FIELD-LEVEL FUNGICIDE EXPOSURE AND REPELLENCY TO HONEY BEES (*APIS MELLIFERA*) DURING ORCHARD BLOOM IN MICHIGAN

By

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ABSTRACT

FIELD-LEVEL FUNGICIDE EXPOSURE AND REPELLENCY TO HONEY BEES (*APIS MELIFFERA*) DURING ORCHARD BLOOM IN MICHIGAN

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Fungicides are often used to manage diseases in Michigan orchards at the same time that bees are providing crop pollination. New research suggests that fungicides have sub-lethal effects for honey bees, such as synergism with other pesticides, decreased immune function, gut microbe interference, and increased larval and colony mortality. Quantifying field-level fungicide exposure to honey bees and understanding how bees interact with orchard crops during bloom is important for developing management practices that protect orchard crops and the bees that provide pollination. At each of three sites (two orchards and one non-orchard), we sampled eight commercial honey bee hives for nurse bees, foragers, larvae, pollen, bee bread, and wax over three consecutive years. Samples were analyzed for the presence of common spring fungicides to assess exposure levels at different time intervals centered around tart cherry bloom. Pollen was identified using DNA sequencing to determine important floral resources and identify possible sources of pesticide exposure. Our results suggest that honey bees that are within foraging range of cherry and apple orchards are exposed to fungicides in the spring, even if the hives are not being rented for pollination. Many of the detected fungicide residues are at levels known to cause negative health effects for honey bees based on previous lab studies. Possible implications on honey bee health based on the detected residue levels are discussed. New and refined best management practices to reduce fungicide exposure to bees during bloom are suggested for orchard growers and beekeepers who rent their hives for pollination services.

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INTRODUCTION

Exposure of honey bees to agricultural fungicides can greatly threaten honey bee health. Understanding how fungicides accumulate in honey bee hives and the health effects caused by this fungicide exposure is crucial for making informed disease management decisions on farms, and protecting honey bees that provide vital pollination services.

Apis mellifera, or the western honey bee, is a eusocial insect of the Apidae family, closely related to orchid bees, bumble bees, and stingless bees (Winston 1991). The western honey bee is thought to have originated in the tropics of Africa, then later migrating to the colder climates of western Asia and Europe (Winston 1991). European settlers dispersed these honey bees around the world to practice beekeeping for wax and honey production, causing *A. mellifera* to have global distribution in modern times (Winston 1991).

Today, largely because of their transportability, honey bees are the most important pollinator of agricultural crops, contributing more than $15 billion in value to the United States economy each year through pollination services (Holdren 2015). However, honey bee colony numbers in the United States have declined 45% over the past 60 years (Johnson et al. 2010). Decreased habitat of wild floral resources, parasites, disease, pesticide exposure, and climate change are all thought to have contributed to these substantial colony losses (Pettis et al. 2013).

Continuing colony losses could be detrimental to the Michigan economy, as honey bee pollination services are vital to the production of many fruit and vegetable crops grown in Michigan. Growers of tree fruit, including cherries and apples, typically rent honey bee hives

from beekeepers to increase pollination services on their farm, which increases fruit yield and quality (Delaplane and Mayer 2000).

There are three main pollinator-dependent orchard crops grown in the northwestern Lower Peninsula of Michigan, sour or tart cherries (*Prunus cerasus)* grown to be flash-frozen or juiced, sweet cherries (*Prunus avium*) grown to be processed into maraschino cherries or for fresh market, and apples (*Malus pumila*) grown for fresh market or processing which includes sweet and hard cider. All are members of the Rosaceae family.

Tart and sweet cherry are thought to have originated in Europe or southwest Asia (Janick 2005). Both are dependent on bee-mediated pollination, but differ in that sweet cherries require cross-pollination with a compatible variety, whereas tart cherries are self-fertile (McGregor 1976). Apples originated in central Asia (Janick 2005), and are also dependent on bees for pollination services, requiring cross-pollination with a compatible cultivar (McGregor 1976).

Michigan is third in terms of overall apple production in the U.S. with 1.175 billion pounds of apples harvested from 35,500 commercial acres across Michigan in 2016 (Michigan Ag Council 2016). Michigan is first in terms of growing tart cherries in the U.S., producing 224 million pounds of tart cherries at a value of $54 million in 2016, as well as 22,600 tons of sweet cherries with a value of $18.2 million (Michigan Ag Council 2016).

Along with good cultural practices, cherry and apple growers use a variety of pesticides to protect their crops from competition from weeds and injury from arthropod pests and diseases. Insecticides are designed to kill insect pests, and therefore pose a significant risk to pollinating insects like honey bees. For this reason, most are restricted from use during bloom in orchards. Bees exposed to high doses of insecticides can be directly killed, but more often bees are exposed to low doses of insecticides that have sub-lethal effects, such as changes in behavior,

cognitive function, or physiology (Johnson 2015). Pyrethroids, organophosphates and neonicotinoids targeting a variety of arthropod pests are the insecticides most commonly associated with direct honey bee kills, sub-lethal effects in larvae and workers, and reproductive effects on the queen (Chauzat et al. 2009, Johnson et al. 2010, Mullin et al. 2010, Gregorc and Ellis 2011, DeGrandi-Hoffman et al. 2013, Sanchez-Bayo and Goka 2014, Zhu et al. 2014, Calatayud-Vernich et al. 2016).

Cherry and apple growers in Michigan also have to protect their crops against high disease pressure. These crops are especially vulnerable to infections in the early spring during bloom, when developing cherry leaf tissue is susceptible to cherry leaf spot (*Blumeriella jaapii*), and apple leaf tissue is susceptible to apple scab infection (*Venturia inaequalis*) (Jenkins 1930, MacHardy 1996). If these diseases are not adequately controlled they will lead to defoliation that can be moderate (in the case of apple scab) to severe (in the case of cherry leaf spot), as well as direct crop damage (apple scab). Defoliation shortens the life of an orchard, and direct damage to fruit makes it unmarketable. To manage these harmful diseases, growers typically use predictive models to drive the application of fungicides to protect vulnerable tissue from infection during bloom and throughout the growing season (MacHardy 1996).

Due to the intense disease pressure in the early spring, fungicide applications are typically necessary during the blooming period, while bees are pollinating the crop. Lab-based acute toxicity studies have previously defined fungicides as relatively safe for use around adult honey bees. However, current research suggests that fungicides may have sub-lethal effects on honey bees at the colony level through a variety of different mechanisms. This literature review will examine what is currently known about the exposure of honey bees to fungicides, as well as the known effects of this exposure on individual honey bees and overall colony health.

LITERATURE REVIEW

Methods

A systematic approach was taken during this literature review to ensure capture of the bulk of all articles relevant to the issue of fungicide exposure of honey bees. Two scientific databases were used, Scopus and Web of Science, to search for 18 possible combinations of keywords relevant to fungicide impacts on honey bees. The keywords used were: "fungicide, fungicides, bees, honey bee, *Apis*", as well as the words "review" and "Michigan" in order to find systematic reviews that have already been conducted on the subject, and any articles of particular relevance to fungicides and bees in Michigan. Relevant listservs and Google Scholar alerts were monitored for newly published articles on the subject.

After the initial search period, a manual exclusion process was implemented where articles that were returned from searches that did not fit certain criteria for inclusion in the review were omitted. These articles were excluded due to one or more of the following reasons: article not in English, specific fungicides not listed, not related directly to *Apis mellifera*, not peer-reviewed, related to chalkbrood or other in-hive fungal diseases, or only the abstract was available. For each article the specific fungicides used, study methods, results, and key conclusions were understood and recorded.

Fungicide Exposure

Sources of Exposure

Honey bees have a large flight range, with workers typically foraging up to 6 km from their colony (Traynor et al. 2016). Due to the immensely diverse nature of Michigan's agriculture, honey bees in Michigan are likely to come into contact with a wide variety of

agrochemicals throughout their flight season (Pettis et al. 2013). Exposure to agrochemicals can happen through many different pathways such as direct spray, contact with treated crops, drift onto nearby floral resources, and water contamination through runoff (Sanchez-Bayo and Goka 2014). Colonies that are rented for pollination of commercial crops are exposed to significantly higher pesticide levels than colonies in honey-production or holding yards, however non-cultivated plants can still serve as a significant route of pesticide exposure for bees (Long and Krupke 2016, Traynor et al. 2016). A study conducted by McArt et al. (2017) suggests that the majority of pesticide risk to bees participating in the mass blooming event of apple comes from non-focal crop pollen sources and pesticides that were not sprayed during the apple blooming period.

Foraging bees can be exposed to fungicides through primary direct/exposure; these foragers then re-enter the colony and bees within the hive undergo secondary exposure through contact with the contaminated foragers (Sponsler and Johnson 2016). When foragers bring pollen and nectar back to the hive it undergoes extensive processing prior to being consumed, such as the modification of pollen into bee bread which is then stored as a food source for larvae when fresh pollen is not available (Sponsler and Johnson 2016). Food storage, processing, and modification can redistribute the pesticides brought to the hive within those fresh food sources, altering the concentration of those pesticides as well as where they accumulate within the hive (Sponsler and Johnson 2016). Previous studies in cherry and apple orchards found bee bread to be the most contaminated food source, when compared to honey and pollen (Kubik et al. 1999). Fungicides account for 94% of all residues in bee bread from hives located in areas dominated by apple orchards (McArt et al. 2017).

High numbers of different pesticide products within a hive have been strongly associated with colony mortality (Traynor et al. 2016). Fungicide residues found in hive pollen stores and in wax comb are significantly higher than residues of herbicides or insecticides (Pettis et al. 2013). The total number of fungicides detected in a hive is positively correlated with honey bee hive disorders and pesticide poisoning (Simon-Delso et al. 2015, Kiljanek et al. 2017).

Levels of Exposure

The common tree fruit fungicides chlorothalonil and captan are among the highest average residue loads found in pollen of all pesticide types (Sanchez-Bayo and Goka 2014). In a broad survey of North American apiaries, 47% of hives tested contained the fungicide chlorothalonil (Mullin et al. 2010). The average level of chlorothalonil found in pollen ranges from 0.0073 ppm to 4.491 ppm (Pettis et al. 2013, Roszko et al. 2016). Maximum detections of chlorothalonil in pollen range from 29 ppm to 98.9 ppm (Mullin et al. 2010, Pettis et al. 2013). Captan is also frequently found in pollen samples, with average residue detections ranging from 0.0029 ppm to 0.9769 ppm (Kubik et al. 2000, Pettis et al. 2013). The maximum detections of captan in pollen range from 10 ppm to 13.8 ppm (Mullin et al. 2010, Pettis et al. 2013). Thiophante-methyl, another common tree fruit fungicide, has also been found in pollen with average residues levels ranging from 0.047 to 0.25 ppm (Kubik et al. 1999, Lambert et al. 2013).

Fewer studies have been done regarding chlorothalonil, captan, or thiophanate-methyl residues accumulation within the bodies of adult bees. Average thiophanate-methyl residues in adult bees range from 0.019 ppm to 0.023 ppm, and maximum residues of 0.077 ppm (Lambert et al. 2013, Kiljanek et al. 2017). Mullin et al. (2010) found the average chlorothalonil within adult bees was 0.10 ppm, and the maximum detected level was 0.878 ppm. The same study

found average captan residues in adult bees was 0.040 ppm, and the maximum detected level was 0.043 ppm (Mullin et al. 2010). The maximum chlorothalonil residue levels found in adult bees were 112-fold lower than the chlorothalonil residues in pollen from the same study (Mullin et al. 2010). The highest detections of captan residues in adult bees were 232-fold lower than the residues in pollen from the same study (Mullin et al. 2010). The differences in residue levels within pollen and adult bees suggest that pollen is a primary source of pesticide exposure to bees, and that they are able to detoxify those pesticides through some mechanism (food storage, digestion, etc.). The accumulation of residues within larvae is highly understudied, and no specific residue levels of chlorothalonil, captan or thiophanate-methyl were available.

Bee bread is another highly understudied hive matrix, especially in regard to the fungicides chlorothalonil and captan. Chlorothalonil was not detected in bee bread samples from healthy hives, nor detected in hives with disorders (dead or diseased colonies) (Simon-Delso et al. 2015). The same study did detect captan in bee bread samples from colonies with disorders at an average of 1.90 ppm. Other studies have also detected captan in bee bread, at average residue levels of 3.43 ppm and 6.39 ppm (Kubik et al. 2000, Pohorecka et al. 2017). Average thiophanate-methyl residues range from 0.034 ppm to 570 ppm (McArt et al. 2017, Pohorecka et al. 2017). Bee bread was found to be the most contaminated hive matrix collected from hives in cherry orchards, compared to honey and pollen, with residues up to 23.6 ppm of the fungicide vinclozolin (Kubik et al. 1999). In fresh bee bread accumulated during apple bloom, 94% of the residues detected were fungicides (McArt et al. 2017).

The total number of pesticide contaminants in both bee bread and wax has been significantly linked to colony mortality (Traynor et al. 2016). Fungicides with particular modes of action increased disproportionately in wax within colonies that died, such as fungicides with

sterol-biosynthesis or multi-site contact activity, which are highly associated with poor colony health (Traynor et al. 2016). In wax, average chlorothalonil residues range from 0.017 ppm to 1.066 ppm, with a maximum recorded detection of 53.7 ppm (Mullin et al. 2010, Wu et al. 2011). The average captan residues in wax range from 0.047 ppm to 3.1 ppm (Mullin et al. 2010, Simon-Delso et al. 2015).

It appears that pollen, bee bread and wax are some of the greatest sources for fungicide exposure to bees. Kubik et al. (1999) suggest that fungicide residues could be chemically modified within the hive and that current detection methods may be underestimating contamination within colonies. They also suggest that the number of fungicide residues within a hive is the main potential stress factor linked to bee disorders (Kubik et al. 1999).

Known Effects of Fungicides

Many studies have demonstrated that bees are commonly exposed to fungicides, and that these fungicides can accumulate in pollen, bee bread, wax, and in the exposed bee's bodies. Although fungicides may not immediately kill bees after exposure, they often have negative sub-lethal effects on bee health.

Developing Bees and Reproduction

Fungicide contamination in developing bees can decrease their survival or cause abnormal development, which can greatly disrupt the productivity and overall health of the entire hive. The queen must consistently be laying eggs that develop into a healthy and high-functioning work-force in order for a colony to remain strong (Winston 1991). Multiple laboratory studies have shown increased mortality of larvae that were orally exposed to diets

containing field relevant doses of chlorothalonil, captan, ziram, and iprodione, with no larvae fed diet with captan, iprodione, or ziram completing development to adulthood at doses of 0.8, 0.8, 0.050 mg/10 g diet, respectively (Mussen et al. 2004, Johnson 2015). Larvae fed diets of 34 mg/L of chlorothalonil suffered 60% mortality (Johnson 2015). Some fungicides, such as chlorothalonil, were found to be significantly more toxic to larvae than adult bees (Zhu et al. 2014). The systemic pesticide pyrazophos, which is used as both a fungicide and an insecticide, increased larval mortality during a 30-day period at the low dose of 0.1 ppm (Ferguson 1987). This level is equal to or higher than the residues present in field-collected pollen, indicating that field-level exposure to contaminated pollen could have a significant effect on larval survival (Ferguson 1987).

Oral exposure to the fungicide Rovral at 0.05 mg/ 10 g of diet caused abnormal pupal development due to a failure to eclose properly (Mussen et al. 2004). Bees developing on pesticide contaminated brood comb experienced delayed development and shortened adult longevity (Wu et al. 2011). Longer development time may provide a reproductive advantage for parasitic mites, and shortened lifespans can cause shifts in hive roles and foraging activity, further weakening the hive (Wu et al. 2011).

In a field-study, hives that were exposed to commercial pollination (and therefore exposed to pesticide applications) were compared to hives kept near “natural” (non-cultivated) forage. This study found that the hives used in commercial pollination during the summer had less brood and fewer adult bees than the hives in natural areas (Meikle et al. 2017). The hives in commercial pollination displayed a reduction in hive temperature control, showing greater temperature variability during summer days, as well as lower overall temperatures during the winter (Meikle et al. 2017). These temperature fluctuations could be a result of having fewer

adult bees to keep the brood nest warm, having possible implications for the over-wintering survival of these colonies.

Queen larvae exposed to pollen containing sub-lethal amounts of both the insecticide chlorpyrifos and the fungicide Pristine (pyraclostrobin and boscalid) showed reduced development and a decrease in successful emergence of adult queens (DeGrandi-Hoffman et al. 2013). Deformed wing virus and black queen cell virus were found in the nurse bees tending to the queens with the contaminated pollen, as well as in the queen larvae (DeGrandi-Hoffman et al. 2013). These results suggest that simultaneous sub-lethal exposure to chlorpyrifos and a fungicide reduces queen development and emergence, possibly due to a compromised immune system in the developing queens.

Nutrition and Digestion

Fungicides accumulated in a bee's food sources can cause a negative impact on bee health and nutrition, such as reductions in food consumption, beneficial gut bacteria, and protein or nutrient digestion (Yoder et al. 2012, Degrandi-Hoffman et al. 2015). Pollen is the sole source of protein for a honey bee colony, and is essential for the growth of the developing bees and adults (Yoder et al. 2012). Honey bees have been shown to reduce their pollen consumption when offered diets treated with fungicides. Bees that were given pollen containing the fungicide Pristine (boscalid and pyraclostrobin) consumed significantly less pollen than bees given non-contaminated pollen (Degrandi-Hoffman et al. 2015).

Fungicide contamination can affect the formation and storage of bee bread, risking the long-term nutrition and food availability for the colony. In one study, bee bread with elevated levels of numerous pesticides was often stored as "entombed pollen" (VanEngelsdorp et al.

2009). In addition to the high pesticide levels, entombed pollen lacked the microbial agents commonly associated with stored pollen (VanEngelsdorp et al. 2009). This entombed pollen was sunken and brick-red in color. Colonies with entombed pollen showed higher rates of mortality (43%) compared to colonies without entombed pollen (20%) (VanEngelsdorp et al. 2009). All entombed pollen samples studied contained extremely high levels of chlorothalonil, with residues 40x higher than non-entombed samples (VanEngelsdorp et al. 2009). In another study, chlorothalonil contamination specifically has been associated with colony mortality. Migratory colonies that did not survive the beekeeping season showed significantly higher residues of chlorothalonil in beebread during the summer months (May-August) when compared to colonies that survived the season (Traynor et al. 2016).

The honey bee gut microbiome is a crucial part of honey bee health. Beneficial bacteria and fungi within the honey bee gut assists with the creation of the vital food source, bee bread, as well as increasing the ability to fend of diseases, such as chalkbrood (Yoder et al. 2013). The functional potential and structure of the honey bee gut bacterial community was significantly altered after exposure to chlorothalonil (Kakumanu et al. 2016). After chlorothalonil exposure, the gut microbiome also demonstrated an increase in putative genes for oxidative phosphorylation, and a decrease in sugar metabolism (Kakumanu et al. 2016). In another study, fungal isolates within the bee bread were significantly reduced as an effect of fungicide contamination, with the number of fungal isolates decreasing with increasing amount of fungicide residues in a dose-response manner (Yoder et al. 2013).

Fungicide exposure can damage sections of a bee's digestive tract, and reduce the subsequent digestion capabilities. For example, larvae that were fed an artificial diet containing the common fungicide myclobutanil experienced 69% cell death within the larval midgut,

compared to only 10% cell death in untreated larvae (Gregorc and Ellis 2011). Bees fed the Pristine-treated pollen had higher concentrations of protein in their hindguts, suggesting that the fungicides in the pollen reduced the digestion of proteins (Degrandi-Hoffman et al. 2015).

Immunity and Disease

Fungicide exposure can increase the prevalence and severity of honey bee diseases, as well as disrupt the bee's ability to withstand and ward off infections. Fungicides increase the probability of infection with the microsporidial pathogen *Nosema. Nosema* infection rates doubled as fungicide loads, primarily chlorothalonil and pyraclostrobin, in bee-collected pollen increased (Pettis et al. 2013, Sanchez-Bayo et al. 2016). Chlorothalonil and pyraclostrobin not only increase the probability of infection, but also significantly reduced bee survival post-infection (Pettis et al. 2013).

Fungicide contaminated pollen fed to bees led to significantly higher deformed wing virus and black queen cell virus titers. Deformed wing virus and black queen cell virus titers were 5x and 2x higher, respectively, in bees fed pollen containing the fungicide Pristine compared to bees fed an uncontaminated diet during a cage study (Degrandi-Hoffman et al. 2015).

Exposure to fungicides alters the expression of various immune-related genes in honey bees. Many pathogen recognition genes were downregulated in pre-pupae exposed to a diet containing 1mg/100 g of prochloraz in a laboratory study, with the most downregulated genes being *spaetzli*, and *defensin* (Cizelj et al. 2016). Six out of 17 genes encoding proteins involved in immune response were upregulated in similarly treated adult honey bees (opposite effect) (Cizelj et al. 2016). Transcript levels of an immune end product, prophenoloxidase-activating enzyme

(PPOact), were significantly elevated in larvae fed pollen with 200 ppm of either chlorothalonil or myclobutanil in a laboratory study (Gregorc et al. 2012). Changes in transcription of pathogen recognition and immune end products may have repercussions for the bee's ability to fight off or survive infections.

