49% of IPM samples (Table 4.2). Imidacloprid concentrations in pollen were significantly higher ($p < 0.001$) in CM colonies (4.76 ± 0.69 ng/g) than in IPM colonies (0.13 ± 0.05 ng/g). Imidacloprid residues were also different across the sampling period ($p = 0.036$) and had a significant interaction effect between sampling period and treatment ($p = 0.030$) (Figure 4.2A). The final sample period (early September) was significantly lower than the first 4 sample periods, while the late August sample had similar levels of imidacloprid to all other sample periods. There was similarly a difference between treatments in both clothianidin ($p < 0.001$) and thiamethoxam ($p < 0.001$), but neither product changed across sampling period ($p = 0.261$ and $p = 0.942$ for clothianidin and thiamethoxam, respectively). Clothianidin concentrations were far higher than any of the other neonicotinoids in both CM (20.79 ± 2.82 ng/g) and IPM hives (4.59 ± 1.09 ng/g).

4.3.3 Landscape composition influenced protein content, but infrequently affected neonicotinoid concentration in pollen

There was a significant negative effect of cropland on the protein content ($p < 0.001$, $R^2 = 0.282$), while there was a significant positive relationship with woodland ($p < 0.001$, $R^2 = 0.268$) 4.3A-B). None of the other landscape categories had any relationship with protein content (Table S4.2). Protein content was positively associated with woodland area of CM colonies ($p = 0.006$, $R^2 = 0.456$) while IPM colonies had no relationship ($p = 0.076$, $R^2 = 0.251$) (Figure 4.3B). While pest management system effected the seasonal average imidacloprid concentration, only semi-natural area was correlated factor ($p = 0.018$) while all other landscape categories had no significance to imidacloprid (Figure 4.4A). Similarly, no landscape categories had any relationship to clothianidin or thiamethoxam, instead pest management treatment was the only factor significantly related to these neonicotinoid concentrations in pollen (Figure 4B-C). There was no relationship with any of the landscape categories in the regression model (Table S4.2). Regression of pollen protein content and pollen toxicity was significant ($p < 0.001$) and pollen with higher toxicity tended to have lower protein content ($R^2 = 0.08$)(Figure 4.5).

4.3.4 Pollen load weight was higher in IPM system, but pollen color was unaffected by any of the variables.

In total, there were 1,168individual pollen loads collected from returning foragers. Color analysis found 133 unique pantone colors collected; however, after removing those that were only infrequently collected (< 1%) there were 26 different colored pollens. Amber Green (n = 105), Golden Brown (n = 56), Ecru Olive (n = 52), and Green Sulphur were the colors collected most often. The color richness (after adjusting for sample size) was not different ($p = 0.885$) between treatments with 6.78 ± 0.36 and 6.47 ± 0.42 colors from CM and IPM colonies, respectively. Similarly, there was no difference in color richness across month sampled ($p = 0.667$) or site ($p = 0.099$). There was a difference ($p = 0.005$) in weight: 10.34 ± 0.29 mg for pollen collected from CM hives compared to 11.84 ± 0.25 mg in IPM hives. Pollen weight was unaffected by month sampled ($p = 0.246$) or site ($p = 0.192$).

4.4 Discussion

Overall, our results demonstrate that there are complexities to connecting landscape context to pollen nutrition and dietary neonicotinoid exposure. When colonies are placed in crops to provide pollination (such as watermelon) there are clear connections to both visitation (Chapter II) and honey bee health (Chapter III) and pesticide inputs applied to the crop. Examining the pollen informs us on the landscape outside our experimental field and whether these smaller-scale changes can lead to changes in foraging behavior. Despite only making up a relatively small portion of the possible foraging range of honey bees, the CM fields with neonicotinoid applications in both corn and watermelon crops had higher levels of these products across the season in collected pollen. With relatively similar surrounding landscapes (Table 4.1) the pollen collected by honey bees was higher in protein throughout the season in IPM colonies (Figure 4.1). Pollen provides nearly all the protein available to a colony, and successful larval development is directly connected to whether sufficient high-quality pollen can support colony growth (Wright *et al.*, 2018). While seasonality did not have a statistical difference on pollen protein ($P = 0.63$) this trend is likely biologically meaningful, demonstrating relative highs and lows to the quality of pollen available. This is supported by previous experiments finding seasonal differences in pollen quality collected by pollinators (Donkersley *et al.*, 2017; Quinlan *et al.*, 2021). The largest driver on pollen nutrition throughout the season is likely the changes in what plants are flowering and attractive to

foraging honey bees. Seasonal collections of pollen in agroecosystems that have identified plant source of honey bee-collected pollen varied greatly depending on when in the season foraging occurred. (Wood *et al.*, 2019; Zawislak *et al.*, 2021).

