Potential Impact Of Neonicotinoid Insecticides On Honey Bees (Apis Mellifera) In Muskmelon Production

Kira L. Nixon
Purdue University

Follow this and additional works at: https://docs.lib.purdue.edu/open_access_theses
Part of the Entomology Commons

Recommended Citation
https://docs.lib.purdue.edu/open_access_theses/358

This document has been made available through Purdue e-Pubs, a service of the Purdue University Libraries. Please contact epubs@purdue.edu for additional information.
This is to certify that the thesis/dissertation prepared

By  Kira L. Nixon

Entitled
POTENTIAL IMPACT OF NEONICOTINOID INSECTICIDES ON HONEY BEES (APIS MELLIFERA) IN MUSKMELON PRODUCTION

For the degree of  Master of Science

Is approved by the final examining committee:

Dr. Ricky E. Foster

Dr. Christian H. Krupke

Dr. Stephen C. Weller

To the best of my knowledge and as understood by the student in the Thesis/Dissertation Agreement, Publication Delay, and Certification/Disclaimer (Graduate School Form 32), this thesis/dissertation adheres to the provisions of Purdue University’s “Policy on Integrity in Research” and the use of copyrighted material.

Dr. Ricky E. Foster

Approved by Major Professor(s): ________________________________

Approved by: Dr. Christian H. Krupke  12/03/2014

Head of the Department Graduate Program  Date
POTENTIAL IMPACT OF NEONICOTINOID INSECTICIDES ON HONEY BEES (APIS MELLIFERA) IN MUSKMELON PRODUCTION

A Thesis
Submitted to the Faculty
of
Purdue University
by
Kira Nixon

In Partial Fulfillment of the
Requirements for the Degree
of
Master of Science

December 2014
Purdue University
West Lafayette, IN
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>v</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>LITERATURE REVIEW</td>
<td>4</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>24</td>
</tr>
<tr>
<td>RESULTS AND DISCUSSION</td>
<td>31</td>
</tr>
<tr>
<td>CONCLUSIONS</td>
<td>37</td>
</tr>
<tr>
<td>LIST OF REFERENCES</td>
<td>50</td>
</tr>
</tbody>
</table>
**LIST OF TABLES**

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1: Summary of published toxicity of neonicotinoids on honey bees</td>
<td>40</td>
</tr>
<tr>
<td>Table 2: Multiple reaction monitoring table with conditions for LC/MS/MS analysis of pesticides</td>
<td>41</td>
</tr>
<tr>
<td>Table 3: Mean soil concentrations for all replications of neonicotinoids and their metabolites sampled from muskmelon fields in the spring</td>
<td>41</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Figure 1: 2013 Imidacloprid (ng/g), Admire Pro</td>
<td>..............................................................................</td>
</tr>
<tr>
<td>Figure 2: 2014 Imidacloprid (ng/g), Admire Pro</td>
<td>..............................................................................</td>
</tr>
<tr>
<td>Figure 3: 2013 Olefin Imidacloprid (ng/g), Admire Pro</td>
<td>..............................................................................</td>
</tr>
<tr>
<td>Figure 4: 2014 Olefin Imidacloprid (ng/g), Admire Pro</td>
<td>..............................................................................</td>
</tr>
<tr>
<td>Figure 5: 2013 5-hydroxyimidacloprid (ng/g), Admire Pro</td>
<td>..............................................................................</td>
</tr>
<tr>
<td>Figure 6: 2014 5-hydroxyimidacloprid (ng/g), Admire Pro</td>
<td>..............................................................................</td>
</tr>
<tr>
<td>Figure 7: 2013 Thiamethoxam (ng/g), Platinum, Actara, FarMore</td>
<td>..............................................................................</td>
</tr>
<tr>
<td>Figure 8: 2014 Thiamethoxam (ng/g), Platinum, Actara, FarMore</td>
<td>..............................................................................</td>
</tr>
<tr>
<td>Figure 9: 2014 Clothianidin (ng/g), Platinum, Actara, FarMore</td>
<td>..............................................................................</td>
</tr>
<tr>
<td>Figure 10: 2013 Acetamiprid (ng/g), Assail</td>
<td>..............................................................................</td>
</tr>
<tr>
<td>Figure 11: 2014 Acetamiprid (ng/g), Assail</td>
<td>..............................................................................</td>
</tr>
<tr>
<td>Figure 12: Imidacloprid, Admire Pro SCB Counts</td>
<td>..............................................................................</td>
</tr>
<tr>
<td>Figure 13: Thiamethoxam, Platinum SCB Counts</td>
<td>..............................................................................</td>
</tr>
<tr>
<td>Figure 14: Thiamethoxam, Actara; Acetamiprid, Assail SCB Counts</td>
<td>..............................................................................</td>
</tr>
<tr>
<td>Figure 15: Thiamethoxam, FarMore SCB Counts</td>
<td>..............................................................................</td>
</tr>
</tbody>
</table>
ABSTRACT