Although many laboratory studies have demonstrated negative immune system responses and increased virus levels after honey bees were exposed to fungicides, there still remains uncertainty when it comes to field-realistic scenarios. Fungicide impacts on infections, viruses, and immunity are unclear at the colony-level due to the narrow laboratory focus of most studies (Collison et al. 2016).

Behavior and Learning

Fungicide exposures that cause a change in bee behavior can have serious implications for both honey bee colony health, and the effectiveness of pollination services. Honey bee hives placed near fields sown with seed-treated corn containing the fungicides fludioxonil and metalaxyl-M showed a significant reduction in foraging behavior post sowing (Tremolada et al. 2010). One study demonstrated that as honey bees were exposed to higher doses of the imidazole fungicide prochloraz, they experienced impaired olfactory learning capabilities (Decourtye et al. 2005). A reduction in olfactory learning and memory can severely disrupt a colony's ability to function properly (Decourtye et al. 2005).

In an effort to reduce honey bee pesticide exposure during crop bloom, attempts to identify chemical repellents have been conducted. The fungicides nitrothal-isopropyl, penconazole, triadimefon, and dodine appear to be promising materials for use as honey bee repellents in apple orchards because they have a high repellency index and they do not damage

pollen germination or predatory mites (Solomon and Hooker 1989). Although the practice of using fungicides as a way to reduce honey bee pesticide exposure may be beneficial to bee health, this has questionable relevance to tree fruit orchards, due to their need for bee pollination. The use of fungicides to repel honey bees may be a useful practice to reduce bee pesticide exposure in crops that have flowers that are attractive to bees but that do not require pollination, such as asparagus (Delaplane and Mayer 2000).

Certain fungicides, such as benomyl, can indirectly impact bee foraging behavior by suppressing below-ground arbuscular mycorrhizal fungi. These fungi are beneficial in enhancing the nutrient capturing ability of plants. Suppression of mycorrhizal fungi through benomyl applications caused a shift in the floral visitors of 23 native grassland plants from large-bodied bees to small-bodied bees and flies, as well as reducing the total number of floral visits per flowering stem (Cahill Jr. et al. 2008).

Pesticide exposure can alter the morphology and development of honey bees. Because honey bees are social insects with a highly-organized caste system within the hive, a change in the morphology and rate of development of individual bees could cause a shift in the demographics of the hive, hence changing colony behavior and efficiency. For example, in a laboratory study on the effects of crop protection products on the morphology of the hypopharyngeal gland, the fungicide captan was found to reduce the size of the glands' acini in treated bees compared to untreated bees (Heylen et al. 2011). Reduced acini in the hypopharyngeal glands of honey bees is typical at the onset of foraging behavior in older bees. The hypopharyngeal gland is important for nurse bees because it is used for feeding larvae, the gland shrinks when bees become foragers and no longer utilize the glands to fulfill the role of a nurse bee. If pesticide exposure leads to premature shrinking of these glands, this could shift bee

behavior from in-hive work to foraging out of the hive, upsetting the balance of worker bee role diversification that maintains productive colonies (Heylen et al. 2011).

Cellular Function

Honey bee cellular function, and their ability to detoxify chemicals can be severely disrupted by fungicide exposure. For example, honey bees fed pollen containing boscalid and/or pyraclostrobin had lower ATP levels in their flight muscles compared to bees fed fungicide-free pollen (Degrandi-Hoffman et al. 2015). Larvae fed pollen treated with 200 ppm myclobutanil had significantly upregulated levels of a hexameric storage protein, Hsp70. This protein is important for developmental physiology and sociobiology of insects, and changes in the levels of this protein could have serious health implications for developing larvae (Gregorc et al. 2012).

Mitochondria that were isolated from the flight muscles of honey bee workers and exposed to the fungicide Pristine (boscalid and pyraclostrobin), experienced inhibition of mitochondrial oxidation rates and higher oxygen consumption than mitochondria isolated from control bees (Campbell et al. 2016). Mitochondria in other tissues may also be negatively affected, possibly conferring health or function problems in the affected honey bee. In another laboratory study, semi-isolated hearts of honey bees were exposed to prochloraz, which caused an immediate alteration of the chronotropic (rate and rhythm) and inotropic (energy/force of muscular contractions) performance of the heart (Papaefthimiou and Theophilidis 2001).

Prochloraz has also been shown to inhibit honey bee thermoregulation (Vandame and Belzunces 1998). Honey bee foragers experienced hypothermia after being exposed to doses of prochloraz and difenoconazole lower than field application rates (Vandame and Belzunces

1998). Changes in thermoregulation could severely impact overwintering survival of honey bees in cold-climates.

Sterol Biosynthesis Inhibiting (SBI) fungicides have been shown to inhibit the P450 mediated detoxification of insecticides, particularly pyrethroids and neonicotinoids, hence increasing the insecticide's toxicity to the honey bee (Glavan and Bozic 2013, Johnson 2015).

Synergism

Fungicides can increase the toxicity of other agrochemicals, or increase in toxicity when combined with other chemicals- a phenomenon called "synergism." These synergistic reactions can lead to higher mortality, increased risk of disease and viruses, reduced ability to metabolize food, decreased cellular function, and a higher risk of hypothermia (Glavan and Bozic 2013).

Many fungicides have been shown to increase the toxicity of miticides applied in the hive by beekeepers to treat for *Varroa destructor* (Johnson et al. 2013). *Varroa destructor* is an external parasitic mite that attacks honey bees, and is currently one of the most serious pests in managed honey bees. (Johnson et al. 2013). *Varroa* weakens colonies directly through the consumption of hemolymph of adult and pupal bees, and indirectly through the vectoring of harmful honey bee viruses (Boecking and Genersch 2008). *Varroa* is certainly one of the factors contributing to the recent onslaught of collapsing honey bee colonies, and therefore the use of miticides in bee hives to control *Varroa* will most likely remain a standard practice for the foreseeable future. The known synergistic reactions between fungicides and these in-hive miticides pose a significant risk to bee health. The fungicide active ingredients pyraclostrobin, chlorothalonil, prochloraz, propiconazole, fenbuconazole, metconazole, and myclobutanil have

all been show to significantly increase the toxicity to honey bees of commonly used miticides (Johnson et al. 2013).

Fungicides can also increase the toxicity of commonly used insecticides, particularly pyrethroids and neonicotinoids (Sanchez-Bayo and Goka 2014). Sterol biosynthesis inhibitors (SBI), ergosterol biosynthesis inhibitors (EBI), and Imidazole fungicides are known to increase the toxicity of neonicotinoids and pyrethroid insecticides (Pilling 1992, Thompson et al. 2014, Kiljanek et al. 2017). EBI fungicides known to increase the toxicity of the pyrethroid insecticide lambda-cyhalothrin are prochloraz, flutriafol, penconazole, imazalil, triadimenol, myclobutanil, triadimefon, and propiconazole (Pilling and Jepson 1993).

Sub-lethal effects increase in severity after a synergistic reaction between two pesticide types. For example, successful queen rearing was significantly reduced when queen larvae were fed pollen containing both the organophosphate insecticide chlorpyrifos and the fungicide Pristine (pyraclostrobin and boscalid) (DeGrandi-Hoffman et al. 2013). This same fungicide-insecticide combination also caused compromised immune systems in developing bees, leading to higher Deformed Wing Virus titers (DeGrandi-Hoffman et al. 2013).

Combined exposure of the insecticide deltamethrin with either prochloraz or difenoconazole fungicides (at 25 g/ha a.i.) inhibited bee's thermoregulation ability and triggered severe hypothermia within the hive (Vandame and Belzunces 1998). This synergistic effect could be a possible explanation for over-wintering survival problems bee hives have been experiencing in cold climates. Prochloraz has also been shown to significantly increase the cardiotoxicity of deltamethrin, making the combined exposure over 100 times more toxic to a semi-isolated (dissected) honey bee heart than exposure to deltamethrin alone (Papaefthimiou and Theophilidis 2001).

The mechanisms of synergism still remain largely unknown, but current research suggests that fungicides increase the toxicity of other chemicals because they inhibit the pathways typically used to detoxify xenobiotics in honey bees (Glavan and Bozic 2013). Specifically, fungicides inhibit the action of cytochrome P450 enzymes that are used in the detoxification of phytochemicals and xenobiotics, as well as the metabolism of certain proteins and flavonols from the honey bee's diet (Mao et al. 2017). Some of the fungicides known to inhibit P450s are carbendazim, difenoconazole, flusilazole, prochloraz, propiconazole, tebuconazole, thiophanate-methyl, myclobutanil, metconazole, and fenbuconazole (Glavan and Bozic 2013). A study by Pilling et al. (1995) analyzed frass expelled from treated honey bees to demonstrate that the fungicide prochloraz inhibits microsomal monoxgenase activity. Prochloraz effectively increased the toxicity of the insecticide lambda-cyhalothrin by delaying the metabolism, detoxification and excretion of the insecticide through inhibiting microsomal oxidation (Pilling et al. 1995).

Simultaneous exposure to fungicides and insecticides can also increase the toxicity of those insecticides through changed behavior, rather than physiological reactions. Pyrethroid insecticides are known to repell honey bees, which decreases the likelihood that bees will visit pyrethroid treated flowers, and reduces the associated exposure risk (Thompson and Wilkins 2003). When fungicides are applied at the same time as pyrethroids, the repellency effect of the pyrethroids is significantly reduced (Thompson and Wilkins 2003). A choice or no-choice cage study revelaed that chlorothalonil, difenconazole and tebuconazole significantly decreased the repellency effect of alpha-cypermethrin, while prochloraz, flusilazole, and propiconazole significantly reduced the repellency effect of lambda-cyhalothrin (Thompson and Wilkins 2003).

These decreases in repellency caused by fungicides can increase the exposure of pyrethroids to bees, greatly increasing the risk of insecticide-related health effects.

Adjuvants and Surfactants

Fungicide formulations often contain "non-active" ingredients, such as adjuvants and surfactants, which have recently been shown to be detrimental to honey bee health and function (Mullin 2015). These supposedly "inert" ingredients in fungicide formulations, can severely disrupt the function of the honey bee's immune system. For example, one study suggests that organosilicone surfactants may aid the movement of pathogens into bee tissues. This is due to the super-penetrating characteristics of the surfactant that can drive the stomatal uptake of even large bacterial-sized mineral particles, therefore putting bees at risk of increased pathogen uptake (Mullin 2015).

Mortality and failed development significantly increased when larvae were exposed to both an organosilicone surfactant and a viral inoculum when compared to larvae exposed to a viral inoculum alone (Fine et al. 2017). The symptoms of death were altered as well; deaths occurred immediately prior to the average day of pupation, and death was most commonly characterized by a failure to evert the imaginal discs. Simultaneous exposure of larvae to organosilicone surfactant and exogenous black queen cell viruses also resulted in higher virus titers, increased mortality, and lower expression of receptors associated with viral defense (Fine et al. 2017). Organosilicone surfactants commonly used with fungicide applications have also been found to cause significant learning and memory impairment when ingested (Ciarlo et al. 2012).

Changes to Plant Physiology

Fungicide applications to particular plant species can also change the physiology of the plant, therefore affecting the plant's viability and potential as a nutrient source for bees (Bartlewicz et al. 2016). When applied to natural populations of *Linaria vulgaris,* the fungicides prothiconazole and tebuconazole were both highly toxic to nectar yeasts within the flowers. The concentrations found to inhibit yeast growth are much lower than what has been found in the field directly after treatment (Bartlewicz et al. 2016). Captan and mancozeb significantly reduced pollen viability within apple blossoms for up to 48 hours, which could have detrimental effects on fruit set, despite there being no reduction in flower visitation rates after application (Fell et al. 1983). If these changes in floral yeast composition and pollen viability after fungicide treatment is true for other plant species, it could have widespread detrimental effects for both bee health, and pollination services.

CONCLUSION

Honey bee exposure to fungicides is common, and these fungicides can accumulate within a variety of hive matrices such as pollen, bee bread, and wax. Fungicide contamination within the hive has been linked to honey bee disorders and overall colony loss. Exposure of honey bees to fungicides can greatly threaten honey bee health through a variety of mechanisms and sub-lethal effects. Fungicides accumulated in honey bee hives can lead to an increase in larvae mortality, and prolonged or abnormal development, which can decrease productivity and overall health of the entire hive. Food sources with accumulated fungicides can cause reductions in overall food consumption, decreased beneficial gut bacteria, and ineffective protein and nutrient digestion in the exposed bees. Fungicide exposure can increase the prevalence and severity of honey bee

diseases, as well as disrupt the bee's ability to withstand and ward off infections. Exposure to fungicides can have detrimental effects on honey bee learning, memory, morphology, and floral visitation, which can lead to decreased honey bee colony survival, as well as reducing a colony's ability to adequately provide pollination services for a crop in bloom. Fungicides can severely disrupt honey bee cellular function and cause detrimental health effects related to gene regulation, oxygen consumption, thermoregulation, and xenobiotic detoxification.

Fungicides can become even more dangerous for honey bees when they are combined with other pesticide types, such as insecticides and miticides, and undergo the process of synergism. These synergistic reactions can lead to higher mortality, increased risk of disease, reduced metabolism, compromised cellular function, and loss of thermoregulation. Adjuvants and surfactants, ingredients typically considered 'inactive' in fungicide formulations, can also have serious health implications for honey bees and significantly increase the danger of the fungicides to which they are added.

The risk for fungicide and other pesticide exposure to honey bees in Michigan is high, due to the diversity of crops grown and the timing of fungicide applications. The generation of crop-specific exposure data will be a valuable first step to understand the potential health risks for bees under current disease management practices, and to determine if those management practices need to be changed to reduce honey bee fungicide exposure.

CHAPTER 2: FIELD-LEVEL FUNGICIDE EXPOSURE TO HONEY BEES DURING ORCHARD BLOOM IN MICHIGAN

INTRODUCTION

Honey bees (*Apis mellifera*) are the most important pollinator of agricultural crops, contributing more than $15 billion in value to the United States economy each year through their pollination services (Holdren 2015). However, honey bee colony numbers in the United States have declined 45% over the past 60 years (Johnson et al. 2010). Continued colony losses could be detrimental to agricultural production and the economy, as honey bee pollination services are vital to the production of many fruit and vegetable crops. Growers of tree fruit, including cherries and apples, typically rent honey bee hives from beekeepers to increase pollination services on their farm, which then increases fruit yield and quality (Delaplane and Mayer 2000). Cherries and apples are both dependent on bee-mediated pollination; sweet cherries and apples require cross-pollination with a compatible variety, whereas tart cherries are self-fertile (McGregor 1976).

Decreased habitat of wild floral resources, parasites, disease, pesticide exposure, and climate change are all thought to have contributed to the substantial honey bee colony losses in the United States (Pettis et al. 2013). Pesticide exposure is a primary concern for the health of honey bees that are used in commercial pollination. Exposure to agrochemicals can happen through many different pathways such as direct spray, contact with treated crops, drift onto nearby floral resources, and water contamination through runoff (Sanchez-Bayo and Goka 2014). Colonies that are rented for pollination of commercial crops are exposed to significantly higher pesticide levels than colonies that are not rented for pollination and instead kept in honey-producing holding yards (Traynor et al. 2016). However, foraging on crop plants is not the only

significant source of pesticide exposure in bees, non-cultivated plants have also been shown to serve as a substantial route of pesticide exposure for bees (Long and Krupke 2016). Cherry and apple growers use a variety of pesticides to protect their crops against competition from weeds and injury from arthropod pests or diseases. Insecticides are designed to kill insect pests, and therefore pose a significant risk to pollinating insects like honey bees. For this reason, most insecticides are restricted from use during bloom in orchards. Bees exposed to high doses of insecticides can be directly killed, but more often bees are exposed to low doses of insecticides with sub-lethal effects, such as changes in behavior, cognitive function, physiology, and reproductive effects on the queen (Johnson 2015, Mullin et al. 2010). A majority of public concern and scientific studies regarding the effects of pesticides on bee health have focused on insecticides, especially neonicotinoids, pyrethroids, and organophosphates.

Tree fruit growers also have to protect their crops against plant pathogens, especially in the early spring during bloom, when developing cherry leaf tissue is susceptible to cherry leaf spot (*Blumeriella jaapi*), and apple leaf tissue is susceptible to apple scab infection (*Venturia inaequalis*) (Jenkins 1930, MacHardy 1996). If these diseases are not adequately controlled they will lead to defoliation, as well as direct crop damage. To manage these harmful diseases, growers typically apply fungicides that protect vulnerable tissues from infection during bloom and throughout the growing season (MacHardy 1996).

On some crops, fungicide applications can overlap with pollination, and while previous lab-based toxicology studies have determined that fungicides are relatively safe for adult honey bees, more recent research suggests that fungicides may have sub-lethal effects on honey bee health through a variety of different mechanisms. Fungicides accumulated in honey bee hives can lead to an increase in larval mortality, and prolonged or abnormal development, which can

decrease productivity and overall health of the entire hive (Ferguson 1987, Mussen et al. 2004, Zhu et al. 2014, Johnson 2015). Food sources contaminated with fungicides can cause reductions in overall food consumption, a decrease of beneficial gut bacteria, and ineffective protein and nutrient digestion in the exposed bees (Yoder et al. 2012, Degrandi-Hoffman et al. 2015). Fungicide exposure can increase the prevalence and severity of honey bee diseases by disrupting the bee's ability to withstand and ward off infections by pathogens such as *Nosema,* deformed wing virus, and black queen cell virus (Pettis et al. 2013, Degrandi-Hoffman et al. 2015, Sanchez-Bayo et al. 2016). Exposure to fungicides may also cause detrimental effects on honey bee foraging behavior, learning, and memory, that can lead to decreased honey bee colony survival as well as a reduction in a colony's ability to adequately provide pollination services for a crop in bloom (Decourtye et al. 2005, Tremolada et al. 2010). Fungicides can severely disrupt honey bee cellular function and gene regulation, oxygen consumption, thermoregulation, and xenobiotic detoxification (Vandame and Belzunces 1998, Gregorc et al. 2012, Glavan and Bozic 2013, Campbell et al. 2016). Exposure of honey bees to fungicides can become even more dangerous to bee health when the fungicides are combined with other pesticide types, such as insecticides and miticides. These combinations cause the chemicals to synergize and increase in toxicity, leading to higher bee mortality, increased risk of disease, reduced metabolism, compromised cellular function, and loss of thermoregulation (Glavan and Bozic 2013).

Foraging bees can be exposed to fungicides directly, while bees inside the hive come into contact indirectly through interactions with the contaminated foragers (Sponsler and Johnson 2016). When foragers bring pollen and nectar back to the hive it undergoes extensive processing prior to being consumed, such as the modification of pollen into bee bread which is then stored as a food source for larvae when fresh pollen is not available (Sponsler and Johnson 2016). Food

storage, processing, and modification can redistribute the pesticides in the hive, altering their concentrations and where they accumulate within the hive (Sponsler and Johnson 2016). An increase in the numbers of different types of pesticides in a hive has been positively correlated with colony mortality (Traynor et al. 2016). Fungicide residues found in hive pollen stores and in wax comb have been significantly higher than residues found of either herbicides or insecticides (Pettis et al. 2013). The total number of fungicides detected in a hive is positively correlated with honey bee hive disorders and pesticide poisoning (Simon-Delso et al. 2015, Kiljanek et al. 2017). In a previous study of honey bee hives in cherry and apple orchards, bee bread was the most contaminated food source, compared to honey and pollen (Kubik et al. 1999).

In conventionally managed tart cherry orchards, fungicides containing the active ingredient chlorothalonil are often applied during bloom to control cherry leaf spot, and captan is typically applied in apple orchards during the early spring to control apple scab. Crop-specific fungicide exposure data is needed for honey bees pollinating tart cherry orchards to understand the potential health risks for bees under current disease management practices, and to determine if those management practices need to be changed to reduce honey bee fungicide exposure. This study's overarching goal is to determine where fungicide residues are found in honey bee hives during cherry pollination, and at what concentration. We predict that hives placed at orchard sites will have higher levels of fungicide contamination that hives in holding yards that are not being rented for pollination. Additionally, we aim to determine if hives that are rented for crop pollination are at a higher risk for fungicide exposure than hives kept in natural areas. The specific objectives of this study are to 1) quantify field-level fungicide residues during tart cherry bloom in nurse bees, foragers, larvae, pollen, bee bread, and wax, 2) determine the important

floral resources that coincide with tart cherry bloom, and 3) refine best management practices to better protect the health of orchard trees, and the bees providing pollination services.

METHODS

Study Area and Design

During the spring of 2015, 2016 and 2017, honey bee colonies were assessed for pesticide residues at each of three sites near Traverse City, Michigan: two sites were tart cherry orchards which contained honey bee colonies being rented for crop pollination, and the third site was an apiary, or "bee yard" used by beekeepers to hold hives in between pollination contracts. A total of 8 honey bee hives per site were sampled each year. While all years of the study consisted of two orchard sites and one bee yard, the exact site locations for each year changed slightly over the course of the study. The hives at the orchard sites were rented by the grower for pollination, and were placed directly adjacent to conventionally managed tart cherry orchards. All cherry orchards in the study were owned and managed by the same grower to maintain consistency. The bee yard site was located in a natural area (86.3-92.5% natural land in 2 km radius) that was semi-isolated from orchard production, and assumed to be less vulnerable to pesticide exposure (Figure 2.1). All of the bee colonies used were owned and managed by the same beekeeper in order to keep the management practices and migratory routes consistent amongst all hives in the study.