Pollen protein was negatively associated with cropland surrounding the colony and conversely had a positive relationship to high woodland area (Figure 4.3). Beekeepers who elect to bring their colonies to the U.S. Northern Great Plains to raise productive colonies have chosen to avoid areas with high amounts of cropland due to poor performance (Otto *et al.*, 2016). These historically underperforming colonies could be experiencing a lack of quality pollen in simplified landscapes. In this experimental design all the colonies' immediate surroundings (watermelon within 6 ha corn field) were the same, but the amount of cropland that composed their foraging range varied from 33.3-88.3% of the landscape (Table 4.1B). More natural landscape types i.e., woodlands provide access to more sources of high-protein pollens and colonies. Even in a diversified farming system there may be insufficient resources to sustain the colony overwinter (Brodschneider and Crailsheim, 2010; St. Clair *et al.*, 2020).Pollen weight collected from +1,000 individual foragers demonstrated that IPM adoption, even when limited to the field the colony resides in, can impact foraging behavior and efficiency. The Pantone system was able to successfully categorize pollen colors, however no seasonal or treatment trends could be seen in the richness of pollen color. This data was likely influenced by the large number of colors rarely found (58% of Pantone colors were found in < 5 instances) that are likely different enough in appearance but from the same plant source, a trend seen in a previous study using Pantone colors to sort pollen (Stoner *et al.*, 2019).

While there was a difference in seasonal neonicotinoid concentration between our treatments, we failed to find any consistent relationships with any of the tested active ingredients and landscape categories or seasonality during this experiment. One exception was a slight decrease in imidacloprid detection in pollen in the final sample (Figure 4.2A). Imidacloprid applied to CM watermelon fields was likely one of its few sources within the foraging radius and the decrease across the season based on the product decay after the single application in later May-early June in the experimental fields. Thiamethoxam and clothianidin are the most commonly used compounds for seed treatments on row crop (Jeschke *et al.*, 2011; Douglas and Tooker, 2015) and landscape analysis finds that both corn and soybeans represent a sizeable portion of the landscape and therefore a large potential source of neonicotinoids and a reason why there were consistently

detected (Figure 4.2B-C). Corn sheds pollen for only a brief period of time (5-8 days) meaning exposure of the active ingredients from NST directly from that crop would be detected in pollen for a similarly short window. The frequent detection of thiamethoxam and clothianidin are therefore likely from a non-crop source that have taken up neonicotinoids and expressed them in the flower tissues. These findings support previous evidence that only a fraction of the applied NST is taken in by the crop (Alford and Krupke, 2017) and resides in the soil (Schaafsma *et al.*, 2016; Anderson and Harmon-Threatt, 2019; Bloom *et al.*, 2021). The residues in the soil can then be taken up by non-crop plants within and around the agricultural fields (Pecenka and Lundgren, 2015; Botias *et al.*, 2016; Long and Krupke, 2016). These findings range from sublethal to honey bees to acutely toxic, however it is important to note that mechanism of neonicotinoid toxicity is dependent on consumption and to be hazardous (in any sense), it will need to be consumed at high enough quantities for any relevant effects to occur. When honey bee foragers take advantage of ephemeral floral resources like weeds on the margins of agricultural fields and collectively can form a significant portion of the colonies diet (Wood *et al.*, 2019; Mogren *et al.*, 2020). These herbaceous weeds could be a source of neonicotinoid exposure, but if these plants bloom for only a brief period they could possibly be missed in sampling period which represents only several "snapshots" of pollen collection during their time in the field. This limitation is not unique to our experiment; other studies have comparable collection periods due to the intensive collection efforts and extensive analytical techniques necessary (Quinlan *et al.*, 2021; Zawislak *et al.*, 2021).

Despite the inability of our findings to correlate trends in landscape composition and pollen, foraging behavior we observed is similar to previous findings. An experiment using anon-honey bee commercial pollinator (*Tetragonula carbonaria*) found that landscape context was unable to predict the source of collected pollen, instead they foraged on "many small" resources that were made up of patches of floral resources that are not reflected in the larger landscape (Wilson *et al.*, 2021). Weedy non-native plants were similarly found to be a major portion of honey bee diets in a landscape dominated by agriculture (Mogren *et al.*, 2020). Bees perform best on diverse plant diets (Keller *et al.*, 2005; Wright *et al.*, 2018) so the tendency to diverse resources is likely reinforced by the improved colony health. A diet comprised of diverse pollen has also been found to improve tolerance to both pathogens (Di Pasquale *et al.*, 2013; Dolezal *et al.*, 2019) and pesticides (Schmehl *et al.*, 2014; Tosi *et al.*, 2017b). Even IPM colonies collected pollen with neonicotinoid residues (Table 4.2), but the higher protein content of the IPM colony pollen could