Nixon, Kira. M.S., Purdue University, December 2014. Potential impact of neonicotinoid insecticides on honey bees (Apis mellifera) in muskmelon production. Major Professor: Dr. Ricky E. Foster.

Honey bees (Apis mellifera) provide pollination services to many agricultural crops, including cucurbits. Neonicotinoids are commonly applied to cucurbits where honey bee colonies are often rented for sufficient pollination and proper fruit set. The goals of this study were to determine the potential impact of neonicotinoid residues on honey bees in muskmelon production and to determine the extent and duration of striped cucumber beetle control among treatments. The neonicotinoids evaluated were imidacloprid and its metabolites imidacloprid olefin and 5-hydroxyimidacloprid, thiamethoxam and its metabolite clothianidin, and acetamiprid. Thiamethoxam applied as a FarMore® seed treatment resulted in a highest mean pollen concentration of clothianidin at 6.48 ng/g. The highest mean pollen concentrations of thiamethoxam when applied as a Platinum® transplant water drench reached 64 ng/g, and the Actara® foliar spray reached 133 ng/g. Imidacloprid applied as an Admire Pro™ transplant water drench reached a mean pollen concentration of 96 ng/g. All of the resulting pollen residue concentrations following these treatments reached levels that have been shown to cause adverse effects on honey bees. The application of the acetamiprid Assail® foliar spray reached a mean pollen concentration of 150 ng/g which is well below the levels that have been shown to cause negative effects on honey bees. The low label rates for both soil drenches of imidacloprid and thiamethoxam provided control comparable to the high label rates. Imidacloprid provided the best protection, while both insecticides maintained populations below the economic threshold for 20 days after application at transplanting whereas the control did not. High label rate foliar sprays of acetamiprid applied as Assail® and thiamethoxam applied as Actara® both provided protection against striped cucumber beetles for 7 days after application when the
control did not. The FarMore® seed treatment did not provide protection against striped cucumber beetles. The results have led to the modification of neonicotinoid product and application method recommendations to growers to maximize insecticide efficacy while minimizing honey bee health risks.
INTRODUCTION

Food production must increase to feed the growing world population which is projected to rise 35% from the 2011 estimate of 6.9 billion to 9.3 billion by 2050 (UN Population Division, 2011). This translates to an estimated 70% food production increase necessary to account for rising incomes and, therefore, higher consumption rates for 1 billion people that are currently underfed (FAO, 2009). Insect pest management is a crucial aspect of food production needed to meet the projected worldwide food requirements. Insects are a major contributor to crop yield losses (Oerke, 2006) and insecticides are an important management component necessary to attain yields that will meet the needs of the growing world population.

The first synthetic organic insecticides appeared in the late 1930’s (Cremlyn, 1978). Organochlorines, including dichlorodiphenyltrichloroethane (DDT), originally used in World War II, were the first of many synthetic insecticides to be used in pest management. Despite its insecticidal effectiveness and broad insect target range, DDT was banned in 1972 due in part to its persistence in the environment and mammals (US EPA, 1972). Organophosphates and carbamates replaced organochlorines as more effective and less persistent compounds with broad spectrum insecticidal properties (Chapalamadugu and Chaudhry, 1992). Organophosphates and carbamates act on the acetylcholinesterase, a vital enzyme in the insect and human nervous systems (Casida, J. E., 1963; Cremlyn, 1978). Although still used today, organophosphates have been increasingly replaced by safer insecticides that are less toxic to mammals (Chambers et al., 2010). Pyrethroids were registered for use as insecticides in the late 1970s (Soderlund, 2010) and with favorable properties of high insecticidal activity and low acute mammalian toxicity, they began replacing organochlorines, organophosphates, and carbamates. Pyrethroids act on the insect nervous system by disrupting sodium ion channels. These synthetic compounds have improved stability compared to that from which they were derived, pyrethrins, and are still used widely today.
Nicotine, an extract from tobacco, has been used for insect control throughout agricultural history (McIndoo, 1916; Tomizawa and Casida, 2009). It is, however, very toxic to mammals and has minimal insecticide activity compared to commercial insecticides used today. Neonicotinoids are synthetic insecticides that were discovered while attempting to find alternative structures for nicotine. Neonicotinoid insecticides are safer for humans and often more effective at managing invertebrate pests than previously used classes of insecticides (Elbert et al., 1990; Tomizawa and Casida, 2003). Neonicotinoids are the fastest growing class of insecticides in the world in terms of usage and market sales as they are registered in over 120 countries and comprised 24% of the insecticide market sales and 80% of all insecticide seed treatment sales in 2008 (Jeschke et al., 2011). Neonicotinoids are often applied by commercial growers for use on cucurbits.