The grower cooperator for this study provided spray records for the tart cherry and apple orchards adjacent to the studied hives. A typical spray program for tart cherry involved 2 fungicide applications before bloom, and 2-4 applications during crop bloom. Common fungicide active ingredients included chlorothalonil, thiophanate-methyl, fenbuconazole,

fluopyram and trifloxystrobin. In apple, between 4 and 6 fungicide applications were made leading up to and during crop bloom, which included the fungicide active ingredients of captan, trifloxystrobin, and mancozeb. Tart cherry orchard sites were between 279 and 498 m from the nearest apple orchard. The bee yard sites were at least 445 m from the nearest apple orchard.

Sample collections and hive-inspections were done at three time intervals surrounding peak-bloom of tart cherry: pre-bloom, bloom, and post-bloom, at which times nurse bees, foragers, and pollen were collected. In addition, bee bread, larvae, and wax were collected once from each hive during peak tart cherry bloom. When tart cherry bloom ended, all hives at the orchard sites were transported by the beekeeper to the same bee yard site for the post-bloom sampling period. Post-bloom samples were not collected in 2016, due to black bear damage at one site and accidental hive removal at another. The accumulated Growing Degree Days (GDD) for the sampling periods of each year are available in Table 2.1 (GDD data obtained from MSU Enviroweather using the Baskerville-Emin calculation method at the default setting of base 42°F beginning January 1 of each year).

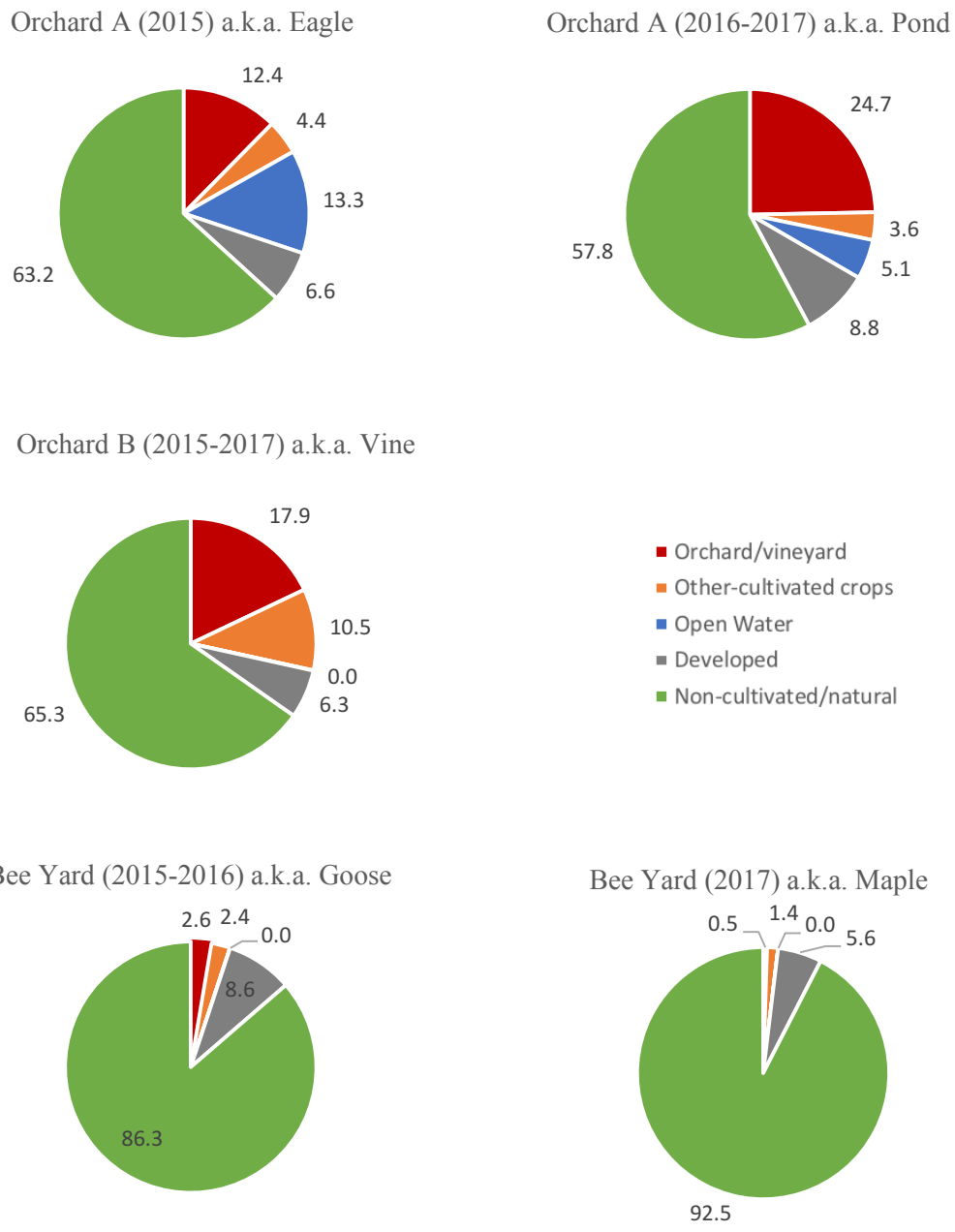

Figure 2.1. Proportions of different landscape categories within a 2-km radius for each orchard and bee yard site used throughout the study. Landscape categories: orchard or vineyard, other cultivated crops, open water, developed land (includes rural and low-intensity developments), and natural or non-cultivated lands.

Table 2.1. Sampling dates and accumulated growing degree days (GDD) for each year and sampling period of the study. GDD accumulation starting on the first (January 1) of each sampling year, with base 42.

Year	Sampling Period	Site(s)	Sampling Dates	GDD
2015	Pre-Bloom	Bee Yard	13-May	385
		Orchards	13-May	347
	Bloom	Bee Yard	20-May	466
		Orchards	20-May	434
	Post-Bloom	Bee Yard	17-Jun	966
		Orchards	17-Jun	966
2016*	Pre-Bloom	Bee Yard	9-May	330
		Orchards	10-May	300
	Bloom	Bee Yard	24-May	492
		Orchards	23-May	466
2017	Pre-Bloom	Bee Yard	9-May	329
		Orchards	10-May	337
	Bloom	Bee Yard	19-May	463
		Orchards	19-May	483
	Post-Bloom	Bee Yard	31-May	643
		Orchards	1-Jun	656

*2016 post-bloom samples were not able to be collected.

Nurse Bee Collection

Nurse bee samples were collected before, during, and after peak orchard bloom in 2015, 2016, and 2017. We began the collection at each hive by removing a brood frame and shaking it until older, non-nurse bees flew off. We then used an open quart sized zip-top bag to gently scoop bees off of the brood frame and into the bag until the bag was ¼ of the way full. Samples were placed in a cooler, then frozen in the laboratory at -20°C, until ready for processing.

Forager Bee Collection

Foragers were sampled only in the 2017 season, before, during, and after peak tart cherry bloom, using insect vacuums (Insect Vacuum 18 Volt Cordless, BioQuip; Rancho Dominguez, CA) to capture any flying bees entering or exiting the hives for 1 min at each hive. Collections were conducted during ideal times for honey bee flight (temperatures above 10° C, low winds). Samples were placed in a cooler, then frozen in the laboratory at -20°C, until ready for processing.

Pollen Collection

Two hives at each site were fitted with pollen traps (10-Frame Superior Pollen Trap, Mann Lake; Hackensack, MN) for pollen collection during the 2016 and 2017 seasons. Pollen was collected during all time intervals (pre-bloom, bloom, post-bloom). At each of the three sampling times, all of the pollen accumulated in the tray was removed from the trap and transferred to a gallon size zip-top bag, which was placed in a cooler, then frozen in the laboratory at -20°C, until ready for processing.

Wax, Larvae, and Food-Stores Collection

Wax samples were collected from each hive during the 2016 and 2017 seasons. In 2016, a fresh drone frame was placed in each hive prior to crop bloom (Green Plastic Drone Comb Frame, Mann Lake; Hackensack, MN). The drone frames were then removed from the hives after the blooming period was over. These frames were individually placed in large plastic bags in a cooler, then frozen at -20°C until ready for processing. We were able to collect fresh wax-

comb from these frames, but the bees did not draw enough comb on the frame for the queen to lay eggs and develop larvae in the time allotted, so no larvae were collected in 2016.

In 2017, no drone frames were inserted in to the hives. Instead, when bloom was near completion, we removed one brood frame from each hive, then placed them individually in large plastic bags in a cooler, then froze the frames at -20°C until they were ready for processing. The collection of these frames provided samples of wax, as well as stored bee-bread, and capped larvae from each hive. Frame dissections were completed in the laboratory, first by scraping empty wax comb that did not contain any brood or bee bread using a sterilized hive-tool (Dadant; Hamilton, IL). Second, freshly-capped larvae that had not yet begun to pupate, and stored bee bread was extracted from each collected frame using sterilized tweezers.

Hive Inspections

Hive inspections were conducted for each hive during every sampling period. Hives were assessed for the status of the queen, the number of frames of bees, beekeeper management (such as re-queening or mite control), and any noticeable pest pressure or other problems. We conducted these hive inspections to ensure that samples were being taken only from healthy and productive hives, and not from weak or non-representative hives. Any hives deemed too weak were exempt from collections during that sampling period.

Sample Preparation and Pesticide Extraction

We tested each sample type for common bloom-time pesticides using the "Quick, Easy, Cheap, Effective, Rugged and Safe" (QuEChERS) method (Wiest et al. 2011). We measured 10g of each nurse bee, forager, larvae, pollen, bee bread and wax sample, except for 2017 wax

samples which were measured to 1g. Weighed samples were transferred to a 118.3 ml graduated glass vial (Qorpak; Bridgeville, PA). Each vial then received the following: 1g sodium chloride (Sigma-Aldrich; St. Louis, MO), 4g magnesium sulfate (Topco Associates; Elk Grove Village, IL), and enough high performance liquid chromatography grade dichloromethane (British Drug Houses, Poole, United Kingdom) to completely cover the sample. Samples were then capped, inverted twice, and placed on an agitator for 30 min. After agitation, samples were stored at 23° C for 2 wks to allow for passive extraction, after which each sample was mixed/mashed with a sterilized muddler and topped off with dichloromethane as needed to ensure the sample remained covered in liquid.

Using a glass funnel with Whatman #1 filter paper (Sigma-Aldrich, St. Louis, MO), and 4g sodium sulfate (British Drug Houses; Poole, United Kingdom), each sample was decanted and filtered into a clean 118.3 ml graduated glass vial (Qorpak; Bridgeville, PA). Samples were then left uncapped in a fume hood until all liquid evaporated (~ 48 hrs).

Once dry, each sample vial received 2ml of acetonitrile (Sigma-Aldrich; St. Louis, MO), was gently swirled, then sonicated for 1 min. The liquid was removed from the jars using a 1250 uL micropipette (Avantor VWR; Center Valley, PA), and placed into a 5ml syringe (Luer-lok tip, Becton Dickinson; Franklin Lakes, NJ) with the plunger removed. Once the liquid was in the syringe, the plunger for the syringe was replaced, and a Polytetrafluorethylene (PTFE) membrane syringe filter (0.45μm pore size, Scientific Equipment of Houston; Navasota, TX) was screwed on to the syringe tip. The liquid was forced through the filter into a glass vial (2ml, Sigma-Aldrich; St. Louis, MO) that was capped, labeled, and placed in the refrigerator at 2.2° C until analysis. Gas chromatography analysis was conducted by the Michigan State University Pesticide Analytical Lab using standard protocols gas chromatography-mass spectrometry (GC-

MS) via the QuEChERS method. Limit of Quantification (LOQ) and Limit of Detection Data (LOD) for each pesticide are listed in Table 2.2.

Table 2.2. Limit of Quantification (LOQ) and Limit of Detection (LOD) for each pesticide analyzed.

Chemical	LOQ (ppm)	LOD (ppm)
Chlorothalonil	0.03	0.01
Captan	0.05	0.02
Thiophanate-Methyl	0.53	0.16
Chlorpyrifos	0.06	0.02
Pendimethalin	0.03	0.01
Simazine	0.07	0.02

Pollen Identification

Pollen was identified from 150g subsamples ground using a mortar and pestle, then transferred to a labeled paper bag. After drying the samples (24 hrs in a drying oven, 60°C), they were transferred into individual whirl-pak bags and shipped to the U.S. Geological Survey (USGS), Leetown Science Center, Kearneysville, WV, for meta-genetic analysis. Molecular and statistical methods followed previously described protocols; DNA extraction from plants (Doyle 1991), PCR amplification, sequencing, and bioinformatics analysis using the lowest-common ancestor approach (Cornman et al. 2015). Because bee yard pollen traps failed in 2016, only 2017 pollen sample identification are reported here. Taxa with less than 1,000 read counts are grouped together as “other” kinds of pollen. Taxa with over 1,000 read counts represent the 10 most common pollen genera collected by honey bees during the study.

Statistical Analysis

Percentages of samples with residue detections were calculated by dividing the number of positive samples (samples with residues above the selected compounds' LOD) by the total number of samples analyzed for the selected compound and multiplying times 100. The average residues were calculated using the arithmetic mean. All samples with no residues detected were considered to be below the selected compound's LOD (Table 2.2), rather than being considered 0 ppm. Statistical analysis was conducted using the frequencies of detections using a standard χ^2 analysis, rather than the levels of the pesticide content. The significance threshold used was 5% throughout all analyses.

RESULTS

Pesticide Exposure

Of the pesticides that were screened, the fungicides chlorothalonil and captan were the most frequently detected in all samples except larvae – individual results by sample type for these fungicides are reported below. The fungicide thiophanate-methyl was detected in only one larval bee yard sample from 2015 (0.07 ppm), and this was the only positive detection of this pesticide in any larval sample throughout the study. The herbicide pendimethalin was detected in all pollen samples from 2016 (max detected 0.12 ppm), but no pendimethalin was detected in any pollen samples from 2017, or in any other sample type throughout the study. The herbicide simazine was never detected. The insecticide chlorpyrifos was only detected in 4 samples throughout the study, all of which were nurse bees. In 2015, nurse bees from the bee yard had chlorpyrifos detected at a maximum of 0.05 ppm, and in 2017 one nurse bee sample from an orchard site had chlorpyrifos residues at 0.08 ppm.

Nurse Bees

The maximum level of chlorothalonil detected in nurse bees was 2.48 ppm from orchard hives, and 0.47 ppm from bee yard hives. No chlorothalonil was detected in nurse bees from the bee yard hives in two out of three years of the study. Tart cherry bloom period coincided with the peak of chlorothalonil residues from orchard hives (Figure 2.2). The maximum level of captan detected in nurse bees from orchard hives was 75.3 ppm, and 47.4 ppm in nurse bees from bee yard hives. No captan was detected in any of the nurse bee samples in 2015. The highest levels of captan residues occurred in pre- and post-bloom samples in 2017 (Figure 2.3).

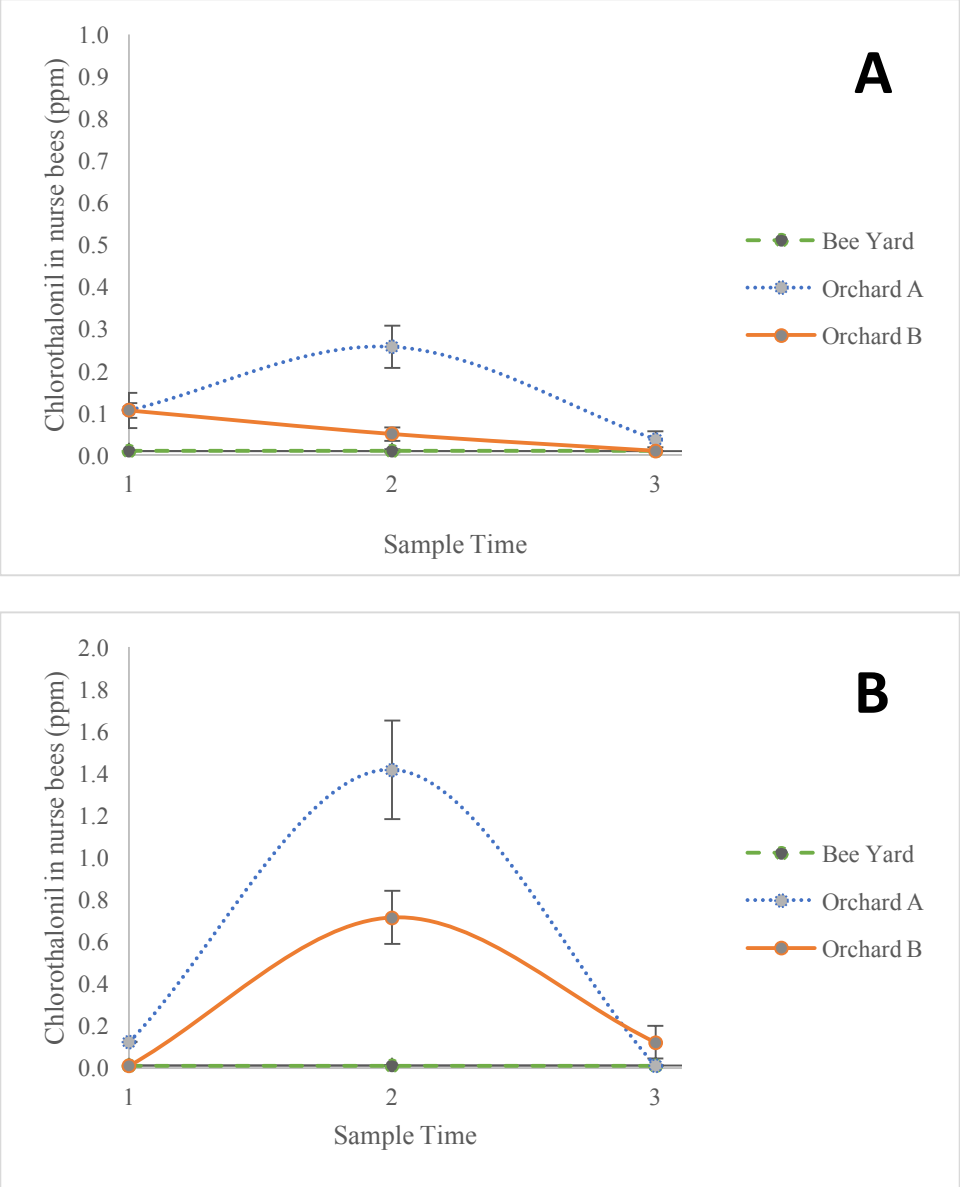

Figure 2.2. Average chlorothalonil (ppm) in nurse bees (± SEM) for 2015 (A) and 2017 (B) at the Bee Yard, Orchard A, and Orchard B sites. Sample time refers to pre-bloom (1), bloom (2), and post-bloom (3) in tart cherry.

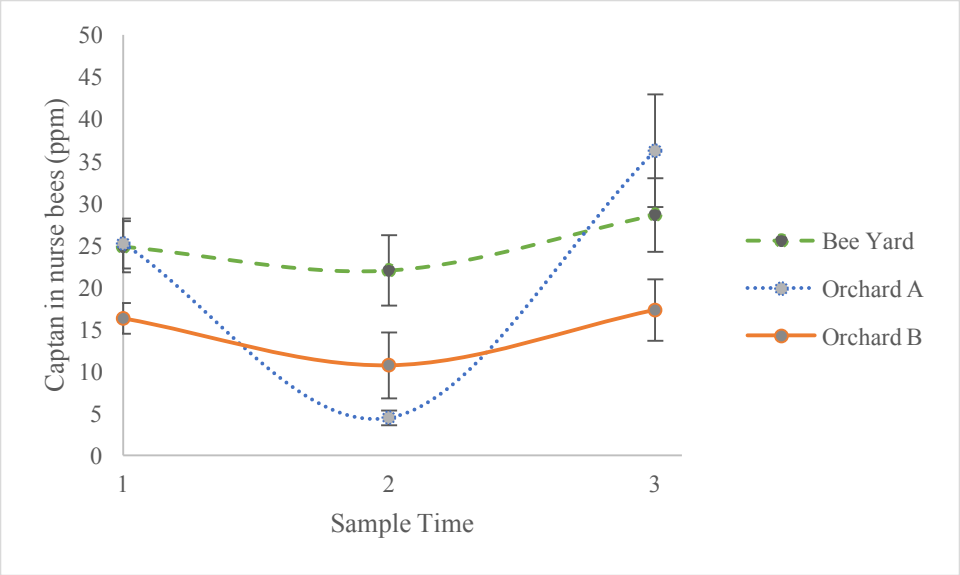

Figure 2.3. Average captan (ppm) in nurse bees (± SEM) for 2017 at the Bee Yard, Orchard A, and Orchard B sites. Sample time refers to pre-bloom (1), bloom (2), and post-bloom (3) in tart cherry.