help mediate the negative effects of chronic neonicotinoid exposure. Pollen samples with higher neonicotinoid concentrations from this experiment tended to have lower protein content (Figure 4.5) which reflects the effect pesticide exposure may be having on foraging behavior. Chronic exposure to insecticides leads to decreased learning and memory in foraging honey bees which result in decreased homing success and survival (van der Sluijs *et al.*, 2013, Fischer *et al.*, 2014, Tison *et al.*, 2017). A high enough loss of foragers within a colony will cause early-recruitment of younger members of the colony to switch to a foraging role, referred to as precocious foragers (Perry *et al.*, 2015). Precocious foragers complete fewer successful foraging flights, take longer during each flight, and are more likely to fail to return to the hive (Perry *et al.*, 2015, Colin *et al.*, 2019). This previously explored phenomenon could be the driver of the trends in pollen quality and contamination in this experiment; precocious foragers could be more frequent in the CM colonies and their less efficient navigation and flight may explain the lower-protein pollen routinely collected by these hives.

It is essential to note that while neonicotinoids represent a large source of insecticide exposure to foraging honey bees, there are co-occurring insecticide stressors both within and outside the experimental fields. Pesticide quantification focused on neonicotinoid residues found in pollen, however the pyrethroid applications to experimental watermelon fields (60 total applications in CM fields and 4 in IPM fields) are an additional hazard that leads to losses to honey bee colonies (Chapter III). Active ingredients from pyrethroid insecticides have previously been found in honey bee-collected pollen (Long and Krupke 2016), and it is likely that residues would accumulate in colonies adjacent to fields where these products are used. There is similarly a possible synergy between the insecticides and fungicides used in all experimental fields, but only presence/absence of the fungicide and pyrethroid active ingredients were reported. It is also possible that pesticides containing active ingredients that were not screened for may have been applied to cropland outside of the manipulated fields, but within the foraging radius of honey bees. Despite these limitations, the ubiquitous use of neonicotinoids in conventional cropping systems represent a significant risk to foraging pollinators and it is important to identify how reducing the use of these products can benefit honey bees and their ecosystem services.

The adoption of IPM has been limited due to a number of factors (Castle *et al.*, 2009; Deguine *et al.*, 2021), but this experiment provides evidence that even local changes can improve conditions for foraging pollinators. Reducing the insecticide inputs not only decrease the toxicity

of food stores within the colony but also appears to boost honey bee foragers' ability to efficiently gather high-quality resources. Pollen quality can be influenced multiple factors, but these findings appear to add to the mounting evidence that insecticide use can impair adequate collection of resources that could lead to decreases in health or even complete hive failure. With beekeepers continue to experience unsustainable annual hive losses it is important to continue to strengthen the understanding on what constitutes favorable environments for honey bees (Smart *et al.*, 2016; Alaux *et al.*, 2017). Expanding the existing study with efforts to monitor amino acid composition, micronutrients and fatty acids could increase our understanding about what how nutrition, landscape, and insecticide exposure interact and impact honey bee health and pollination services.

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Table 4.1: Summary of the landscape surrounding all hives within a 1 km (A) and 3 km (B) radius from 2018-2020. All land-use types that make up less than 0.1% of the total area were omitted and the remaining 18 land-use types were consolidated into the listed categories.

A.

1 km	CM		IPM	
Landscape category	Average (%)	Range (%)	Average (%)	Range
Total cropland	60.1	34.8-82.2	61.3	23.1-93.6
Corn	24.5	5.3-52.7	32.5	2.7-42.3
Soybean	31.1	8.1-47.2	34.5	6.2-54.0
Wheat	4.6	0.0-14.4	5.3	0-17.1
Watermelons	0.2	0.0-2.3	0.0	0.0
Hay/Alfalfa	2.0	0.0-7.8	1.2	0.0-3.9
Developed	7.8	3.1-19.4	5.9	0.9-13.8
Woodland	21.0	3.3-55.0	24.6	0.2-72.6
Semi-natural	8.5	3.2-14.2	6.7	0.3-23.1

B.

3 km	CM		IPM	
Landscape category	Average (%)	Range (%)	Average (%)	Range
Total cropland	64.6	39.7-88.3	62.9	33.3-82.1
Corn	29.3	6.2-46.2	23.4	5.8-38.7
Soybean	32.8	24.7-42.2	34.6	17.4-45.0
Wheat	2.2	0.0-4.2	4.6	0.0-10.5
Watermelons	0.4	0.0-2.7	0.3	0.0-1.6
Hay/Alfalfa	1.0	0.3-1.8	1.1	0.2-2.4
Developed	7.1	3.2-10.3	6.7	3.1-10.9
Woodland	19.9	3.0-42.9	21.1	4.9-55.3
Semi-natural	7.0	3.7-13.2	7.7	3.1-17.8

Table 4.2: Neonicotinoid insecticides were frequently detected in honey bee-collected pollen from hives within both a conventional management (CM) and integrated pest management (IPM) system. Concentrations below limit of detection (0.0275, 0.0235, and 0.0056 ng/g for clothianidin, imidacloprid, and thiamethoxam, respectively) were used for the minimum value on all ranges.