Foragers

The maximum level of chlorothalonil detected in foragers returning to the orchard hives was 73.2 ppm, but no chlorothalonil was detected in foragers returning to bee yard hives (0.01 ppm LOD, Table 2.2). The tart cherry bloom period coincided with the peak of chlorothalonil residues from orchard hives in foragers (Figure 2.4). The maximum level of captan detected in foragers returning to the orchard hives was 54.7 ppm, and 9.2 ppm in foragers returning to the bee yard hives. At Orchard A, captan residues in foragers peaked during bloom, which was in contrast to the captan detection trends in nurse bees (Figure 2.5). Orchard B had high residues pre-bloom, but dropped to undetectable levels during and post-bloom. Residues of captan in foragers from the bee yard were low to undetectable in the bee yard site (Figure 2.5).

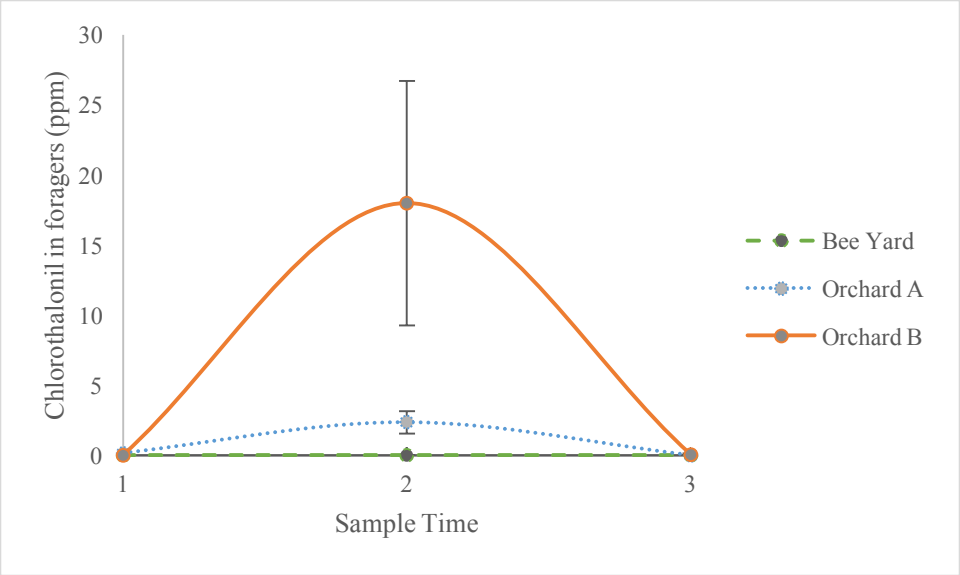

Figure 2.4. Average chlorothalonil (ppm) in foragers (± SEM) for 2017 at the Bee Yard, Orchard A, and Orchard B sites. Sample time refers to pre-bloom (1), bloom (2), and post-bloom (3) in tart cherry.

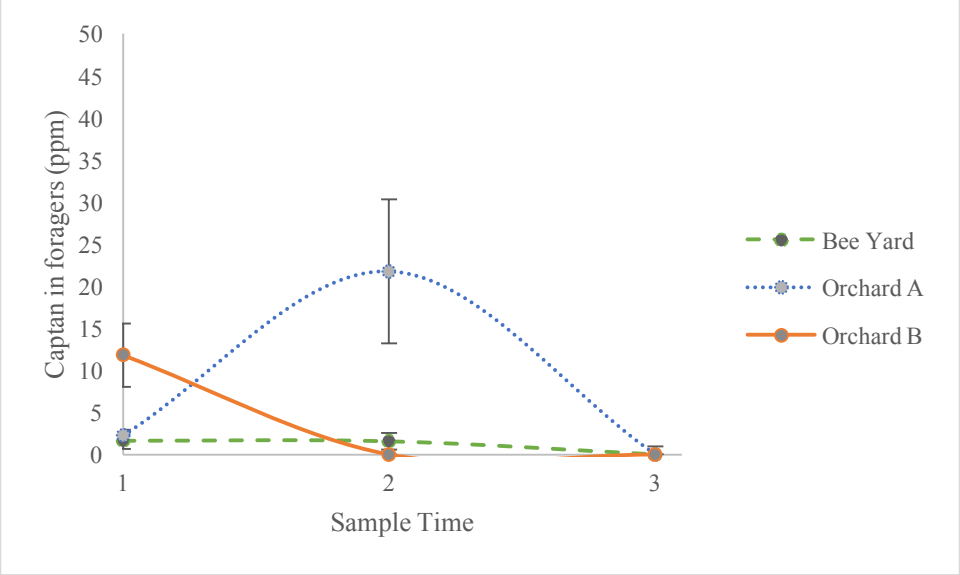

Figure 2.5. Average captan (ppm) in foragers (± SEM) for 2017 at the Bee Yard, Orchard A, and Orchard B sites. Sample time refers to pre-bloom (1), bloom (2), and post-bloom (3) in tart cherry.

Pollen

Chlorothalonil (Figure 2.6) and captan (Figure 2.7) were detected in all but one of the pollen samples throughout the study, at maximum levels of 108.8 and 25.8 ppm, respectively. In 2017, when we had pollen samples from both orchard and bee yard sites, chlorothalonil residue levels from the orchard samples were 7 times greater than bee yard samples (Table 2.3). Similar levels of captan were detected in the pollen samples from the orchard and bee yard sites, with numerically higher levels from bee yard samples (Figure 2.7).

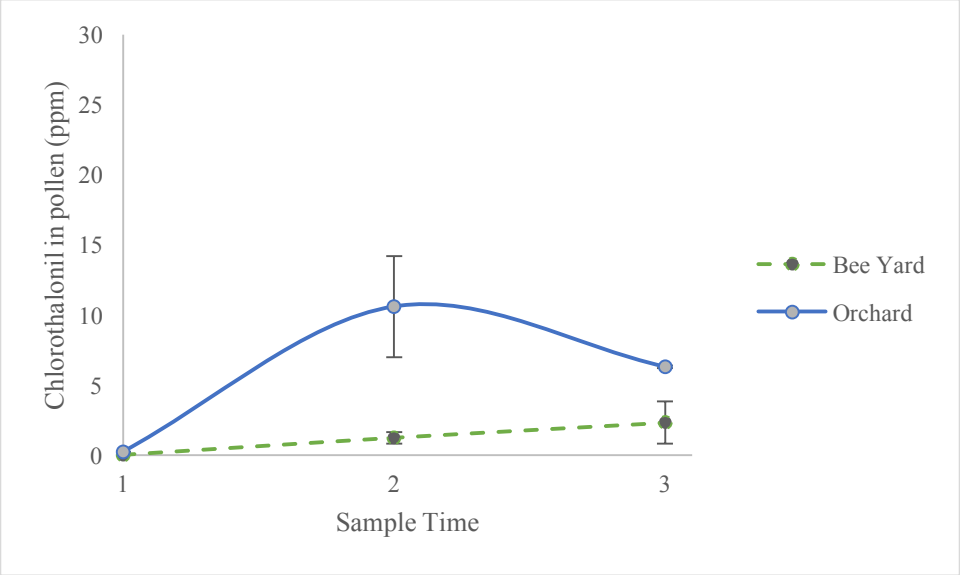

Figure 2.6. Average chlorothalonil (ppm) in pollen (± SEM) for 2017 at the Bee Yard and Orchard A sites. Sample time refers to pre-bloom (1), bloom (2), and post-bloom (3) in tart cherry.

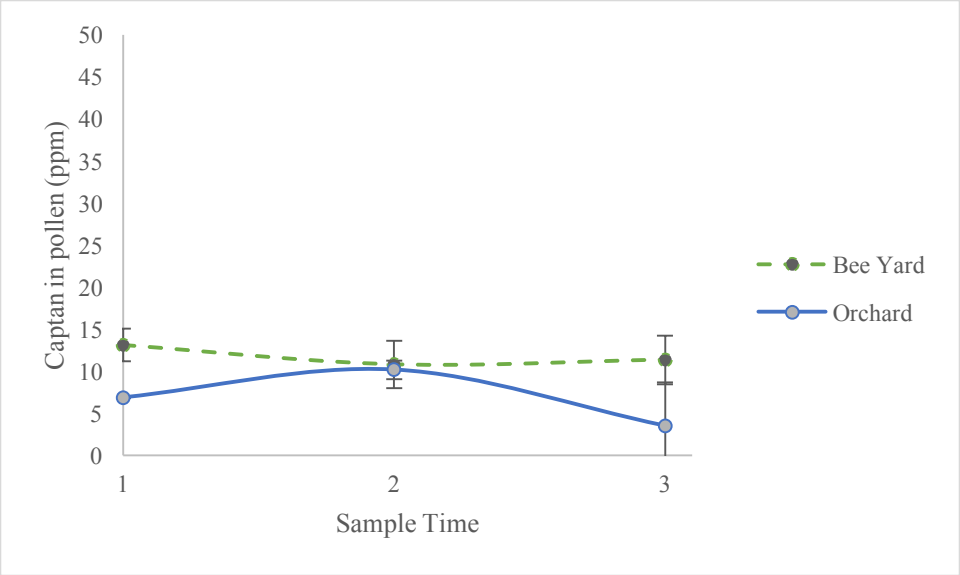

Figure 2.7. Average captan (ppm) in pollen (± SEM) for 2017 at the Bee Yard, Orchard A, and Orchard B sites. Sample time refers to pre-bloom (1), bloom (2), and post-bloom (3) in tart cherry.

Wax

The maximum level of chlorothalonil detected in wax was 0.67 ppm from orchard hives, and 0.07 ppm from bee yard hives (Figure 2.8). No captan was detected in any wax samples (Table 2.4).

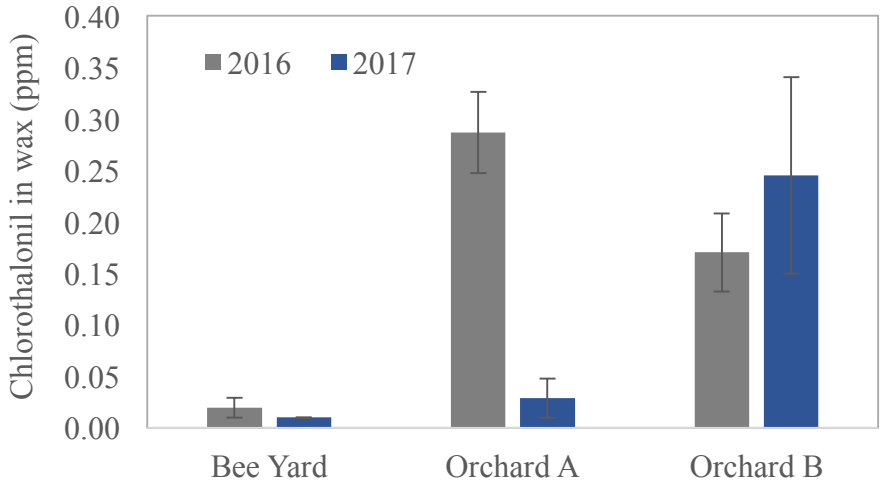

Figure 2.8. Average chlorothalonil (ppm) in wax (± SEM) for 2016 and 2017 at the Bee Yard, Orchard A, and Orchard B sites during bloom in tart cherry.

Bee Bread

The maximum level of chlorothalonil detected in bee bread was 10.3 ppm from orchard hives, and 0.85 ppm from bee yard hives (Figure 2.9). Captan was higher in the bee bread samples from the bee yard (30.9 ppm max) than in bee bread samples from the orchard (20.3 ppm max) (Figure 2.9).

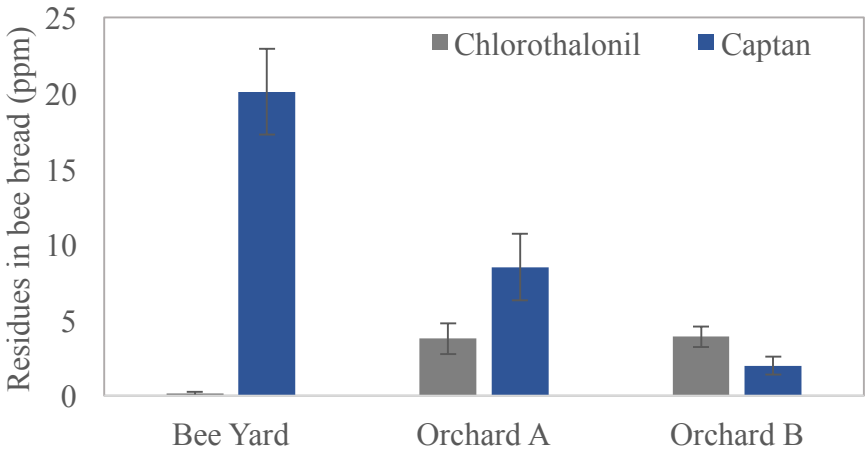

Figure 2.9. Average chlorothalonil and captan residues (ppm) in bee bread (± SEM) from 2017 at the Bee Yard, Orchard A, and Orchard B sites during bloom in tart cherry.

Table 2.3. Average and maximum detections of chloro. (chlorothalonil) and captan for each sample type collected before, during, and after tart cherry bloom in 2015, 2016, and 2017.

Sample Type	Year	Location	Timing	Avg. Chloro. ± SEM (ppm)	Max. Chloro. (ppm)	Avg. Captan ± SEM (ppm)	Max. Captan (ppm)
Nurse bees	2015	Bee Yard	Pre-Bloom	0.01 ± 0.00	ND	0.02 ± 0.00	ND
			Bloom	0.01 ± 0.00	ND	0.02 ± 0.00	ND
			Post-Bloom	0.01 ± 0.00	ND	0.02 ± 0.00	ND
		Orchard A	Pre-Bloom	0.11 ± 0.04	0.32	0.02 ± 0.00	ND
			Bloom	0.26 ± 0.05	0.58	0.02 ± 0.00	ND
			Post-Bloom	0.04 ± 0.02	0.15	0.02 ± 0.00	ND
		Orchard B	Pre-Bloom	0.11 ± 0.02	0.13	0.02 ± 0.00	ND
			Bloom	0.05 ± 0.02	0.14	0.02 ± 0.00	ND
			Post-Bloom	0.01 ± 0.00	ND	0.02 ± 0.00	ND
	2016	Bee Yard	Pre-Bloom	0.01 ± 0.00	ND	2.57 ± 2.56	17.91
			Bloom	0.08 ± 0.17	0.47	1.32 ± 0.89	6.51
		Orchard A	Pre-Bloom	0.13 ± 0.08	0.33	0.02 ± 0.00	ND
			Bloom	0.01 ± 0.00	ND	0.02 ± 0.00	ND
		Orchard B	Pre-Bloom	0.11 ± 0.05	0.43	0.02 ± 0.00	ND
			Bloom	0.01 ± 0.00	ND	3.04 ± 3.03	24.22
	2017	Bee Yard	Pre-Bloom	0.01 ± 0.00	ND	24.84 ± 3.05	36.1
			Bloom	0.01 ± 0.00	ND	22.00 ± 4.18	47.4
			Post-Bloom	0.01 ± 0.00	ND	28.58 ± 4.38	40
		Orchard A	Pre-Bloom	0.12 ± 0.00	0.54	25.21 ± 2.97	35.8
			Bloom	1.42 ± 0.24	2.48	4.49 ± 0.87	7.94
			Post-Bloom	0.01 ± 0.00	ND	36.21 ± 6.71	75.3
		Orchard B	Pre-Bloom	0.01 ± 0.00	ND	16.29 ± 1.85	21.9
			Bloom	0.72± 0.13	1.42	10.71 ± 3.94	31.8

Table 2.3. (cont.)

Sample Type	Year	Location	Timing	Avg. Chloro. ± SEM (ppm)	Max. Chloro. (ppm)	Avg. Captan ± SEM (ppm)	Max. Captan (ppm)
			Post-Bloom	0.12 ± 0.08	0.57	17.30 ± 3.65	32.4
Foragers	2017	Bee Yard	Pre-Bloom	0.01 ± 0.00	ND	1.65 ± 1.09	8.85
			Bloom	0.01 ± 0.00	ND	1.59 ± 1.17	9.22
			Post-Bloom	0.01 ± 0.00	ND	0.02 ± 0.00	ND
		Orchard A	Pre-Bloom	0.16 ± 0.13	1.04	2.26 ± 0.68	4.16
			Bloom	2.37 ± 0.81	7.17	21.78 ± 8.57	54.7
			Post-Bloom	0.01 ± 0.00	ND	0.02 ± 0.00	ND
		Orchard B	Pre-Bloom	0.01 ± 0.00	ND	11.83 ± 3.80	23.9
			Bloom	17.99 ± 8.73	73.20	0.02 ± 0.00	ND
			Post-Bloom	0.07 ± 0.06	0.53	0.02 ± 0.00	ND
Pollen	2016	Orchard A	Pre-Bloom	41.98 ± 16.20	58.18	5.99 ± 0.18	0.59
			Bloom	16.27 ± 2.32	18.59	13.61 ± 1.60	7.6
		Orchard B	Pre-Bloom	13.99 ± 0.00	13.99	22.99 ± 0.00	22.99
			Bloom	65.70 ± 43.10	108.80	0.40 ± 12.18	25.8
	2017	Bee Yard	Pre-Bloom	0.01 ± 0.00	ND	13.10 ± 1.94	6.13
			Bloom	1.24 ± 0.41	1.58	10.80 ± 2.81	24.4
			Post-Bloom	2.32 ± 1.51	3.83	11.37 ± 2.88	12.3
		Orchard A	Pre-Bloom	0.24 ± 0.00	0.24	6.89 ± 0.00	4.85
			Bloom	10.60 ± 3.60	21.10	10.19 ± 1.09	7.75
			Post-Bloom	6.31 ± 0.11	6.41	3.55 ± 5.11	16.6

ND = no detections.

Table 2.4. Average and maximum detections of chlorothalonil and captan for each sample type collected on frames during tart cherry bloom in 2015, 2016, and 2017.

Sample Type	Year	Location	Avg. Chlorothalonil ± SEM (ppm)	Max. Chlorothalonil (ppm)	Avg. Captan ± SEM (ppm)	Max. Captan (ppm)
Larvae	2015	Bee Yard	0.01 ± 0.00	ND	0.02 ± 0.00	ND
		Orchard A	0.01 ± 0.00	ND	0.02 ± 0.00	ND
		Orchard B	0.01 ± 0.00	ND	0.02 ± 0.00	ND
	2017	Bee Yard	0.01 ± 0.00	ND	0.02 ± 0.00	ND
		Orchard A	0.01 ± 0.00	ND	0.02 ± 0.00	ND
		Orchard B	0.01 ± 0.00	ND	0.02 ± 0.00	ND
Wax	2016	Bee Yard	0.02 ± 0.01	0.07	0.02 ± 0.00	ND
		Orchard A	0.29 ± 0.04	0.43	0.02 ± 0.00	ND
		Orchard B	0.17 ± 0.04	0.31	0.02 ± 0.00	ND
	2017	Bee Yard	0.01 ± 0.00	ND	0.02 ± 0.00	ND
		Orchard A	0.03 ± 0.02	0.16	0.02 ± 0.00	ND
		Orchard B	0.25 ± 0.10	0.67	0.02 ± 0.00	ND
Bee Bread	2017	Bee Yard	0.13 ± 0.12	0.85	20.10 ± 2.83	30.9
		Orchard A	3.76 ± 1.01	10.30	8.49 ± 2.20	20.3
		Orchard B	3.89 ± 0.67	7.84	1.99 ± 0.58	5.15

ND = no detections.

Pollen Identification

Similar kinds of pollen were collected by bees from the bee yard and orchard hives during the study, however the proportion of these pollens differed by site and sample time (Figure 2.10). For example, the proportion of *Prunus* pollen increased from 14% in the pre-bloom samples to 51% in the post-bloom samples from bee yard hives, but decreased from 85% of the pre-bloom samples down 10% of the post-bloom samples from hives placed next to tart cherry orchards. The *Prunus* genus includes tart cherry, sweet cherry (blooms earlier than tart cherry), and wild black cherry (blooms after tart cherry) (USDA NRCS 2018). *Acer* and *Taraxacum,* which bloom April-June and April-September, respectively, made up a majority of the pollens found in the bee yard pollen traps during the pre-bloom and bloom periods. Acer comprised 70% and *Taraxacum* 66% of all the pollens collected during that time, but dropped to a total of only 5% during post-bloom. In contrast, *Acer* and *Taraxacum* made up only 14% of pollen samples from hives next to orchards during pre-bloom, 19% during bloom, and 35% post-bloom. *Salix, Malus*, and *Brassica* (most species bloom in May) were important sources of pollen during and post-bloom in the orchard hives. *Quercus* pollen was collected often during post-bloom at the bee yard and orchard sites. Pollen from *Malus,* which blooms after tart cherry, was collected in trace amounts in the bee yard hives, and only comprised of at most 7% of the proportion of pollen in orchard hives.

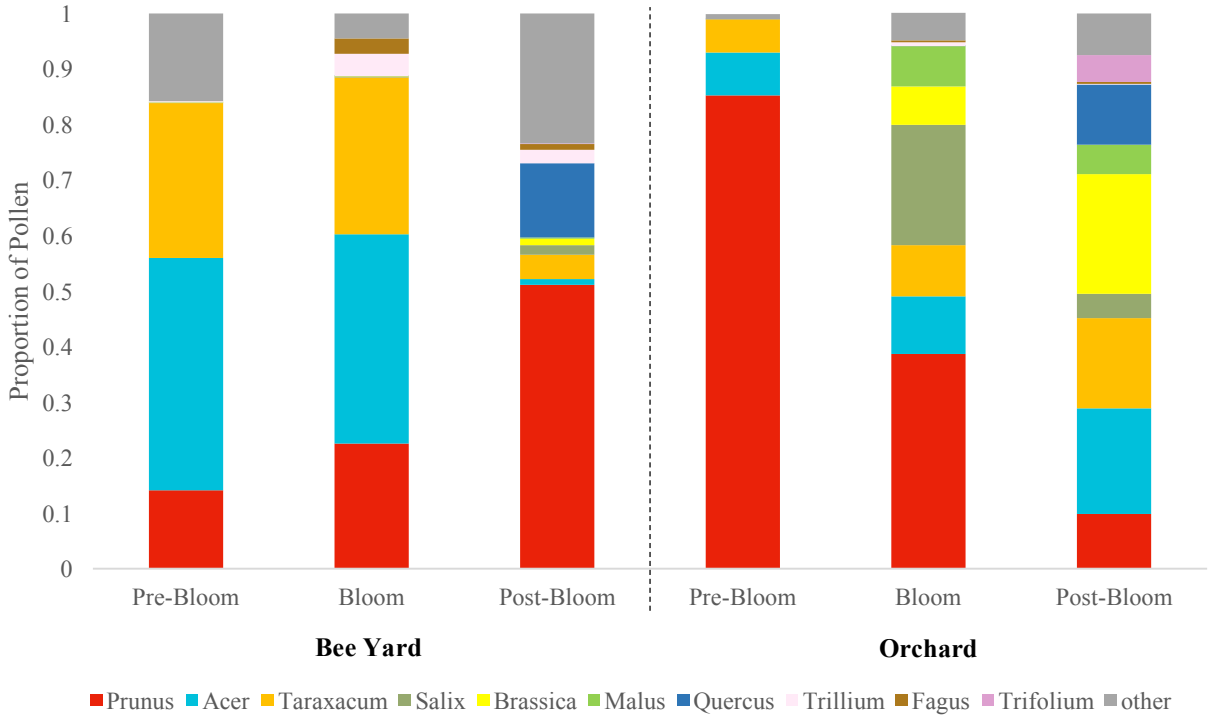

Figure 2.10. Composition of pollen collected from bee yard and orchard hives before, during, and after tart cherry bloom in 2017.

DISCUSSION

Field-level Fungicide Exposure

The results from this study indicate that tart cherry and apple orchards are an attractive floral resource for honey bees in the spring and that orchards can serve as a source of significant fungicide exposure both to bees pollinating the crop, and to bees in holding yards nearby. Samples from the orchard hives generally had higher detection frequencies and higher average fungicide levels compared to samples collected from the bee yard hives. Increasing concerns about fungicides and honey bee health is particularly relevant to fruit production in the Great Lakes region of the United States because of the high fungal disease pressure during bloom while bees are providing vital pollination services. In order to effect change in grower practices

that could reduce pesticide exposure in honey bees, we first needed to quantify the level of exposure occurring under realistic field conditions.

Pollen samples contained the most frequent detections of fungicides and often had the highest residue levels compared to other sample types in this study, as well as in previous studies. For example, prior nation-wide surveys of hives kept by migratory beekeepers have shown chlorothalonil to be among the pesticides most commonly found in pollen (Mullin et al. 2010, Sanchez-Bayo and Goka 2014). The average level of chlorothalonil found in pollen from past studies ranged from 0.0073 ppm (Roszko et al. 2016) to 4.491 ppm (Pettis et al. 2013,) with maximum levels ranging from 29 ppm (Pettis et al. 2013) to 98.9 ppm (Mullin et al. 2010). In this study, average chlorothalonil in pollen samples from bee yard hives ranged between 1.24 to 2.32 ppm, but pollen from the orchard hives ranged between 0.24 to 65.7 ppm, with a max detection of 108.8 ppm. The range of chlorothalonil residues in pollen detected in this study is similar to the chlorothalonil residues detected in past studies. Our pollen samples from 2016 are among the highest levels previously recorded, while the 2017 pollen samples are more similar to the lowest averages recorded. Captan has also been frequently found in pollen samples in previous studies, with average residue detections from ranging from 0.43 ppm (Mullin et al. 2010) to 2.99 ppm (Kubik et al. 2000), and maximum recorded detections ranging from 10 ppm (Mullin et al. 2010) to 13.8 ppm (Pettis et al. 2013). Our study showed higher averages of captan than what has previously been observed, with average levels in pollen up to 22.99 ppm, and a maximum detection of 25.8 ppm.

We detected higher levels of the fungicides chlorothalonil and captan in adult honey bees compared with the study by Mullin et al. (2010) in which they found maximum chlorothalonil residues of 0.88 ppm and maximum captan residues of 0.043 ppm. In our study, where we tested

nurse bees separately from foragers, we found maximum detections of 2.5 ppm chlorothalonil and 75.3 ppm captan in nurse bees, and 73.2 ppm chlorothalonil and 54.7 ppm captan in foragers during bloom. The present study is the first to our knowledge to analyze residue levels of foragers and nurse bees separately. In 2017, foragers had higher average levels of chlorothalonil than nurse bees, with averages ranging from 0.07 to 17.99 ppm in foragers and only 0.12 to 1.42 ppm in nurse bees. The opposite was observed for captan residues in 2017, with nurse bees having higher average residues ranging from 4.49 to 36.2 ppm and forager residue averages ranging only from 1.59 to 21.78 ppm. The difference between forager and nurse bee residues for chlorothalonil and captan may relate to the different exposure routes, processing, and pharmacokinetics of the two pesticides.

Pollen samples had a very high frequency of positive fungicide detections throughout the study, with 90% of pollen samples containing chlorothalonil residues, and 100% of pollen samples containing captan residues. In contrast, the adult bee samples had much lower frequency of detections, with only 48% of adult bee samples containing chlorothalonil residues, and 51% with detectable captan residues. The maximum residues of chlorothalonil detected in pollen samples were also higher than the residue levels detected in nurse bees and foragers. The high residue levels in pollen samples and lower residues in adult bees suggest that pollen is a primary source of pesticide exposure to bees, and that adults are somehow able to at least partially detoxify the pesticides after they are brought in to the hive, possibly through food processing and storage, digestion, metabolism, P450 activity, etc. (Vannette et al. 2015, Gong and Diao 2017). Fungicide residues were still frequently detected in adult bees, however, suggesting that the bees are not able to completely detoxify the fungicides they are exposed to and that some fungicide accumulation within their bodies does occur during bloom. Recent studies have demonstrated

that compared to other insects, honey bees have fewer numbers of detoxifying genes, which then makes them more susceptible to the toxic effects of most fungicides and insecticides commonly used in agriculture (Gong and Diao 2017).

Bee bread is a highly-understudied hive matrix, especially in regard to the fungicide chlorothalonil. In one study that investigated fungicide residues in bee bread, chlorothalonil was not detected in bee bread samples from healthy hives, nor in bee bread from hives with disorders (dead or diseased colonies) (Simon-Delso et al. 2015). In the same study, captan was detected in bee bread from colonies with disorders at an average of 1.90 ppm. Other studies have also detected captan in bee bread, at average residue levels of 3.43 ppm and 6.39 ppm (Kubik et al. 2000, Pohorecka et al. 2017). Our average detections in bee bread were similar to these studies in our orchard hives (5.2 ppm), but much higher than these past findings in our bee yard hives (20.1 ppm). Kubik et al. (1999) screened for a different set of fungicides in hives next to cherry orchards and found that bee bread was the most contaminated hive matrix over honey and pollen. McArt et al. (2017) found that fungicides accounted for 94% of the pesticide residues detected in fresh bee bread accumulated during apple bloom.

Previous studies have found average chlorothalonil residues in wax range from 0.02 ppm (Wu et al. 2011) to 1.07 ppm (Mullin et al. 2010), with a maximum recorded detection of 53.7 ppm (Mullin et al. 2010). Our detections in wax had similar average levels, with a much lower maximum of only 0.67 ppm. Although our study found no detectable levels of captan in wax samples, other studies have found captan in wax with residues ranging from 0.05 ppm (Mullin et al. 2010) to 3.1 ppm (Simon-Delso et al. 2015). Traynor et al. (2016) found that the number of fungicides with multi-site activity, which includes both chlorothalonil and captan, increased significantly in the wax of colonies that died or experienced queen death.

Not surprisingly, samples from the orchard sites had higher detection frequencies and higher average fungicide levels compared to samples collected from the bee yard. Chlorothalonil residues were higher in samples from orchard sites compared to the bee yard in every sample type collected, except larvae (which had no chlorothalonil detections at any site). For example, there were no chlorothalonil detections in foragers collected from the bee yard during any time, contrasted with 100% of foragers from the orchard sites at bloom having chlorothalonil detections (max. 73.2 ppm). The same trend of higher detection frequencies and average residue levels in orchard hives was also true for captan. The only exception to this trend was in 2017 when the bee yard pollen contained higher captan levels than the pollen from orchard sites, which then coincided with higher captan residues in bee bread and nurse bee samples from the bee yard during bloom that year. The higher captan residues in the bee yard site in 2017 may be attributed to non-orchard crops nearby or home owner applications, as captan is commonly used for a wide range of agricultural commodities, lawns, and ornamentals (CDMS 2018).

The only pesticide residue found in larvae in our study was thiophanate-methyl, which was found in only one larvae sample throughout the study. Thiophanate-methyl was not detected in any other sample type during the study period. The low residues detected in larvae samples could be attributed to the timing of the larvae collection. In this study, brood frames were removed during peak tart cherry bloom, so the pesticides being brought in to the hive during bloom may not have been processed fast enough to make it into the developing larvae's food source prior to being collected. Thiophanate-methyl is a systemic fungicide registered for use in stone fruit and apples, and it is commonly tank mixed with captan when applied. Thiophanate methyl is labeled for brown rot control in cherries, and although this is a common disease problem during pre-bloom and bloom, the product is seldom used in Michigan orchards due to a

high incidence of fungicide resistance (Michigan Fruit Management Guide 2018). Systemic pesticides are often considered to pose a greater exposure risk for bees because they can be translocated through the plant and may accumulate in the plant's nectar and pollen resources. It is possible that pollinator safety discussions in pesticide education programs has encouraged a shift away from the use of thiophanate-methyl in cherries prior to the flowering period as an attempt to minimize exposure risk to bees through systemic exposure. Grower education and surveys may be a useful tool to determine if the risk to bees has influenced the reduced use of systemic pesticides in orchards prior to and during bloom.

Previous studies have found high levels of insecticide residues in honey bee hives (Chauzat et al. 2009, Mullin et al. 2010). These studies were in non-orchard crops, or at other times during the season. Chlorpyrifos was the only insecticide screened in this study, and was detected in only 3 nurse bee samples from the bee yard in 2015, with average detection of 0.02 ppm, and a maximum of 0.05 ppm. This organophosphate insecticide is often used on apple trees during pre-bloom to protect against an early season apple insect pest complex that includes San Jose scale, rosy apple aphid, oblique-banded leafroller, and dogwood borers (Calatayud-Vernich et al. 2016). Chlorpyrifos is only registered for post-bloom foliar use in tart cherries (Michigan Fruit Management Guide 2018), so exposure likely occurred from interactions with nearby apple orchards. In Michigan, insecticides are largely restricted or discouraged from use during orchard bloom due to concerns about pollinator exposure (Michigan Fruit Management Guide 2018). Throughout our study, insecticide applications were only made in the tart cherry orchards adjacent to our study hives during the post-bloom period. Not surprisingly, our study found the chlorpyrifos exposure to be very low for bees during apple and cherry bloom in Michigan. It would be beneficial to also look at exposure risk for the other early spring insecticides that are

commonly applied at petal fall in orchards, such as thiamethoxam, phosmet, and lambda-cyhalothrin (all of which were applied by our grower-cooperator shortly after bloom at some point during the study period).

The herbicide pendimethalin was found in 100% of the pollen samples collected from orchards in 2016, but not in any other sample type or year. Herbicide applications are often made in the early-spring to kill weed species that may compete with the crop for vital resources. The rates of pendimethalin were very low in our study. This is in opposition to findings from other studies that have found other herbicide residues to be common in honey bee hives. The direct and sub-lethal health effects of these products need to be further investigated in future studies (Bogdanov 2006, Mullin et al. 2010).

Implications for Bee Health

The levels of chlorothalonil detected in this study often exceeded the levels known to cause negative health effects in previous toxicology studies. For example, Pettis et al. (2013) found that *Nosema* infections increased in bees that consumed pollen with high fungicide loads, with chlorothalonil residues in pollen of only 4.49 ppm coinciding with increased susceptibility to Nosema infection and a decreased ability to withstand the infection. Another study observed a dramatic increase in larval mortality when larvae were reared on a diet containing chlorothalonil at 34 ppm, compared to adult honey bee mortality (Zhu et al. 2014). Although 34 ppm in pollen is a rather high dose, many of the pollen samples in our study contained chlorothalonil residues above this level, with our maximum detections reaching as high as 108.8 ppm. Food contaminated with as little as 0.01 ppm chlorothalonil (same as our study's LOD) fed to honey bees in vitro was shown to significantly alter the structure and functional potential of the

bacterial community within the honey bee gut (Kakumanu et al. 2016). Increases in disease susceptibility and larval mortality, as well as changes to the functional potential of the honey bee bacterial community, can have serious repercussions for bee health. The levels of chlorothalonil detected in this study present a serious risk to honey bees from those health effects under current disease management during spring orchard bloom.

In addition to these risks, chlorothalonil residues in bee bread have been associated with a phenomenon called "entombed pollen", first described by VanEngelsdorp et al. (2009). Entombed pollen is characterized as bee bread that is sunken and brick-red in color, with high pesticide levels and low levels of the microbial agents commonly associated with stored pollen. High levels of chlorothalonil residues were found in 100% of entombed pollen samples previously studied, with residues at an average of 1.35 ppm. The chlorothalonil residues in entombed pollen were 40 times higher than the chlorothalonil in non-entombed samples (VanEngelsdorp et al. 2009). In our study, all but two of the average chlorothalonil residues in positive pollen samples were higher than the levels formerly found in entombed pollen. The chlorothalonil residues in bee bread from orchard sites were also higher than the residues found in entombed pollen, with averages of 3.76-3.89 ppm. Colonies with entombed pollen have been shown to have higher rates of mortality (43%) compared to colonies without entombed pollen (20% mortality) (VanEngelsdorp et al. 2009), suggesting that the chlorothalonil residues found in the present study may lead to an increase in entombed pollen and eventual colony mortality. However, we did not observe any entombed pollen during this study.

The levels of captan detected also exceeded the levels known to cause a variety of negative health effects on honey bees. Pettis et al. (2013) found that *Nosema* infections increased in bees that consumed pollen with captan residues of only 0.98 ppm. The amount of captan in

pollen that was associated with this increased susceptibility to *Nosema* is well below the average captan levels in pollen detected in our study. In a laboratory study conducted by Mussen et al. (2004), no larvae completed development to adulthood after being fed a diet containing a very high dose of 80 ppm captan. Although the dose of captan administered in that study was higher than the levels of captan we observed in pollen and bee bread samples, the severity of the larval response to captan is highly concerning. Future studies should investigate the effects of field-relevant captan doses on larval development.

Environmental Influences on Exposure

Yearly variability in weather and disease pressure determine the need for growers to make fungicide applications, and therefore impact the potential fungicide exposure risk for bees. The spring of 2017 was particularly rainy. As a consequence, more protective fungicide applications were made by our grower cooperator before and during bloom that season, and fungicide residue levels detected were higher on average than the residues detected in 2015 or 2016 (with chlorothalonil residues in pollen from orchards in 2016 being the only exception). Disease pressure during each sampling season was determined using disease models on the MSU Enviroweather website (2018). Our study showed higher residue levels of captan in nurse bees during periods of higher apple scab disease pressure. For example, in 2017 moderate disease pressure for apple scab was reported immediately before the pre-bloom and post-bloom sampling periods. This increase in disease pressure coincided with higher residues of captan in nurse bee samples during pre-bloom and post-bloom across all sites, and lower residues during tart cherry bloom when apple scab risk was low. We also observed lower chlorothalonil detections when disease models predicted there was “no risk” of cherry leaf spot infection. For example, in 2016

when there was no risk for cherry leaf spot during tart cherry bloom, this corresponded with no chlorothalonil detections in nurse bees during bloom at orchard sites. Fruit growers in Michigan use these models to determine when fungicide applications are absolutely necessary and avoid sprays whenever there is not a risk of infection. It may also be beneficial for beekeepers to utilize these disease models to assess the current season's potential pesticide exposure risks within particular crops, and to develop pollination contracts and management strategies based on that potential risk.

The pollen identification revealed that *Prunus*, *Acer, Taraxacum*, *Salix, Brassica*, *Malus*, *Quercus, Trillium, Fagus,* and *Trifolium* are important floral resources for honey bees in the early spring. Weeds on the orchard floor, such as *Taraxacum* (dandelion), *Brassica* (wild mustards), and *Trifolium* (clover), could be a possible non-crop route of fungicide exposure for honey bees. For instance, we observed a high percentage of dandelion pollen in samples from the bee yard that also contained high levels of captan. These same pollen samples from the bee yard contained little to no apple pollen, suggesting that the captan may have drifted onto dandelions within the nearest orchard leading to a high level of exposure for the bee yard bees, despite their low-visitation of apple flowers. One of the best management practices that growers can implement to reduce this potential hazard is to mow or otherwise remove flowering weeds from the orchard floor prior to applying plant protectants.

The high percentage of *Prunus* pollen from orchard samples collected during the pre-bloom period is likely from the nearby sweet cherry orchards that bloom slightly earlier than tart cherry trees. A surprisingly high percentage of *Prunus* pollen was also returned at the bee yard during the post-bloom sampling period. We assume that this pollen came from non-cultivated *Prunus* species, such as the Black Cherry (*Prunus serotina).* Black Cherry trees are known to

flower in late May to early June, which coincides with the post-bloom sampling period (USDA NRCS 2018).

Access to diverse flower resources early in the growing season may help to reduce the negative health effects associated with pesticide exposure, improve colony health, and lead to increased pollination efficiency. Colwell et al. (2017) found a significant negative correlation between floral diversity and pollen hazard quotients, suggesting that higher floral diversity can reduce the severity of honey bee pesticide exposure. The quality and diversity of pollen can shape honey bee physiology and greatly influence bee nutrition and health (Pasquale et al. 2013). Growers can plant or conserve flowering species that bloom in the early spring to provide honey bees with access to non-sprayed flowers and a diverse diet while they are being rented in orchards for pollination services.

Future Research

More research is necessary to understand the best ways to balance the need for orchard disease management and the need for healthy bee hives for pollination. We now know the specific exposure rates that bees experience just prior to, during, and just after tart cherry bloom in Michigan. Future studies should investigate how honey bee health directly relates to the fungicide accumulation within hives being rented for orchard pollination. Many management recommendations are currently made to growers in an attempt to reduce bee pesticide exposures from bloom-time applications. Some of these recommendations include spraying at night when bees are inactive, placing hives in buffered locations (behind tree lines on hill sides), avoiding applications at particular percentages of peak bloom, and carefully calibrating sprayers (May et al. 2015). It would be valuable to quantify the fungicide residues in bee hives under these

suggested strategies and compare those levels to the residues found in this study under current standard orchard management practices.

During apple bloom, bacterial infections such as fire blight (*Erwinia amylovora*) are also a common disease management concern for growers. Despite the high risk of fire blight being spread during bloom, little attention has been paid to the effects of antibiotic sprays used to control this disease on honey bee health. It would be interesting to investigate the health impact of antibiotic sprays on bees, how much antibiotic accumulates in hives, and if those detected residue levels have dangerous sub lethal effects on honey bee health.

Native pollinators, such as bumble bees and solitary bees, supplement honey bee pollination in many specialty crops, and are therefore also exposed to a wide range of pesticides that can have detrimental effects on their health (Hladik et al. 2016). The number of studies that have examined the risks of fungicides for native bees are few compared with the number of honey bee studies on the subject. The reactions of honey bees to fungicides cannot be extrapolated to native bees, as the small number of native bee species that have been studied have been shown to respond differentially to fungicides than honey bees (Biddinger et al. 2013, Sgolastra et al. 2017). Future studies also need to include other bees and pollinators as model species in pesticide exposure studies and risk assessments.