Year	Conventional			IPM		
	Percent detection (30)	Median (ng/g)	Range (ng/g)	Percent detection (30)	Median (ng/g)	Range (ng/g)
			Imidacloprid			
2018	86.7%	2.70	< LOD-38.45	13.3%	< LOD	< LOD-1.78
2019	76.7%	2.54	< LOD-24.42	6.7%	< LOD	< LOD-3.06
2020	80%	2.75	< LOD- 27.05	6.7%	< LOD	<LOD-1.73
			Clothianidin			
2018	86.7%	13.69	< LOD-88.56	23.3%	< LOD	< LOD-33.73
2019	90%	12.14	< LOD-99.37	43.3%	< LOD	< LOD-67.75
2020	76.7%	8.21	< LOD-135.26	36.7%	< LOD	< LOD-37.65
			Thiamethoxam			
2018	50%	0.05	< LOD-3.07	13.3%	< LOD	< LOD-0.93
2019	43.3%	< LOD	< LOD-3.62	16.7%	< LOD	< LOD-0.96
2020	63.3%	0.39	< LOD-4.16	23.3%	< LOD	< LOD-1.58

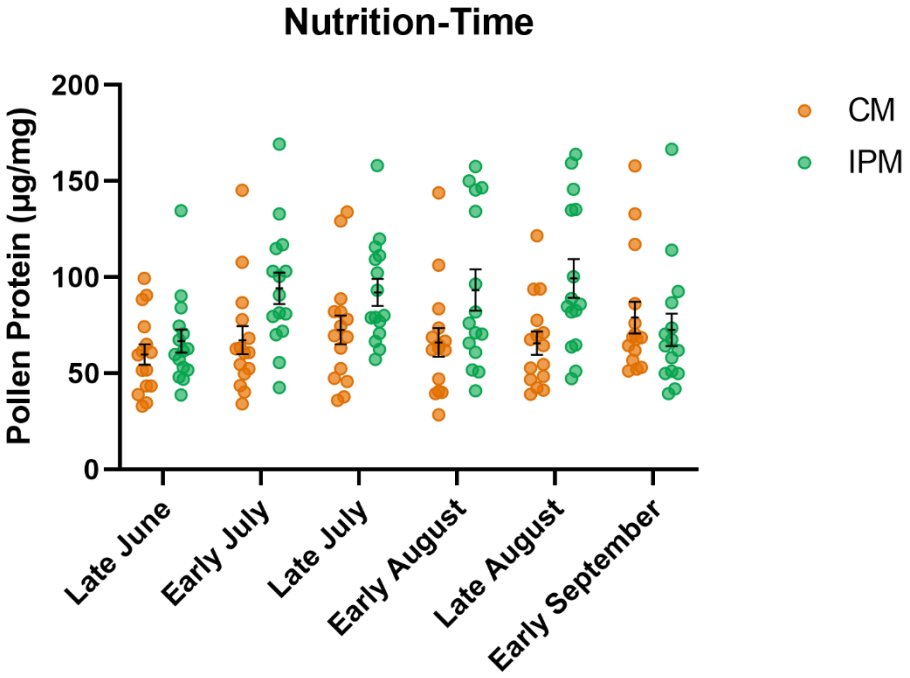

Figure 4.1: The protein content of honey bee collected pollen during the collection period from 2018-2020. Each treatment/time cluster of points (n = 15) is made up of 5 different sites sampled in each of the 3 years at approx. the same time during the year. Black whiskers within the plot show the average ± SEM of all points within each cluster.