CONCLUSION

Orchards are an attractive floral resource for honey bees in the spring, so even if hives are not placed directly in an orchard, foragers will still visit orchards and bring fungicide residues back to their hives. Our study suggests honey bee hives that are within foraging range of orchards are exposed to fungicides in the early spring, even if the hives are not being rented for

pollination. The residue levels detected in this study exceed levels previously associated with negative health effects for bees, such as increased disease susceptibility, increased larval mortality, changes in the gut microbiome, and overall colony loss. Weather and disease pressure influence the number of fungicide applications made, and therefore play a significant role in the exposure risks for bees. Growers need to utilize disease models to reduce sprays as much as possible, only spraying when disease pressure is high enough to warrant an application that could be potentially dangerous for bees. Disease models might also be utilized by beekeepers to assess potential exposure risks for hives on pollination contracts. Growers can plant or conserve flowering tree species outside of orchards to provide pollinators access to non-sprayed flowers and a more diverse diet during the crop-pollination period. Access to diverse floral resources early in the season may help improve colony health and lead to increased pollination efficiency. Pollen analysis results indicate that a combination of pollens from cultivated and wild *Prunus*, and *Malus*, woodland plants common to Michigan such as *Acer*, *Salix*, *Quercus*, *Fagus*, and *Trillium*, and herbaceous perennials commonly found in orchard floors or as cover crops, such as *Taraxacum*, *Brassica*, and *Trifolium* are important resources for honey bees in the early spring. Pesticide drift onto nearby flowering weeds is a likely source of fungicide contamination. Removing flowering weeds from the orchard floor could reduce the amount of pesticides returned to the hive in pollen from non-cultivated floral resources. Providing access to nearby floral resources that are protected from sprays also has potential to greatly increase the health of rented bees and reduce the negative health effects associated with pesticide exposure. We strongly suggest that growers mow dandelions down before applying any plant protectants to their orchards, and that they provide non-sprayed floral resources nearby to boost colony strength.

There are still many gaps in our understanding of how pesticide exposure relates to honey bee colony health at the field level. These suggested strategies may help to reduce exposure risk for bees, but the struggle to balance pest management and pollinator health will continue. Further research is needed on how to best balance crop disease management with the need for pollination services by honey bees.

CHAPTER 3: REPELLENCY AND ATTRACTION EFFECTS OF BLOOM-TIME FUNGICIDES ON HONEY BEES IN APPLE ORCHARDS

INTRODUCTION

Honey bees (*Apis mellifera*) contribute more than $15 billion in value to the United States economy each year through pollination services, making them the most important pollinator of agricultural crops (Holdren 2015). Growers of tree fruit, including cherries and apples, typically rent honey bee hives from beekeepers to increase pollination services on their farm, which increases fruit yield and quality (Delaplane and Mayer 2000). Apples are particularly dependent on bees because they require cross-pollination with a compatible apple variety. Inadequate pollination can result in premature fruit abscission, or deformed and unmarketable fruit (McGregor 1976).

Over the past 60 years, honey bee colony numbers in the United States have declined by 45% (Johnson et al. 2010). Factors implicated in the decline include sociological and economic drivers associated with an aging cohort of beekeepers and low honey prices, as well as a number of environmental factors such as parasites, diseases, habitat loss, pesticide exposure, and climate change (Pettis et al. 2013). Pesticide exposure is a primary concern for the health of honey bees that are used in commercial pollination. Bees can be exposed to agrochemicals through many different pathways such as direct spray, contact with treated crops, drift onto nearby floral resources, and water contamination through runoff (Sanchez-Bayo and Goka 2014). Because honey bee pollination services are vital to the production of many fruit and vegetable crops, continued colony losses could be detrimental to agricultural production and the economy.

During orchard bloom in North America east of the Mississippi River, growers commonly use fungicides to protect their crops from key diseases that can either infect newly

emerged green tissue or cause blossom blight. Apple scab (*Venturia inaequalis*) is an economically important disease of apples with a primary infection period that overlaps with bloom. Unprotected tissue will lead to secondary infections that cause defoliation, as well as damaged and unmarketable fruit when under severe disease pressure (Sutton et al. 2014). Early-spring fungicide applications to control apple scab frequently occur while bees are actively pollinating the crop.

Fungicides were previously defined as relatively safe for use around adult honey bees based on acute exposure lethality studies. Current research, however, suggests that exposure of honey bees to fungicides can have sub-lethal effects that threaten honey bee health at the colony level in a variety of ways, including abnormal larval development (Mussen et al. 2004, Zhu et al. 2014), a decrease of beneficial gut bacteria (Yoder et al. 2013), an increase in the impact of honey bee diseases (Pettis et al. 2013), and detrimental effects on honey bee foraging behavior, learning, and memory (Decourtye et al. 2005). Honey bee foragers must evaluate potential food resources in the field and make important decisions about their quality in terms of the colony's collective needs, thus the preferences and behaviors of foragers can have a remarkable impact on the nutrition and health of their hive (Liao et al. 2017). Orchards in bloom provide high quality pollen and nectar resources and are therefore attractive to honey bee foragers, however honey bees visiting these orchards are likely to encounter fungicide residues that may be problematic.

In an effort to reduce honey bee pesticide exposure in croplands, previous researchers have attempted to identify chemical repellents, some of which include fungicides. For example, in the late 1980s, the fungicides Pallitop (nitrothal-isopropyl), Topas (penconazole), Bayleton (triadimefon), and Melprex (dodine) showed promise for use as honey bee repellents in apple orchards (Solomon and Hooker 1989). However, these products are no longer registered for use

in apples, or have been discontinued by the manufacturer due to US Environmental Protection Agency regulations related to farm worker safety (Michigan Fruit Management Guide 2018). Not all fungicides show repellency effects; for example, in semi-field, free-flight experiments, bees displayed a preference for feeding on sugar water containing the fungicide chlorothalonil over sugar water alone (Liao et al. 2017). This preference for chlorothalonil was indicated by both consumption ratios and visitation frequency ratios at an extremely low concentration of only 0.0005 ppm chlorothalonil (Liao et al. 2017). The preference for chlorothalonil-treated food may explain the high frequency at which chlorothalonil is found as a hive contaminant (Mullin et al. 2010, Sanchez-Bayo and Goka 2014). The use of fungicides to repel honey bees may be a useful practice to reduce bee pesticide exposure in crops that have flowers attractive to bees, but do not require pollination, such as asparagus (Delaplane and Mayer 2000). However, in crops dependent on bee-mediated pollination, the use of fungicides may interfere with pollination when the fungicides are repellent, or there may be an increase of fungicide exposure in honey bee hives when fungicides are either attractive or not repellent to honey bees.

Post-bloom, fungicides are often tank-mixed with insecticides. Pyrethroid insecticides on their own are known to repel honey bees, but when combined with certain fungicides, they can significantly reduce this repellency effect (Thompson and Wilkins 2003). A choice or no-choice cage study revealed that the fungicides chlorothalonil, difenoconazole and tebuconazole significantly reduced the repellency effect of the insecticide alpha-cypermethrin, while the fungicides prochloraz, flusilazole, and propiconazole significantly reduced the repellency effect of the insecticide lambda-cyhalothrin (Thompson and Wilkins 2003). These decreases in repellency caused by fungicides could increase the exposure of pyrethroids to bees, greatly increasing the risk of insecticide-related health effects.

The objective of this study was to determine if three fungicides commonly used to control apple diseases during bloom in Michigan have either a repellency effect, an attraction effect, or no apparent effect on honey bee foraging behavior. Examining how these fungicides impact honey bee foraging behavior will help enhance our understanding of 1) the potential risks of honey bee fungicide exposure during apple bloom, 2) the potential for loss of pollination services if fungicides are repellent, or 3) if fungicides may be used intentionally to repel bees in an attempt to reduce pesticide exposure during apple bloom. Understanding the impact that bloom-time fungicide applications have on honey bee foraging behavior will be crucial for refining best management practices to better protect the health of orchard trees, and the bees providing pollination services.

METHODS

Three replicates of four treatments, three fungicides and a water-only control, were applied to a single block planting of apple cultivar "Jonamac" located at the Michigan State University Trevor Nichols Research Center near Fennville, Michigan. Manzate Pro Stick (mancozeb) was applied at 6 lbs of formula per acre. Captan 80WDG (captan) was applied at 5 lbs of formula per acre, and Merivon (fluxapyroxad and pyraclostrobin) was applied at 5.5 oz of formula per acre. The water-only treatment was applied using the same gallons per acre rate and sprayer calibration used for the fungicides treatments.

To account for the honey bee hive placement, the orchard block was divided into thirds and the treatments were assigned according to a 'Latin Square' design, each consisting of 10 trees by 3 rows, with the outer rows acting as buffers, and the middle row used for observations. Observations were conducted exactly 24 hours after the treatments were applied, which is the

labeled re-entry interval for the materials used. Four observers simultaneously counted the number of honey bees visiting the middle ten trees in each plot for 2 minutes. Observers were not told what color flags corresponded to each treatment in order to avoid observer bias. The observation period occurred around 4:00 pm under conditions favorable to honey bee foraging (sunny, light wind, 18.9° C). An Analysis of Variance (ANOVA) test was conducted using R 3.4.3 statistical software in order to determine if the number of bees observed in each treatment plot was significantly different than the number of bees observed in other fungicide treatment plots or the control plots.

RESULTS

No significant differences were observed in the number of honey bees foraging on apple blossoms 24 hours after being treated with commonly used fungicides to control the fungal disease apple scab across all treatments ($F= 0.138$, $P= 0.934$). The average number of bees observed during the 2-minute sampling for each treatment were: 8 bees in control plots, 9.3 bees in plots treated with Manzate Pro Stik, 10 bees in plots treated with Captan 80WDG, and 9 bees in plots treated with Merivon (Figure 3.1). Neither a repellency effect nor an attraction effect was observed, thus treatments had no apparent effect on honey bee foraging behavior.

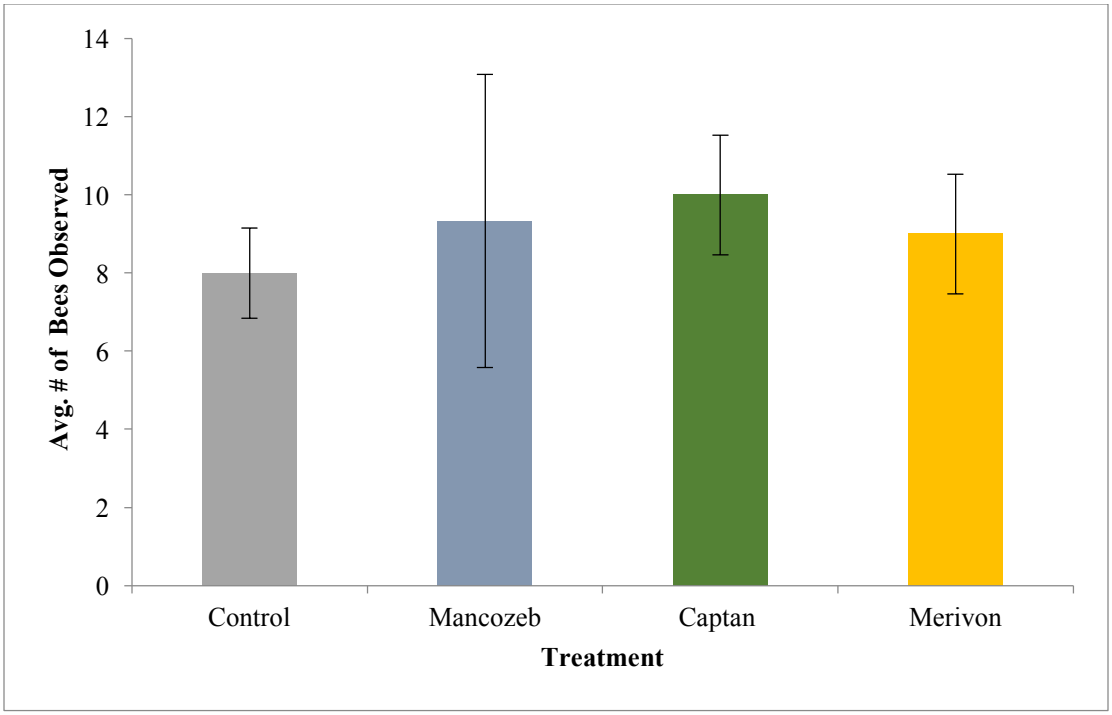

Figure 3.1. The average number of honey bees observed (± SEM) for each treatment during the 2-minute observation period.

DISCUSSION

The number of bees foraging on treated flowers in this study suggest that the fungicide treatments of Captan 80WDG, Manzate Pro Stik, and Merivon do not have either a repellent or an attractant effect on honey bee foraging behavior. Based on these results, these products are not expected to interfere with honey bee foraging when applied to prevent apple scab infection during bloom. The use of captan and mancozeb, however, have been shown to significantly reduce pollen viability within apple blossoms for up to 48 hours post-application, so even if honey bees are moving pollen around within 24 hours post-application, the pollen they are moving may not be viable, which can then interfere with fruit set (Fell et al. 1983). It is also likely that because honey bee visitation was not reduced 24 hours after these fungicide

treatments, residues of these materials on flowers that were open during the application will end up in honey bee hives when they are picked up by foraging bees.

Fungicide exposure to bees is potentially problematic given that the active ingredients captan and pyraclostrobin, which are in two of our treatments, have been associated with negative honey bee health effects in laboratory studies. Captan has been associated with an early reduction in the size of the acini in the hypopharyngeal gland in treated bees compared to untreated bees (Heylen et al. 2011). The hypopharyngeal gland is important for nurse bees because it is used for feeding larvae, but normally as a worker bee ages the gland shrinks, which triggers these bees to become foragers. Hence, if captan exposure leads to premature shrinking of these glands, this could shift bee behavior from in-hive work to foraging out of the hive, upsetting the balance of worker bee role diversification that maintains productive colonies (Heylen et al. 2011). In addition, multiple laboratory studies have shown increased mortality of larvae that were orally exposed to diets containing captan, with no larvae completing development to adulthood when fed captan-laced diets (Mussen et al. 2004, Johnson 2015). Pyraclostrobin, an active ingredient in Merivon, has been associated with a reduction in development and successful emergence of adult honey bee queens (DeGrandi-Hoffman et al. 2013). Pyraclostrobin in bee-collected pollen has also been correlated with a significant increase in the probability of *Nosema* infection (Pettis et al. 2013).

In order to gain a better understanding of how fungicide applications affect honey bees being rented for pollination, it would be beneficial to quantify the field-level fungicide residues that accumulate in the rented hives during apple bloom. These field-level residue rates could then be used to understand how the health of hives being rented for apple pollination directly relates to the concurrent fungicide exposure. It would also be beneficial to study the potential repellency

effect that fungicides have on native pollinators, such as bumble bees and solitary bees. These native pollinators supplement honey bee pollination in apple and other specialty crops, and therefore may also be exposed to a wide range of pesticides that can have detrimental effects on their health (Hladik et al. 2016). The number of studies that have examined the risks of fungicides for native bees are few compared with the number of honey bee studies on the subject.

CONCLUSION

The fungicides Captan 80WDG (captan), Manzate Pro Stik (mancozeb), and Merivon (fluxapyroxad and pyraclostrobin) are not repellent to bees, nor did they increase visitation to apple blossoms when applied to apple orchards in bloom. In order to avoid high levels of honey bee exposure, applications of these products during apple bloom should occur only when there is a high risk of disease. The potential negative impacts to honey bee health from exposure to fungicides may lead to an overall decline in colony health, and possible reduction in pollination efficiency of the effected colonies. Fungicide applications can also cause changes to apple pollen viability, and if this is true for other plant species or fungicide formulations, it could have widespread detrimental effects for both bee health and pollination efficiency. Potential strategies to reduce honey bee pesticide exposure during bloom include spraying at night when bees are inactive, placing hives in buffered locations (behind tree lines on hill sides), avoiding applications at particular percentages of peak bloom, and carefully calibrating sprayers (May et al. 2015). More research is needed to understand how fungicide applications during crop bloom influence the behavior and health of bees providing vital pollination services, and to develop new strategies to reduce pesticide exposure for bees.

CHAPTER 4: CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

Declines in honey bee health in recent years pose a threat to the production of pollinator-dependent crops, such as tart cherries and apples. Possible reasons for this decline include habitat loss, parasites, disease, and pesticide exposure. Bees that are rented for commercial pollination are at particular risk for pesticide exposure-related health effects. In tart cherry and apple orchards, insecticides are typically restricted from use during crop bloom, in order to reduce the potential exposure of pollinating bees to insecticides. This is not the case for fungicides. Cherry and apple trees are particularly susceptible to fungal infections in the early spring, so fungicide applications are common during bloom while bees are out foraging and visiting crop flowers, increasing the likelihood that bees pollinating tree fruit orchards will be exposed to potentially hazardous fungicides. Based on results of lethality studies alone, fungicides were considered relatively safe for use around adult honey bees. However, current research suggests that fungicides may cause sub-lethal effects on honey bees at the colony level through a variety of different mechanisms including abnormal larval development, a decrease of beneficial gut bacteria, an increase in the impact of honey bee diseases, and detrimental effects on honey bee physiology, foraging behavior, learning and memory. Understanding how fungicides accumulate in honey bee hives during tart cherry and apple bloom is crucial for making informed disease management decisions on farms, and protecting the health of honey bees that provide vital pollination services.

Our work suggests honey bee hives that are within foraging range of cherry and apple orchards are exposed to fungicides in spring, even if the hives are not being rented for pollination. Chlorothalonil and captan were the most frequently detected pesticides in honey bee hives; chlorothalonil was detected in all sample types except larvae, and captan was frequently

detected in nurse bees, foragers, pollen and bee bread, but was never detected in wax or larvae samples. Exposure of insecticides and herbicides is relatively low for bees during tart cherry bloom, compared to fungicides. Insecticides and herbicides were rarely detected in any sample type, and when they were detected it was at very low levels (near the LOD).

Pollen analysis results indicate that a combination of the following pollens are important resources for honey bees in the early spring: cultivated and wild *Prunus* and *Malus*, woodland plants such as *Acer*, *Salix*, *Quercus*, *Fagus*, and *Trillium*, and herbaceous perennials commonly found in orchard floors such as *Taraxacum*, *Brassica*, and *Trifolium.* Pesticide drift onto nearby flowering weeds is a likely source of fungicide contamination, as indicated by the high prevalence of *Taraxacum* pollen and captan residues in pollen samples from hives not being rented for pollination.

The residue levels detected in this research exceed the levels previously associated with negative health effects for bees, such as increased disease susceptibility, increased larval mortality, changes in the gut microbiome, and overall colony loss. The levels of fungicides detected in hives from this study under current disease management during spring orchard bloom present a serious risk to honey bees from sub-lethal health effects.

We also found that the fungicides Captan 80WDG (captan), Manzate Pro Stik (mancozeb), and Merivon (fluxapyroxad and pyraclostrobin) were not repellent to bees, nor did they increase bee visitation over the water-only control when applied to apple orchards in bloom. These products are not expected to interfere with honey bee foraging when applied to prevent apple scab infection during bloom and they would also not be effective in deterring bees from visiting treated orchard. As a consequence, the potential negative impacts to honey bee health

from exposure to these fungicides may lead to an overall decline in colony health, and a possible reduction in pollination efficiency of the effected colonies.

Quantification of the fungicide residues accumulated during orchard bloom in honey bee hives has illuminated the need for better management practices, and indicated some improvements that could be made to current strategies. For example, when comparing our data to disease models obtained from MSU Enviroweather, we have shown that bees have a higher risk for fungicide exposure when there is a greater environmental risk of disease development in orchards. This demonstrates that growers are utilizing the Enviroweather service to determine when fungicide sprays are necessary, and that the models for disease risk may be predictive of the amount of fungicide residues accumulating in the nearby honey bee hives. Beekeepers may be able to use the pest-forecasting models on Enviroweather to make informed decisions about the exposure risks for their bees in specific crops under current weather conditions. Another management strategy to reduce pesticide exposure is to remove flowering weeds from the orchard floor and surrounding landscapes prior to the application of plant protectant products. Growers can also plant or conserve early-spring flowering species near orchards to provide for non-sprayed flowers and a diverse diet during the pollination period. Access to a variety of pesticide-free floral resources will help to reduce the negative health effects associated with pesticide exposure, improve colony health, and lead to increased pollination efficiency. Other strategies that can be implemented to reduce pesticide exposure to bees include: making applications when bees are inactive, placing hives in buffered locations (behind tree lines or on hillsides), and carefully calibrating sprayers.

The findings from this research have led to a greater understanding of crop and region-specific pesticide exposure risk to honey bees. We now know the specific levels of fungicides

accumulating in honey bee hives being rented for pollination of tart cherry in Michigan. Using these results, we were able to relate the real-world exposure rates to past toxicological studies and determine that honey bees are at risk of sub-lethal health effects from fungicide exposure experienced during tart cherry and apple bloom. This information has allowed us to begin the process of refining best management practices and has drawn Michigan tree fruit producers' attention to the risks that fungicides may pose to bees during crop bloom.

Future studies should investigate how honey bee health directly relates to the fungicide accumulation within hives being rented for orchard pollination. It would be valuable to quantify fungicide exposure and monitor hive health simultaneously throughout the entire growing season. We have proposed many strategies that growers and beekeepers can implement in an attempt to reduce pesticide exposure for bees, it is now necessary to quantify the fungicide residues in bee hives under these new suggested strategies and compare those levels to the residues found in this study under current standard orchard management practices to determine if the proposed strategies actually lead to a significant reduction in pesticide exposure.

This study specifically looked at bee hives being rented for tart cherry production. It would also be beneficial for future studies to quantify the field-level fungicide residues that accumulate in the rented hives during bloom in other bee-dependent crops in Michigan, such as apple. We found that commonly-used fungicides in apples during bloom did not have a repellency effect on honey bee foragers. The next step of this research should be to investigate what effects the use of other current and commonly used fungicides may be having on honey bee foraging behavior. For instance, past studies have shown that chlorothalonil-laced sugar water is more attractive to honey bee foragers than unlaced sugar water, so it would be interesting to test whether this attraction effect translates into increased pollinator visitation during tart cherry

bloom after field applications of chlorothalonil. Because honey bees are not the only important pollinators for specialty crops in Michigan, it would also be beneficial to determine if fungicides have a repellency/attraction effect on native pollinators, such as bumble bees and solitary bees.

Despite the high risk of bacterial infections on flower blossoms, such as fire blight, little attention has been paid to the effects of antibiotic sprays on honey bee health. It would be interesting to investigate the health impact of antibiotic sprays on bees, how much antibiotic accumulates in hives, and if those detected residue levels have dangerous sub lethal effects on honey bee health. Research on antibiotic sprays would provide a more complete picture of how overall disease management in orchards influences bee health.

This thesis represents the first steps in understanding fungicide exposure in tree fruit crops in Michigan. The struggle to balance pest management and pollinator health will continue. Future research should focus on crops-specific strategies to best coordinate crop disease management with the need for pollination services by honey bees.

APPENDICES

APPENDIX A

RECORD OF DEPOSITION OF VOUCHER SPECIMENS

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: _____2018-02_____

Author and Title of thesis:
Jacquelyn Albert
Field-level Fungicide Exposure and Repellency to Honey Bees During Orchard Bloom in Michigan

Museum(s) where deposited:
Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Specimens:

Family	Genus-Species	Life Stage	Quantity	Preservation
Apidae	*Apis mellifera*	adult	5	pinned

APPENDIX B

FUNGICIDE USES AND POSSIBLE HEALTH EFFECTS

Table A.1. Registration status in Michigan tree fruit of the fungicides listed in literature review (Chapter 1) and their known health effects on honey bees.

	Registered for use?			**Known negative health effects:**						
Fungicide Active Ingredients	**Tart Cherry**	**Sweet Cherry**	**Apple**	**Cognition**	**Development & Reproduction**	**Nutrition & Digestion**	**Immune System**	**Cellular Function**	**Synergism**	**Notes**
Benomyl				x						Not registered for use in tree fruit.
Boscalid	yes	yes	yes	-	x	x	x	x	x	
Captan	yes	yes	yes	x	x	x	x	x	x	
Carbendazim									x	Not registered for use in tree fruit.
Chlorothalonil	yes	yes			x	x	x		x	
Difenoconazole	yes		yes					x	x	
Dodine	yes	yes	yes	x						
Fenbuconazole	yes	yes	yes						x	
Fludioxonil			yes	x						May only be used at post-harvest in apple.
Flusilazole					-				x	Not registered for use in tree fruit.
Flutriafol									x	Not registered for use in tree fruit.
Imazalil									x	Not registered for use in tree fruit.
Iprodione	yes	yes		-	x				x	
Mancozeb			yes	-	-	x			x	
Metalaxyl	yes	yes	yes	x						
Metconazole	yes	yes							x	

Table A.1. (cont.)

Fungicide Active Ingredients	Registered for use?			Known negative health effects:						Notes
	Tart Cherry	Sweet Cherry	Apple	Cognition	Development & Reproduction	Nutrition & Digestion	Immune System	Cellular Function	Synergism	
Myclobutanil	yes		yes		-	x	x	x	x	
Nitrothal-Isopropyl				x						Not registered for use in tree fruit.
Penconazole				x					x	Not registered for use in tree fruit.
Prochloraz				x			x	x	x	Not registered for use in tree fruit.
Propiconazole	yes	yes							x	
Prothiconazole						x				Not registered for use in tree fruit.
Pyraclostrobin	yes	yes	yes	-	x	x	x	x	x	
Pyrazophos					x		-			Not registered for use in tree fruit.
Tebuconazole	yes	yes	yes	-		x			x	
Thiophanate-methyl	yes	yes	yes	-		-			x	
Triadimefon				x					x	Not registered for use in tree fruit.
Ziram	yes	yes	yes		x					

Notes: "x" indicates a known effect. "-" indicates no effect found in previous studies. Blank spaces indicate no findings/studies reported

APPENDIX C

2016 GIS CDL LANDSCAPE CLASSIFICATIONS

Table A.2. Reclassification of CDL Class Names from Arc GIS to fit into desired landscape categories (developed, non-cultivated/natural, open water, orchard/vineyard, and other cultivated crops.

CDL Class Name	Reclassification
Developed/High Intensity	Developed
Developed/Low Intensity	Developed
Developed/Med Intensity	Developed
Developed/Open Space	Developed
Barren	Non-cultivated/Natural
Deciduous Forest	Non-cultivated/Natural
Evergreen Forest	Non-cultivated/Natural
Fallow/Idle Cropland	Non-cultivated/Natural
Grassland/Pasture	Non-cultivated/Natural
Herbaceous Wetlands	Non-cultivated/Natural
Mixed Forest	Non-cultivated/Natural
Shrubland	Non-cultivated/Natural
Woody Wetlands	Non-cultivated/Natural
Open Water	Open Water
Apples	Orchard/Vineyard
Cherries	Orchard/Vineyard
Grapes	Orchard/Vineyard
Alfalfa	Other cultivated crops
Barley	Other cultivated crops
Christmas Trees	Other cultivated crops
Clover/Wildflowers	Other cultivated crops
Corn	Other cultivated crops
Cucumbers	Other cultivated crops
Dry Beans	Other cultivated crops
Oats	Other cultivated crops
Peas	Other cultivated crops
Rye	Other cultivated crops
Soybeans	Other cultivated crops
Sunflower	Other cultivated crops
Winter Wheat	Other cultivated crops

APPENDIX D

MSU ENVIROWEATHER GROWING DEGREE DAY ACCUMULATION

Table A.3. 2015 Benzonia Weather Station growing degree-day data [base: 42].

Date	Max Air Temp.	Min Air Temp.	Baskerville Emin	Baskerville Emin Sum (GDD)
5/1/15	64.3	33.7	8.9	202.1
5/2/15	71.6	48.2	17.9	220
5/3/15	77.7	52.8	23.2	243
5/4/15	68	50.1	17	260
5/5/15	70.9	43.3	15.1	275
5/6/15	74.6	47.2	18.9	294
5/7/15	83.2	58.9	29.1	323
5/8/15	74.8	54.8	22.8	346
5/9/15	57.7	46	9.9	356
5/10/15	62.7	44.5	11.6	368
5/11/15	65.4	47.2	14.3	382
5/12/15	50.6	38.4	3.3	385
5/13/15	42.5	39.9	0.1	385
5/14/15	64.6	35.4	9.3	395
5/15/15	61.6	47.1	12.3	407
5/16/15	77.5	49.9	21.7	429
5/17/15	81.5	58.5	28	457
5/18/15	47.5	45.7	4.6	461
5/19/15	46.6	31.4	1.1	462
5/20/15	53.1	27.6	3.3	466
5/21/15	60.2	39	8.1	474
5/22/15	62.7	31.7	7.8	482
5/23/15	72.2	37.8	13.6	495
5/24/15	74.6	53.7	22.1	517
5/25/15	74.4	58.1	24.2	542
5/26/15	74.3	57.1	23.7	565
5/27/15	66.2	52.4	17.3	582
5/28/15	79	54.9	24.9	608
5/29/15	79	60.2	27.6	635
5/30/15	46.5	39.1	1.6	637
5/31/15	65.3	36.9	10	647
6/1/15	68.1	44.6	14.4	661

Table A.3. (cont.)

Date	Max Air Temp.	Min Air Temp.	Baskerville Emin	Baskerville Emin Sum (GDD)
6/2/15	67.3	41.4	12.4	674
6/3/15	73	49.7	19.4	693
6/4/15	74.6	54.6	22.6	716
6/5/15	61	47.5	12.2	728
6/6/15	57.2	52.5	12.8	740
6/7/15	70.2	54.5	20.4	761
6/8/15	73.5	51.8	20.6	782
6/9/15	73.3	50	19.6	801
6/10/15	74.9	54.9	22.9	824
6/11/15	69.1	51.8	18.4	843
6/12/15	72	54.6	21.3	864
6/13/15	65.3	50.8	16	880
6/14/15	71.6	60.1	23.9	904
6/15/15	76.5	55.2	23.9	928
6/16/15	69	50.1	17.5	945
6/17/15	77.5	48.4	20.9	966

Table A.4. 2015 NWMHRC Weather Station growing degree-day data [base: 42].

Date	Max Air Temp.	Min Air Temp.	Baskerville Emin	Baskerville Emin Sum (GDD)
5/1/15	65.1	34.5	9.4	169
5/2/15	73.2	46	17.6	187
5/3/15	79.6	53.8	24.7	212
5/4/15	67.2	47	15.1	227
5/5/15	68.3	42.4	13.1	240
5/6/15	72.8	44	16.4	256
5/7/15	84.9	57.2	29	285
5/8/15	78.2	50.4	22.3	308
5/9/15	57.2	43.7	8.5	316
5/10/15	56.6	42.6	7.6	324
5/11/15	64.1	44.1	12.1	336
5/12/15	53.5	36.7	4.4	340
5/13/15	57.7	35.6	6.2	347

Table A.4. (cont.)

Date	Max Air Temp.	Min Air Temp.	Baskerville Emin	Baskerville Emin Sum (GDD)
5/14/15	66	35.7	10.1	357
5/15/15	58.3	45.2	9.8	366
5/16/15	74.1	44.4	17.3	384
5/17/15	81	54.1	25.5	409
5/18/15	76.1	45.2	18.6	428
5/19/15	47.2	33.1	1.4	430
5/20/15	56.1	30.4	4.7	434
5/21/15	64.2	40.5	10.5	444
5/22/15	56.9	35.1	5.7	450
5/23/15	73.1	39.8	14.7	465
5/24/15	77.5	55.8	24.6	490
5/25/15	74.2	55.7	22.9	513
5/26/15	78.5	61.1	27.8	540
5/27/15	66.7	55.7	19.2	560
5/28/15	81.4	55.5	26.4	586
5/29/15	78.1	59.8	26.9	613
5/30/15	66.5	39.1	11.2	624
5/31/15	61	38.4	8.3	632
6/1/15	67.6	39.9	12	644
6/2/15	70.8	43.7	15.3	660
6/3/15	78.2	47.7	21	681
6/4/15	75.4	52.6	22	703
6/5/15	63	47.5	13.2	716
6/6/15	70.1	44.5	15.3	731
6/7/15	72.4	53.7	21	752
6/8/15	71.5	53.8	20.7	773
6/9/15	76.8	52.6	22.7	796
6/10/15	71.3	57.4	22.3	818
6/11/15	65.5	53	17.3	835
6/12/15	72	53.2	20.6	856
6/13/15	65.1	50.5	15.8	872
6/14/15	74.7	61.4	26.1	898
6/15/15	76.6	59.6	26.1	924
6/16/15	66.1	51.9	17	941
6/17/15	77.1	51.2	22.2	963

Table A.5. 2016 Benzonia Weather Station growing degree-day data [base: 42].

Date	Max Air Temp.	Min Air Temp.	Baskerville Emin	Baskerville Emin Sum (GDD)
5/1/16	54.7	42	6.4	264.5
5/2/16	63.6	35.5	8.9	273
5/3/16	63.9	37.5	9.5	283
5/4/16	45.9	39.3	1.4	284
5/5/16	60.7	34.8	7.4	292
5/6/16	75.1	38.4	15.2	307
5/7/16	64.6	40.8	10.8	318
5/8/16	61.2			
5/9/16	70.8	36.2	12.5	330
5/10/16	63.7	46.4	13	343
5/11/16	75.8	53.2	22.5	366
5/12/16	63.9	54.1	17	383
5/13/16	57.4	42.4	7.9	391
5/14/16	45.8	33	0.9	392
5/15/16	48.9	31.4	1.9	394
5/16/16	62.7	37.8	9	403
5/17/16	61.6	33.9	7.7	410
5/18/16	63.6	34.5	8.7	419
5/19/16				
5/20/16	72.4	39.5	14.2	433
5/21/16	77.9	44.1	19	452
5/22/16	73.9	44.9	17.4	470
5/23/16	75.7	52.9	22.3	492

Note: A blank means there is missing data from this weather station

Table A.6. 2016 NWMHRC Weather Station growing degree-day data [base: 42].

Date	Max Air Temp.	Min Air Temp.	Baskerville Emin	Baskerville Emin Sum (GDD)
5/1/16	54.7	41.4	6.1	212
5/2/16	64.2	35.7	9.2	221
5/3/16	67.4	39.7	11.9	233
5/4/16	48	39.3	2.3	236
5/5/16	59.6	36.5	7.2	243
5/6/16	79.2	40	17.8	261
5/7/16	65.2	43.4	12.3	273
5/8/16	58.3	39.3	7.2	280
5/9/16	66.3	36	10.3	290
5/10/16	59.9	43.4	9.6	300
5/11/16	75.3	49.7	20.5	321
5/12/16	66.5	52.6	17.6	340
5/13/16	58.2	40.9	7.7	346
5/14/16	45.1	34.7	0.7	347
5/15/16	49.5	32.9	2.3	349
5/16/16	61.3	39.4	8.8	358
5/17/16	62.1	35.4	8.2	366
5/18/16	64.2	36.3	9.4	375
5/19/16	72.6	38.2	13.9	390
5/20/16	73.2	41.4	15.3	404
5/21/16	77.1	47.4	20.3	425
5/22/16	72.8	47.4	18.1	443
5/23/16	80.3	49.5	22.9	466

Table A.7. 2017 Benzonia Weather Station growing degree-day data [base: 42].

Date	Max Air Temp.	Min Air Temp.	Baskerville Emin	Baskerville Emin Sum (GDD)
5/1/17	59.7	37.1	7.4	295
5/2/17	45.2	38.4	1	296
5/3/17	56.5	33	5.2	301
5/4/17	65.2	36.2	9.8	311
5/5/17	55.1	38.9	5.6	317
5/6/17	53.5	34.5	4.1	321
5/7/17	46.3	31.9	1	322
5/8/17	52.4	29.8	3.2	325
5/9/17	54.8	26.5	3.8	329
5/10/17	63.7	39.5	10	339
5/11/17	69	47.4	16.2	355
5/12/17	64.7	44.3	12.5	368
5/13/17	70.9	41.8	14.3	382
5/14/17	61.9	42.6	10.3	392
5/15/17	75.9	39.9	16.1	408
5/16/17				
5/17/17	82.1	69.3	33.7	442
5/18/17	73.9	38	14.6	457
5/19/17	58.2	37.8	6.8	463
5/20/17	65.7	36.6	10.1	474
5/21/17	66.9	48.1	15.5	489
5/22/17	64.4	44.6	12.5	502
5/23/17	66.9	43	12.9	514
5/24/17	63.6	51.7	15.7	530
5/25/17	67.3	50.1	16.7	547
5/26/17	67.4	47.1	15.2	562
5/27/17	74.7	48.1	19.4	582
5/28/17	67.4	53.6	18.5	600
5/29/17	67	48.4	15.7	616
5/30/17	63.8	47.4	13.6	629
5/31/17	65.1	45.7	13.4	643
6/1/17	68.5	42.9	13.7	656

Note: A blank means there is missing data from this weather station

Table A.8. 2017 NWMHRC Weather Station growing degree-day data [base: 42].

Date	Max Air Temp.	Min Air Temp.	Baskerville Emin	Baskerville Emin Sum (GDD)
5/1/17	60.9	36.7	7.9	288
5/2/17	47.5	36.6	1.8	290
5/3/17	60.8	36.2	7.7	297
5/4/17	66.5	37.2	10.7	308
5/5/17	54.1	36.6	4.7	313
5/6/17	52.8	34.5	3.8	316
5/7/17	45	33.2	0.7	317
5/8/17	51	32.3	2.8	320
5/9/17	56.5	29.4	4.8	325
5/10/17	69	39.6	12.6	337
5/11/17	68.1	45.5	14.8	352
5/12/17	67.8	46.4	15.1	367
5/13/17	65	41.2	11.2	378
5/14/17	60.2	43.7	10	388
5/15/17	73	38.8	14.3	403
5/16/17	81.9	52.4	25.1	428
5/17/17	83.1	69.9	34.5	462
5/18/17	76.5	38.1	15.9	478
5/19/17	55.7	37.7	5.6	484
5/20/17	65.6	40.9	11.3	495
5/21/17	70.1	48.8	17.4	513
5/22/17	64.3	45.9	13.1	526
5/23/17	64.9	43.8	12.4	538
5/24/17	68.6	49.5	17.1	555
5/25/17	64.6	51.4	16	571
5/26/17	70.4	47.5	17	588
5/27/17	76.9	50.5	21.7	610
5/28/17	67.5	54.6	19	629
5/29/17	67.6	50.2	16.9	646
5/30/17	64.7	49.1	14.9	661
5/31/17	62.6	45	11.8	672
6/1/17	69.2	42.1	13.7	686

APPENDIX E

CHI-SQUARED TABLES

Table A. 9. χ^2 values comparing the number of positive chlorothalonil residue detections at bee yards with number of positive chlorothalonil detections at orchard sites for each sample type in 2015, 2016, and 2017.

Sample Type	Year	Timing	df	χ^2	*P*
Nurse	2015	Pre-Bloom	1	7.2	< 0.01
		Bloom	1	19.08	< 0.001
		Post-Bloom	1	0.96	> 0.05
	2016	Pre-Bloom	1	2.795	> 0.05
		Bloom	1	6.86	< 0.01
	2017	Pre-Bloom	1	1.714	> 0.05
		Bloom	1	24	< 0.001
		Post-Bloom	1	1.14	> 0.05
Wax	2016	Bloom	1	14.7	< 0.001
	2017	Bloom	1	3.16	< 0.05
Bee Bread	2017	Bloom	1	18.55	< 0.001
Foragers	2017	Pre-Bloom	1	1.09	> 0.05
		Bloom	1	24	< 0.001
		Post-Bloom	1	0.54	> 0.05
Pollen	2017	Pre-Bloom	1	3	< 0.05
		Bloom	1	.	> 0.05
		Post-Bloom	1	.	> 0.05

. = no χ^2 value (all samples from both treatments had detectable residues)

Table A.10. χ^2 values comparing the number of positive captan residue detections at bee yards with number of positive captan detections at orchard sites for each sample type in 2015, 2016, and 2017.

Sample Type	Year	Timing	df	χ^2	*P*
Nurse	2016	Pre	1	2.389	> 0.10
		Bloom	1	1.71	> 0.10
	2017	Pre	1	.	> 0.05
		Bloom	1	2.4	> 0.10
		Post	1	0.52	> 0.10
Bee Bread	2017	Bloom	1	.	> 0.05
Foragers	2017	Pre	1	1.342	> 0.10
		Bloom	1	0.8	> 0.10
		Post	1	.	> 0.05
Pollen	2017	Pre	1	.	> 0.05
		Bloom	1	.	> 0.05
		Post	1	.	> 0.05

. = no χ^2 value (all samples from both treatments had detectable residues)

APPENDIX F

MSU ENVIROWEATHER CHERRY LEAF SPOT DISEASE MODELS

Table A.11. 2015 cherry leaf spot disease model from NWMHRC weather station.

Start of wetting period	End of wetting period	Duration (Hrs.)	Avg Temp (F)	Rainfall (in.)	Cherry Leaf Spot	Progress towards infection
5/10 9-10PM	5/12 3-4PM	Wet: 30; Span: 43	49	0.22	Moderate	138%
5/15 2-3AM	5/16 9-10AM	Wet: 29; Span: 32	48.9	0.35	Low	121%
5/17 6-7PM	5/17 8-9PM	Wet: 3; Span: 3	72.9	0.32	None	40%
5/24 8-9PM	5/25 1-2PM	Wet: 16; Span: 18	58.9	1.6	Moderate	197%
5/26 Midnight-1AM	5/26 2-3AM	Wet: 3; Span: 3	63.2	0.06	None	57%
5/26 5-6PM	5/26 10-11PM	Wet: 6; Span: 6	65.6	0.31	Low	117%
5/27 Noon-1PM	5/27 3-4PM	Wet: 4; Span: 4	57.5	0.03	None	45%
5/29 Noon-1PM	5/29 1-2PM	Wet: 2; Span: 2	67.6	0.11	None	34%
5/29 11PM - Midnight	5/30 1-2PM	Wet: 15; Span: 15	51.8	0.25	None	102%
6/7 8-9AM	6/9 11AM-Noon	Wet: 39; Span: 52	57.4	0.6	High	439%
6/11 4-5PM	6/12 11AM-Noon	Wet: 20; Span: 20	55.4	0.55	Moderate	182%
6/13 5-6PM	6/15 10-11AM	Wet: 31; Span: 42	63.9	0.27	High	570%
6/18 3-4PM	6/18 4-5PM	Wet: 2; Span: 2	60.6	0.03	None	31%

Table A.12. 2016 cherry leaf spot disease model from NWMHRC weather station.

Start of wetting period	End of wetting period	Duration (Hrs.)	Avg Temp (F)	Rainfall (in.)	Cherry Leaf Spot	Progress towards infection
5/12 10-11AM	5/13 5-6AM	Wet: 10; Span: 20	54.8	0.01	None	87%
5/13 6-7PM	5/14 8-9AM	Wet: 9; Span: 15	43.6	0.1	None	12%
5/26 1-2AM	5/26 8-9AM	Wet: 7; Span: 8	64	0.22	Low	133%
5/27 4-5PM	5/28 4-5AM	Wet: 13; Span: 13	66.6	0.93	Moderate	257%
6/1 10-11AM	6/1 Noon-1PM	Wet: 3; Span: 3	57.5	0.06	None	32%

Table A.13. 2017 cherry leaf spot disease model from NWMHRC weather station.