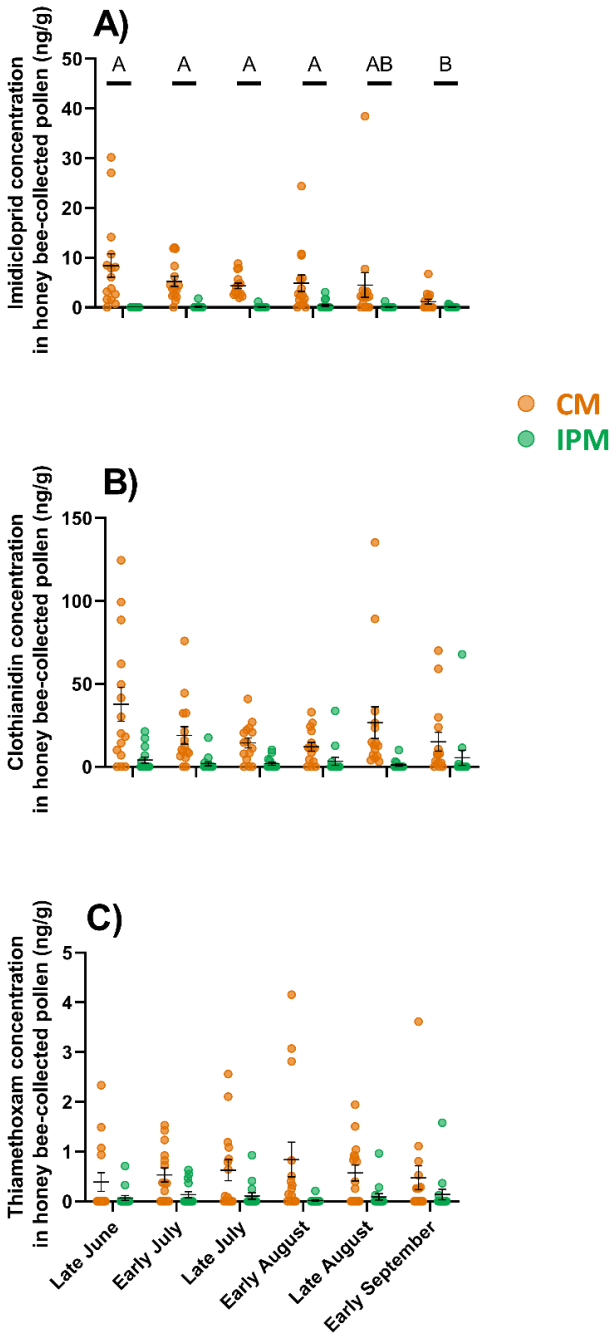

Figure 4.2: The area devoted to capped brood was higher in honey bee colonies within IPM fields. Points in the violin plots were the average area of capped brood from the front and back of 4 frames within each hive body. Each point is the mean value of total brood area from two colonies at each site (n = 5) from 2018-2020 from July, August, and September, and only 2019-2020 in June. Solid lines within violin plot represent 50th percentile (median), with lower and upper dashed lines indicating 25th and 75th percentiles, respectively.

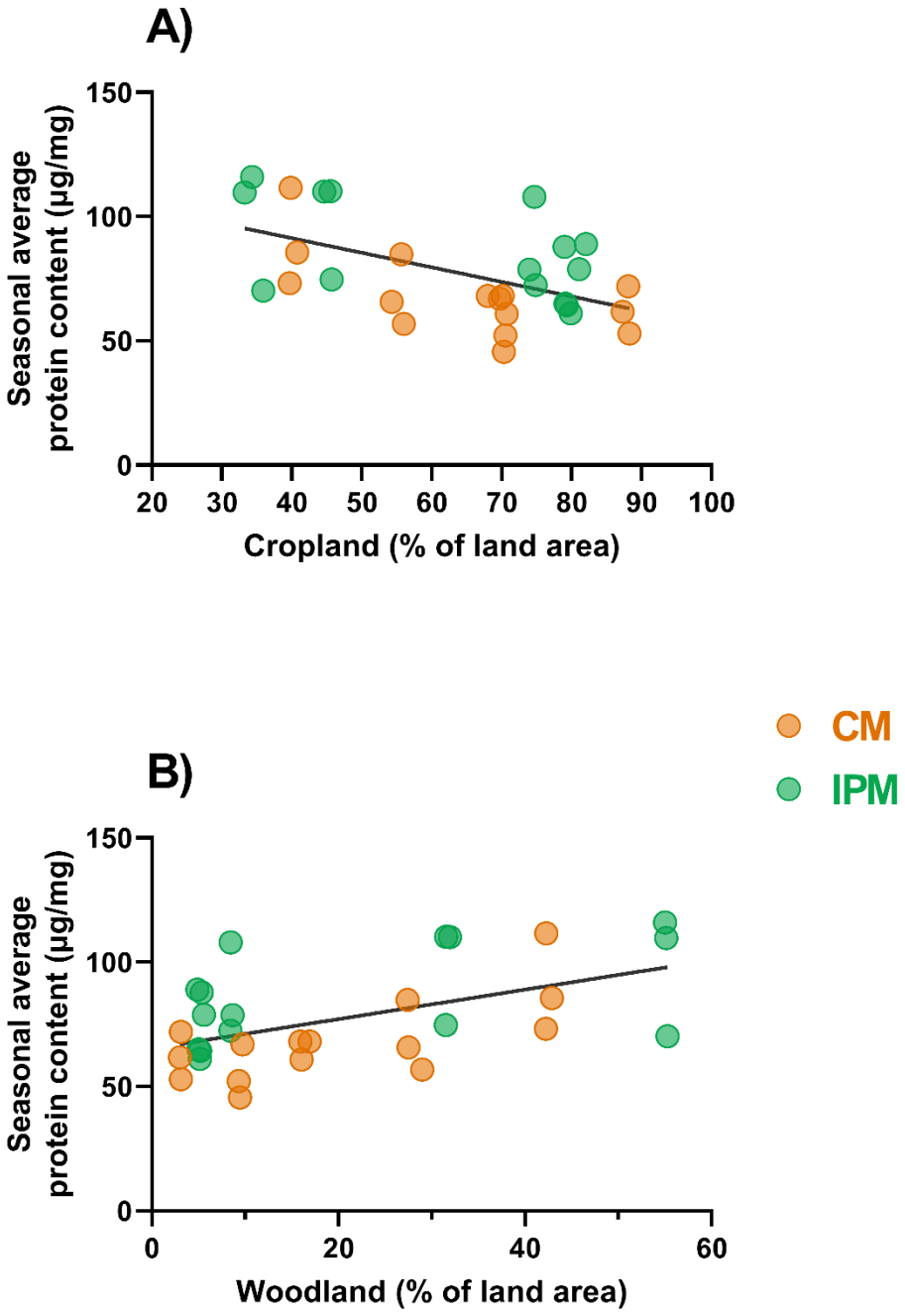

Figure 4.3: Relationship between the percent of cropland and woodland within 3 km of colonies and the average protein content in collected pollen throughout the season. Each point is the average across the season (n = 6 sampling periods) from all experimental fields from 2018-2020. Showing a regression line indicates a significant trend for that set of data ($p < 0.05$).

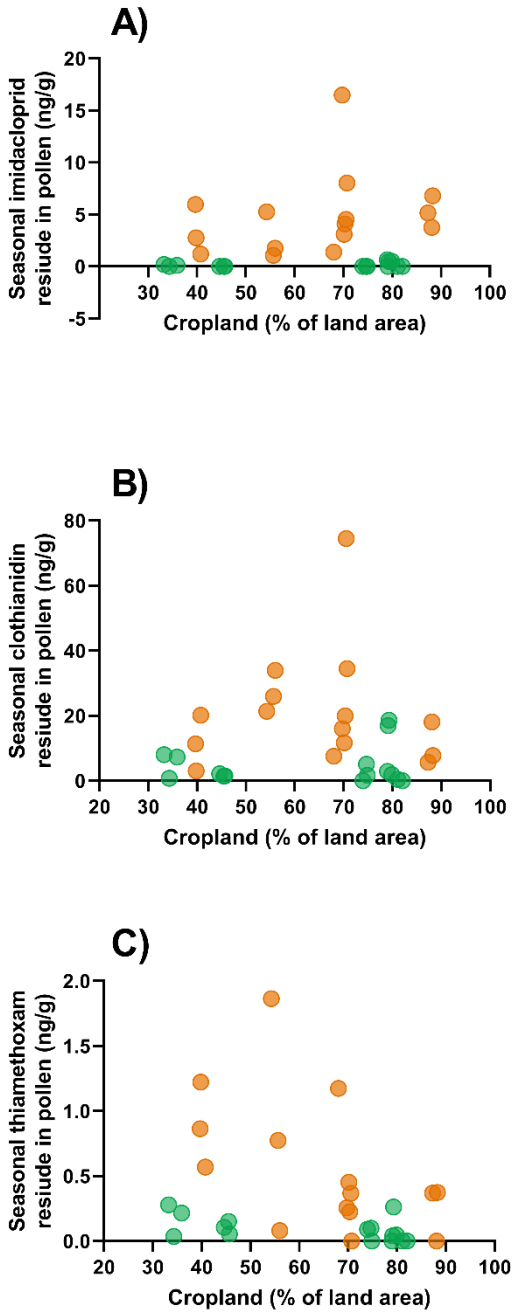

Figure 4.4: Relationship between cropland with 3 km of samples honey bee hives and the concentration of neonicotinoids in honeybee-collected pollen. Each point is average of the season's neonicotinoid concentration (n = 6) at each field from 2018-2020.