Start of wetting period	End of wetting period	Duration (Hrs.)	Avg Temp (F)	Rainfall (in.)	Cherry Leaf Spot	Progress towards infection
5/13 9-10AM	5/13 11AM-Noon	Wet: 2; Span: 3	48.7	0.08	None	9%
5/18 Midnight-1AM	5/18 3-4AM	Wet: 4; Span: 4	67	0.08	None	56%
5/21 9-10AM	5/21 10-11AM	Wet: 2; Span: 2	51	0.02	None	12%
5/22 8-9AM	5/22 9-10AM	Wet: 2; Span: 2	46.4	0.02	None	7%
5/22 11PM - Midnight	5/24 11AM-Noon	Wet: 28; Span: 37	51.2	0.67	Moderate	163%
5/25 3-4AM	5/26 9-10AM	Wet: 24; Span: 31	53	0.03	Moderate	173%
5/28 Noon-1PM	5/29 7-8AM	Wet: 14; Span: 20	54.4	0.69	Low	115%
5/30 5-6PM	5/31 11PM - Midnight	Wet: 3; Span: 7	55.1	0.01	None	27%
5/31 9-10AM	5/31 11AM-Noon	Wet: 3; Span: 3	51.1	0.03	None	17%

APPENDIX G

MSU ENVIROWEATHER APPLE SCAB DISEASE MODELS

Table A.14. 2015 apple scab disease model from NWMHRC weather station.

Start of wetting period	End of wetting period	Duration (Hrs.)	Avg Temp (F)	Rainfall (in.)	Apple Scab (leaf)	Wet hrs @ avg temp for 1st infection	Progress towards infection
4/19 9-10PM	4/21 9-10AM	Wet: 30; Span: 37	41.4	0.49	Light (Symptoms appear: 5/8)	26	121%
4/21 9-10PM	4/22 3-4AM	Wet: 7; Span: 7	33.3	0.02	None	48	6%
4/23 11AM-Noon	4/23 11AM-Noon	Wet: 1; Span: 1	29.8	0.01	None	--	0%
5/4 5-6AM	5/4 10-11AM	Wet: 6; Span: 6	54.7	0.09	None	11.5	52%
5/9 2-3AM	5/9 Noon-1PM	Wet: 10; Span: 11	46.1	0.07	None	16	60%
5/10 9-10PM	5/12 3-4PM	Wet: 30; Span: 43	49	0.22	Heavy (Symptoms appear: 5/25)	14.5	203%
5/15 2-3AM	5/16 9-10AM	Wet: 29; Span: 32	48.9	0.35	Moderate (Symptoms appear: 5/28)	15	198%
5/17 6-7PM	5/17 8-9PM	Wet: 3; Span: 3	72.9	0.32	None	9	30%
5/24 8-9PM	5/25 1-2PM	Wet: 16; Span: 18	58.9	1.6	Moderate (Symptoms appear: 6/4)	10	158%

Table A.14. (cont.)

Start of wetting period	End of wetting period	Duration (Hrs.)	Avg Temp (F)	Rainfall (in.)	Apple Scab (leaf)	Wet hrs @ avg temp for 1st infection	Progress towards infection
5/26 Midnight-1AM	5/26 2-3AM	Wet: 3; Span: 3	63.2	0.06	None	9	33%
5/26 5-6PM	5/26 10-11PM	Wet: 6; Span: 6	65.6	0.31	None	9	67%
5/27 Noon-1PM	5/27 3-4PM	Wet: 4; Span: 4	57.5	0.03	None	10	39%
5/29 Noon-1PM	5/29 1-2PM	Wet: 2; Span: 2	67.6	0.11	None	9	22%
5/29 11PM - Midnight	5/30 1-2PM	Wet: 15; Span: 15	51.8	0.25	Light (Symptoms appear: 6/10)	13	115%
6/7 8-9AM	6/9 11AM-Noon	Wet: 39; Span: 52	57.4	0.6	Heavy (Symptoms appear: 6/18)	10	371%
6/11 4-5PM	6/12 11AM-Noon	Wet: 20; Span: 20	55.4	0.55	Moderate (Symptoms appear: 6/22)	11	180%
6/13 5-6PM	6/15 10-11AM	Wet: 31; Span: 42	63.9	0.27	Heavy (Symptoms appear: 6/23)	9	344%
6/18 3-4PM	6/18 4-5PM	Wet: 2; Span: 2	60.6	0.03	None	9.5	21%

Table A.15. 2016 apple scab disease model from NWMHRC weather station.

Start of wetting period	End of wetting period	Duration (Hrs.)	Avg Temp (F)	Rainfall (in.)	Apple Scab (leaf)	Wet hrs @ avg temp for 1st infection	Progress towards infection
4/21 5-6AM	4/22 8-9AM	Wet: 28; Span: 28	47.8	0.09	Moderate (Symptoms appear: 5/11)	15	179%
4/24 1-2PM	4/26 7-8AM	Wet: 34; Span: 43	41.6	0.84	Light (Symptoms appear: 5/13)	26	141%
5/3 10-11PM	5/4 Noon-1PM	Wet: 15; Span: 15	42.2	0.32	None	23	65%
5/12 10-11AM	5/13 5-6AM	Wet: 10; Span: 20	54.8	0.01	None	11.5	88%
5/13 6-7PM	5/14 8-9AM	Wet: 9; Span: 15	43.6	0.1	None	21	44%
5/26 1-2AM	5/26 8-9AM	Wet: 7; Span: 8	64	0.22	None	9	78%
5/27 4-5PM	5/28 4-5AM	Wet: 13; Span: 13	66.6	0.93	Moderate (Symptoms appear: 6/5)	9	144%
6/1 10-11AM	6/1 Noon-1PM	Wet: 3; Span: 3	57.5	0.06	None	10	29%

Table A.16. 2017 apple scab disease model from NWMHRC weather station.

Start of wetting period	End of wetting period	Duration (Hrs.)	Avg Temp (F)	Rainfall (in.)	Apple Scab (leaf)	Wet hrs @ avg temp for 1st infection	Progress towards infection
4/10 8-9AM	4/12 7-8AM	Wet: 38; Span: 48	39.3	0.14	Light (Symptoms appear: 5/1)	33	121%
4/15 5-6AM	4/17 7-8AM	Wet: 35; Span: 51	48.6	0.72	Heavy (Symptoms appear: 5/6)	15	231%
4/20 3-4AM	4/21 9-10AM	Wet: 31; Span: 31	40	0.7	Light (Symptoms appear: 5/12)	29	104%
4/27 2-3AM	4/27 8-9AM	Wet: 7; Span: 7	58.1	0.15	None	10	69%
4/28 5-6AM	4/28 7-8AM	Wet: 3; Span: 3	36.8	0.01	None	48	7%
4/30 4-5AM	5/2 3-4PM	Wet: 50; Span: 60	39.8	0.92	Moderate (Symptoms appear: 5/18)	33	163%
5/13 9-10AM	5/13 11AM-Noon	Wet: 2; Span: 3	48.7	0.08	None	15	14%
5/18 Midnight-1AM	5/18 3-4AM	Wet: 4; Span: 4	67	0.08	None	9	44%
5/21 9-10AM	5/21 10-11AM	Wet: 2; Span: 2	51	0.02	None	13	15%
5/22 8-9AM	5/22 9-10AM	Wet: 2; Span: 2	46.4	0.02	None	16	13%
5/22 11PM - Midnight	5/24 11AM-Noon	Wet: 28; Span: 37	51.2	0.67	Heavy (Symptoms appear: 6/3)	13	211%

Table A.16. (cont.)

Start of wetting period	End of wetting period	Duration (Hrs.)	Avg Temp (F)	Rainfall (in.)	Apple Scab (leaf)	Wet hrs @ avg temp for 1st infection	Progress towards infection
5/25 3-4AM	5/26 9-10AM	Wet: 24; Span: 31	53	0.03	Moderate (Symptoms appear: 6/6)	12	197%
5/28 Noon-1PM	5/29 7-8AM	Wet: 14; Span: 20	54.4	0.69	Light (Symptoms appear: 6/8)	11.5	121%
5/30 5-6PM	5/31 11PM - Midnight	Wet: 3; Span: 7	55.1	0.01	None	11	27%
5/31 9-10AM	5/31 11AM-Noon	Wet: 3; Span: 3	51.1	0.03	None	13	23%

APPENDIX H

CHERRY BAY ORCHARDS SPRAY RECORDS

Table A.17. Pesticide application records in apple plantings for 2015 from our grower cooperator at Cherry Bay Orchards. 2015 is only year with available spray records for apple.

Date	Chemical	Class	Rate	Unit	Target
5/11/15	Captan 80 WDG	Fungicide	1.5	lb	Apple Scab
	Flint	Fungicide	1.25	oz	Powdery Mildew
	Koverall	Fungicide	1.5	lb	Apple Scab
5/16/15	Captan 80 WDG	Fungicide	1.5	lb	Apple Scab
	Roper DF	Fungicide	1.5	lb	Apple Scab
	Topsin M WSB	Fungicide	0.5	lb	Powdery Mildew
5/20/15	Glyphosate Plus	Herbicide	25.4	oz	Weeds
	Prowl H2O	Herbicide	42.2	oz	Weeds
	Simazine 90DF	Herbicide	1.6	lb	Weeds
5/23/15	Captan 80 WDG	Fungicide	1.5	lb	Apple Scab
	Roper DF	Fungicide	1.5	lb	Apple Scab
	Topsin M WSB	Fungicide	0.5	lb	Powdery Mildew
5/26/15	Captan 80 WDG	Fungicide	1.5	lb	Apple Scab
	Harbour	Antiobiotic	0.5	lb	FireBlight
	Mycoshield	Antiobiotic	0.5	lb	FireBlight
	Roper DF	Fungicide	1.5	lb	Apple Scab
	Topsin M WSB	Fungicide	0.5	lb	Powdery Mildew
6/1/15	Captan 80 WDG	Fungicide	1.5	lb	Apple Scab
	Flint	Fungicide	1.25	oz	Powdery Mildew
	Roper DF	Fungicide	1.5	lb	Apple Scab
6/8/15	Carbaryl 4L	Growth Regulator	25.6	oz	Thin Fruit
	Maxcel	Growth Regulator	41	oz	Thin Fruit
	Pomaxa	Growth Regulator	3.2	oz	Thin Fruit
6/9/15	Nealta	Insecticide	6.8	oz	Mites
	Reaper 0.15EC	Insecticide	9.6	oz	Mites
	Rimon 0.83EC	Insecticide	12.8	oz	Coddling Moth
	Captan 80 WDG	Fungicide	1.5	lb	Apple Scab
	Flint	Fungicide	1.25	oz	Powdery Mildew
	Nealta	Insecticide	6.8	oz	Mites
	Reaper 0.15EC	Insecticide	9.6	oz	Mites
	Rimon 0.83EC	Insecticide	12.8	oz	Coddling Moth
6/16/15	Captan 80 WDG	Fungicide	1.5	lb	Apple Scab

Table A.17. (cont.)

Date	Chemical	Class	Rate	Unit	Target
	Carbaryl 4L	Growth Regulator	48	oz	Thin Fruit
	Rally 40 WSP	Fungicide	3	oz	Powdery Mildew
6/17/15	Carbaryl 4L	Growth Regulator	23	oz	Thin Fruit
	Pomaxa	Growth Regulator	4.3	oz	Thin Fruit

Table A.18. Pesticide application records in tart cherry for 2015 from our grower cooperator at Cherry Bay Orchards.

Date	Chemical	Class	Rate	Unit	Target
5/13/15	Echo 90DF	Fungicide	3.5	lb	Leaf Spot
	Topsin M WSB	Fungicide	1	lb	Eu. Brown Rot
5/25/15	Amine 2,4D	Herbicide	12.5	oz	Weeds
	Glyphosate Plus	Herbicide	30.1	oz	Weeds
5/28/15	Belt SC	Insecticide	2	oz	OBLR
	Echo 90DF	Fungicide	3.5	lb	Leaf Spot
	Topsin M WSB	Fungicide	1	lb	Eu. Brown Rot
6/4/15	Badge X2	Fungicide	12	oz	Leaf Spot
	Falgro 4L	Growth Regulator	2	oz	
	Imidan 70W	Insecticide	1	lb	Plum Curculio
	Rally 0 WSP	Fungicide	3	oz	Powdery Mildew
6/9/15	Badge X2	Fungicide	12	oz	Leaf Spot
	Falgro 4L	Growth Regulator	2	oz	
	Imidan 70W	Insecticide	1	lb	Plum Curculio
	Rally 0 WSP	Fungicide	3	oz	Powdery Mildew
6/17/15	Actara	Insecticide	2.75	oz	Plum Curculio
	Badge X2	Fungicide	12	oz	Leaf Spot
	Gem 500SC	Fungicide	1.6	oz	Powdery Mildew

Table A.19. Pesticide application records in tart cherry for 2016 from our grower cooperator at Cherry Bay Orchards.

Date	Chemical	Class	Rate	Unit	Target
5/17/16	Indar 2F	Fungicide	3.2	oz	Eu. Brown Rot
	Bravo Ultrex	Fungicide	2.7	lb	Leaf Spot
5/22/16	Captan 80WDG	Fungicide	1.25	lb	Brown Rot
	Luna Sensation	Fungicide	2.8	oz	Leaf Spot
5/31/16	Imidan 70W	Insecticide	1	lb	Plum Curculio
	Lambda-Cy	Insecticide	1.25	oz	Green Fruitworm
	Luna Sensation	Fungicide	2.8	oz	Leaf Spot
	Bravo Ultrex	Fungicide	2.7	lb	Leaf Spot
6/1/16	Amine, 2 4D	Herbicide	11.1	oz	Weeds
	Glyphosate Plus	Herbicide	27.8	oz	Weeds

Table A.20. Pesticide application records in tart cherry for 2017 from our grower cooperator at Cherry Bay Orchards.

Date	Chemical	Class	Rate	Unit	Target
5/12/17	LI 700	Adjuvant	0.8	oz	Adjuvant
	Topsin M WSB	Fungicide	0.75	lb	Mildew
	Bravo Ultrex	Fungicide	1.9	lb	Leaf Spot
5/18/17	Echo 90DF	Fungicide	1.75	lb	Leaf Spot
	Topsin M WSB	Fungicide	0.75	lb	Eu. Brown Rot
	SuperSpread 700	Adjuvant	0.8	oz	Adjuvant
5/25/17	Echo 90DF	Fungicide	1.75	lb	Leaf Spot
	Topsin M WSB	Fungicide	0.75	lb	Eu. Brown Rot
	SuperSpread 700	Adjuvant	0.8	oz	Adjuvant
6/1/17	20-20-20 Moraleaf	Foliar	1.9	lb	Foliar
	Captan 80WDG	Fungicide	1.1	lb	Brown Rot
	Luna Sensation	Fungicide	2.8	oz	Leaf Spot
	SuperSpread 700	Adjuvant	0.8	oz	Adjuvent
	Besiege	Insecticide	4.5	oz	OBLR

APPENDIX I

PLANT GENERA IN POLLEN RETURNED FROM GENETIC ANALYSIS

Table A.21. Results from the genetic analysis of 2017 pollen samples collected from the bee yard pollen traps.

	Pre-Bloom		Bloom				Post-Bloom		
Date	**5/9/17**		**5/16/17**		**5/19/17**		**5/31/17**		**Total**
Hive No.	**1**	**8**	**1**	**8**	**1**	**8**	**1**	**8**	**Reads**
Top 10 Pollen Genera									
Prunus	1,761	532	1,006	2,466	831	1,826	2,215	1,499	12,136
Acer	4,405	2,362	829	7,549	603	1,285	53	23	17,109
Taraxacum	1,338	3,199	523	3,826	1,249	2,088	228	92	12,543
Salix	4	1	4	6	1	3	83	42	144
Brassica	3	2	4	6	10	5	38	48	116
Malus	1	-	-	7	4	10	7	4	33
Quercus	-	-	-	2	-	2	2	973	979
Trillium	38	-	52	9	711	310	2	170	1,292
Fagus	-	-	106	103	345	202	6	75	837
Trifolium	-	-	-	-	-	-	1	-	1
Total Top 10	7,550	6,096	2,524	13,974	3,754	5,731	2,635	2,926	45,190
Other Genera									
Amelanchier	-	-	-	-	-	-	-	-	-
Barbarea	-	-	-	-	-	-	-	2	2
Betula	1	3	2	22	2	-	-	-	30
Carya	-	-	-	-	-	1	-	-	1
Claytonia	725	56	-	1	-	-	-	5	787
Crataegus	-	-	-	-	-	-	-	-	-
Elaeagnus	-	-	-	-	-	-	4	5	9

Table A.21. (cont.)

	Pre-Bloom		Bloom				Post-Bloom		
Date	**5/9/17**		**5/16/17**		**5/19/17**		**5/31/17**		**Total**
Hive No.	**1**	**8**	**1**	**8**	**1**	**8**	**1**	**8**	**Reads**
Other Genera cont.									
Erodium	-	-	-	-	-	-	-	-	-
Erythronium	3	-	-	-	-	-	-	1	4
Fragaria	-	-	-	-	-	-	1	51	52
Fraxinus	-	2	-	-	13	62	3	4	84
Krigia	-	-	-	-	-	-	1	-	1
Lonicera	-	-	-	-	-	1	395	78	474
Melilotus	-	-	-	-	-	4	-	-	4
Morus	-	-	-	-	-	-	-	-	-
Ostrya	32	2	36	160	8	1	11	46	296
Picea	-	-	-	-	-	-	2	6	8
Pinus	-	-	-	-	-	-	32	-	32
Pyrus	-	-	-	-	-	1	-	-	1
Rumex	-	-	-	-	-	-	53	9	62
Sambucus	-	1	-	-	-	2	-	-	3
Solidago*	-	790	-	-	-	-	-	-	790
Sorbus	-	-	-	-	-	-	-	120	120
Viburnum	-	-	-	-	-	-	-	-	-
Viola	-	-	-	-	-	-	-	-	-
Total Other	761	854	38	183	23	72	502	327	2,760

* = sample time does not match with plant phenology, genera is most-likely misidentified

Table A.22. Results from the genetic analysis of 2017 pollen samples collected from the orchard pollen traps.

	Pre-Bloom	Bloom				Post-Bloom		
Date	**5/10/17**	**5/15/17**		**5/18/17**		**5/31/17**		**Total**
Hive No.	**1**	**1**	**5**	**1**	**5**	**1**	**5**	**Reads**
Top 10 Pollen Genera								
Prunus	28,518	11,610	11,809	4,959	13,768	1,472	895	73,031
Acer	2,586	3,767	3,868	332	3,246	4,509	86	18,394
Taraxacum	1,999	4,779	3,834	538	980	2,575	1,347	16,052
Salix	5	130	5,096	4,337	13,975	527	521	24,591
Brassica	1	4	160	3,050	4,353	2,770	2,419	12,757
Malus	-	53	312	1,305	6,210	558	724	9,162
Quercus	-		2	7	20	2,543	81	2,653
Trillium	-	23	544	79	115	2	13	776
Fagus	-	97	164	18	20	2	96	397
Trifolium	-	-	-	-	-	585	575	1,160
Total Top 10	33,109	20,463	25,789	14,625	42,687	15,543	6,757	158,973
Other Genera								
Amelanchier	-	24	5	11	-	-	-	40
Barbarea	-	-	-	2	-	18	1	21
Betula	-	47	54	6	19	11	-	137
Carya	-	-	-	-	-	-	-	-
Claytonia	-	-	-	-	-	3	1	4
Crataegus	-	-	-	-	-	-	1	1
Elaeagnus	-	-	-	-	-	33	31	64
Erodium	-	36	-	7	4	2	2	51
Erythronium	-	-	-	-	-	-	-	-

Table A.22. (cont.)

	Pre-Bloom	Bloom				Post-Bloom		
Date	**5/10/17**	**5/15/17**		**5/18/17**		**5/31/17**		**Total**
Hive No.	**1**	**1**	**5**	**1**	**5**	**1**	**5**	**Reads**
Fragaria	-	-	1	-	-	4	1	6
Other Genera cont.								
Fraxinus	-	-	39	12	83	9	7	150
Krigia	-	-	-	-	-	-	-	-
Lonicera	1	1	1	2	3	91	161	260
Melilotus	-	-	-	-	1	-	-	1
Morus	-	-	-	-	1	-	-	1
Ostrya	55	49	116	6	-	10	60	296
Picea	-	46	3	17	39	2	1	108
Pinus	-	-	-	-	-	-	-	-
Pyrus	-	1	3	18	1	-	-	23
Rumex	-	-	-	-	-	2	38	40
Sambucus	-	-	2	37	54	73	-	166
Solidago*	-	-	-	-	-	-	-	-
Sorbus	-	-	1	-	-	-	-	1
Viburnum	-	-	1	-	-	-	-	1
Viola	-	-	5	-	21	-	-	26
Total Other	56	204	231	118	226	258	304	1,397

* = sample time does not match with plant phenology, genera are most-likely misidentified

Note: The pollen trap on hive #5 malfunctioned during pre-bloom

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