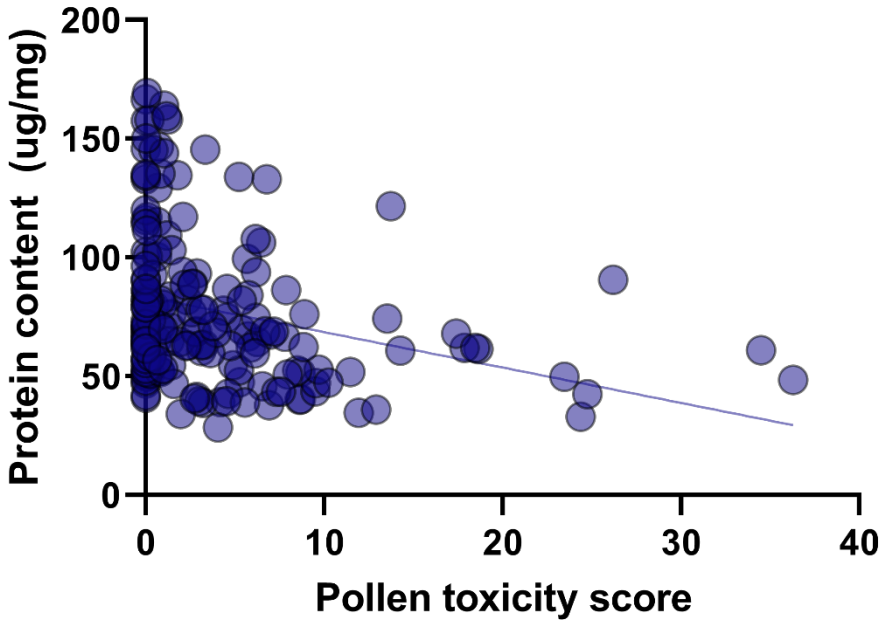

Figure 4.5: Regression of toxicity and protein content of all pollen samples collected from 2018-2020 (n = 180). Pollen toxicity was calculated by (Σ (neonicotinoid concentration * (1/oral LD50)) to incorporate the concentration and relative honey bee toxicity of all neonicotinoid insecticides used during this experiment. Tread line shows the linear relationship of all points ($R^2 = 0.080$).

4.6 Supplemental Information

SUPPLEMENTAL TABLES

Table S4.1. Land classification categories based on CDL data taken from USDA NASS. Consolidated categories were used to pool together similar land cover types.

Landscape Classification	Consolidated Category	Average percent 1 km	Average percent 3 km
Corn	Cropland	23.7	25.3
Soybeans	Cropland	31.5	33.6
Winter wheat	Cropland	5.2	3.6
Watermelon	Cropland	0.1	0.3
Alfalfa	Alfalfa/hays	0.8	0.7
Other hay	Alfalfa/hays	0.4	0.3
Open space	Developed	4.6	4.8
Developed low intensity	Developed	1.4	1.5
Developed med intensity	Developed	0.2	0.4
Developed high intensity	Developed	0.1	0.1
Deciduous forest	Forest	23.4	20.5
Evergreen forest	Forest	0.1	0.2
Woody wetlands	Forest	0.2	0.2
Shrubland	Semi-natural	0.3	0.2
Grassland or pastures	Semi-natural	7.2	6.0
Herbaceous wetlands	Semi-natural	0.1	0.1
Open water	Semi-natural	0.7	1.2

Table S4.2. A. Two-sample T-tests comparing the landscape categories between the field under conventional management (CM) and integrated pest management (IPM) system.

Landscape Category 1 km	df	t	*P*
Cropland	28	-0.15	0.887
Alfalfa/hay	28	1.11	0.276
Developed	28	1.00	0.326
Woodland	28	-0.41	0.683
Semi-natural	28	1.79	0.084

Landscape Category 3 km	df	t	*P*
Cropland	28	0.26	0.797
Alfalfa/hay	28	-0.495	0.624
Developed	28	0.442	0.662
Woodland	28	-0.20	0.845
Semi-natural	28	0.50	0.620

B. General linear mixed models of sampling time (1-6) and treatment treated as fixed effects, year and site were a random effects.

Response Variable	Explanatory Variable(s)	df	F	*P*
Protein Analysis	Treatment	1,22	20.02	< **0.001**
	Sample Date	5,22	2.49	0.063
	Treatment*Sample Date	5,22	2.46	0.061
Imidacloprid	Treatment	1,22	157.88	< **0.001**
	Sample Date	5,22	2.92	**0.036**
	Treatment*Sample Date	5,22	3.06	**0.030**
Clothianidin	Treatment	1,22	52.59	< **0.001**
	Sample Date	5,22	1.40	0.261
	Treatment*Sample Date	5,22	1.15	0.364
Thiamethoxam	Treatment	1,22	34.77	< **0.001**
	Sample Date	5,22	0.24	0.942
	Treatment*Sample Date	5,22	0.54	0.742

Response Variable	Landscape Category	*P*
Protein Content	Treatment	< **0.001**
	Cropland	< **0.001**
	Alfalfa/hay	0.638
	Developed	0.181
	Woodland	< **0.001**
	Semi-natural	0.954
Imidacloprid	Treatment	< **0.001**
	Cropland	0.669
	Alfalfa/hay	0.416
	Developed	0.147
	Woodland	0.953
	Semi-natural	**0.018**
Clothianidin	Treatment	< **0.001**
	Cropland	0.431
	Alfalfa/hay	0.144
	Developed	0.874
	Woodland	0.711
	Semi-natural	0.247

Thiamethoxam	Treatment	< **0.001**
	Cropland	0.432
	Alfalfa/hay	0.246
	Developed	0.515
	Woodland	0.362
	Semi-natural	0.115

C. Generalized linear mixed models with month, site, and treatment as fixed effects, year is a random effect.

Response Variable	Explanatory Variable(s)	df	F	*P*
Pollen Color Richness	Treatment	1,60	0.66	0.419
	Sample Date	3,60	0.52	0.667
	Site	4,60	2.05	0.099
Pollen Weight	Treatment	1,60	8.67	**0.005**
	Sample Date	3,60	1.42	0.246
	Site	4,69	1.58	0.192

CHAPTER 5. CONCLUSION AND FUTURE WORKS

Integrated pest management (IPM) provides growers with a paradigm to successfully management pests while protecting their crop yield and ensuring profitability of their operation. Management practices to a field influence all the organisms in the field (either directly or indirectly) and when a practice is implemented with considerations for only the targeted pest, there can be deleterious effects to other species. To ensure and IPM system is effective and provide service to growers the complex of pests threatening the crop must be considered in context with any beneficial insects present in the environment. Commonly the beneficial community is considered natural enemy species that control pest populations, but when a crop is dependent on insect pollination there is an additional factor for growers to consider; one tied directly to yield.

The concepts and principles of IPM explored in this dissertation such as pest scouting and economic thresholds are neither new nor novel practices. Instead, these are long-established techniques that have been increasingly removed from conventional agriculture. The desire to provide real-world evidence to support IPM motivated the work within this dissertation; a proof-of-concept that IPM can benefit growers. Economic thresholds and crop injury levels should continue to be explored for different agriculture systems and even modified for crops where pollination is necessary and vulnerable to insecticides. As new insecticide products are developed and become used in conventional insecticide systems it is important to understand their effect on beneficial insects.

Pollinating insects fill an essential role in agriculture; yield of many economically important crops is improved by, or completely dependent on insect pollinators visiting flowers and transferring crop pollen. This service can be compromised if pest management practices create an environment that is too hazardous to persist. such as insecticides. Watermelon fields are one of the crops that is entirely reliant on frequent insect visitors with fertile pollen to sufficiently achieve complete pollination. Watermelon growers are pressured to also protect their crop from insects, their associated pathogens, in ways that extend to maintaining cosmetic appeal of fruit to maintain marketability.

Chapter II demonstrated that pest populations rarely reach economically damaging levels in watermelons; weekly scouting was sufficient at monitoring pests and allowed for a timely insecticide application if necessary. Replacing prophylactic neonicotinoid applications and

calendar sprays with IPM significantly decreased the insecticide residue in the soil and plant tissues, including the floral tissues. The lack of insecticidal residues and decreased overall environmental toxicity of the IPM fields clearly benefited pollinators. Pollinator visits (Chapter II), managed pollinator colony growth (Chapter III), and foraging efficiency (Chapter IV) was all improved in IPM fields. The increase in pollination services was reflected in increased watermelon yield despite higher overall pest abundances. The struggle to navigate the costs/benefits falls to the grower, but this study system shows that if pest scouting is employed then improvements to pollination can dramatically improve.

While wild bees were repeatedly found as key contributors to yield, the integration of honey bees and bumble bees as managed pollinators placed into agricultural fields merited further exploration to their health in fields adopting IPM practices. Both species showed improved colony weight gain, higher reproductive growth and lower mortality when IPM was applied, factors that led to greater overwintering success in honey bees. This is especially important to consider as beekeepers in recent years have experienced high annual colony mortality which has led to increased hive rental fees to specialty crop growers. If beekeeper losses continue or worsen, this already sizeable cost to growers could increase. IPM can assist these issues by 1) improving the abundance and diversity of the wild pollinator community, and 2) improve the health of managed honey bees.

This work adds to the growing evidence that current ways that insecticides are applied, such as NST or calendar sprays, are not corresponding to existing pest pressures. Neonicotinoids as seed treatments are used ubiquitously in current crop systems and in this study, similar to several previous experiments, failed to provide any economic advantage to growers. This asynchronous pest/management response has huge potential to non-target exposure and resistance development that could create an entirely new suite of challenges to growers. Use of NSTs in this study didn't provide any yield benefit to corn fields despite the non-NST fields being free of any crop rotation or transgenic protection, two additional practices an IPM approach would incorporate; leaving few scenarios where use of NSTs would be effective.

Collectively, this work serves as a proof of concept that IPM can be integrated in a successful multi-crop system. Other crops in different regions of the world will have different parameters, pest pressures, and pollination requirements, but using pest biology, environmental conditions, and dynamic management tactics can still be combined to form an effective IPM

program. The transition is not instant and requires incorporating grower knowledge, an understanding of pest biology, and previous research into a set of practices that growers would be willing and able to adopt. Future work should strive to create a set of pest management practices that follow the foundations of IPM. Specifically, for Midwest's specialty crop growers we should work to strengthen the information flow from research to growers to highlight the benefit of an IPM approach and disseminate the tools necessary for adoption. Cost of crop production must be measured against the value added to yield from both pest reduction and pollination services. Changes to policy and recommendations will be driven an increasing number of growers demanding for changes to way insecticides are marketed and incorporated into a pest management program.