Although neonicotinoids have excellent insecticidal properties against pests, they are also highly or moderately toxic to honey bees (US EPA, 2012). Honey bees provide pollination services to many agricultural systems, including cucurbits. The presence of neonicotinoid residues in the pollen and nectar of cucurbit flowers may threaten honey bee health. Concern for this economically important pollinator has led to research evaluating the possible connection between neonicotinoid use and honey bee population decline. Neonicotinoid risk has been evaluated in in many toxicity studies regarding lethal, sublethal, and visual effects yet very few studies have published the realistic field concentrations of these toxins. Cresswell (2011) recognized the need for more studies to determine field realistic concentrations of neonicotinoids in pollen and nectar.

Dively and Kamel (2012) studied the pollen concentrations of imidacloprid, dinotefuran, and thiamethoxam with 9 treatments applied to pumpkins, Cucurbita pepo L., with different application methods at different times. The highest mean levels of imidacloprid were 80.2 ng/g in pollen for the split treatment in transplant water and drip irrigation. Mean thiamethoxam pollen residues reached 68.0 ng/g and 95.2 ng/g in the transplant-drip irrigation split treatment and in the treatment of two foliar sprays, respectively. Stoner and Eitzer (2012) studied neonicotinoid concentrations in pollen and nectar of summer and winter squash, Cucurbita pepo L. Concentrations ranged between 5 and 35 ng/g in pollen and 5 and 20 ng/g in nectar, encompassing all residues from treated plants. The mean concentrations of both insecticides
used at label rates over the course of the study for pollen were $14\pm8$ ng/g imidacloprid and $12\pm9$ ng/g thiamethoxam.

These studies provide information for their respective commodities. Although all cucurbits have similar properties, one key difference between muskmelon and pumpkin or squash is that muskmelon have smaller flowers. No studies have been conducted to determine the content of neonicotinoids in muskmelon. As a widely produced agricultural crop, and one for which pollination services in the form of rented honey bee hives is often required, it is important to determine the most effective pest management treatment for muskmelons while minimizing potential negative impacts on honey bees.

The goals of this study were to determine the potential impact of neonicotinoid insecticides on honey bees in muskmelon production and to determine the extent and duration of striped cucumber beetle control among treatments. Quantifying the potential neonicotinoid impact on honey bees was accomplished in two steps. First, the residue concentrations in muskmelon pollen were determined for several recommended neonicotinoids applied at high and low label rates. The delivery methods for the neonicotinoids included seed treatments, bedding tray applications, transplant soil drenches, and foliar sprays. Secondly, the field residue concentrations were coupled with known honey bee toxicological sensitivity to determine the health risk associated with such neonicotinoids and delivery methods. The neonicotinoids evaluated were imidacloprid and its metabolites imidacloprid olefin and 5-hydroxyimidacloprid, thiamethoxam and its metabolite clothianidin, and acetamiprid.
Neonicotinoid Insecticides

Neonicotinoids are the fastest growing class of insecticides in the world (Jeschke et al., 2011). The first neonicotinoid, imidacloprid, was sold in the 1990s and several more have since entered the market. Within the neonicotinoid class of insecticides, the seven active ingredients commercially sold today are imidacloprid, thiacloprid, thiamethoxam, nitenpyram, acetamiprid, clothianidin, and dinotefuran. Chloronicotinyls and thianicotinyls comprise the two subclasses of neonicotinoids (Laurino et al., 2011). The first generation neonicotinoids imidacloprid, thiacloprid, nitenpyram, and acetamiprid comprise the subclass chloronicotinyl (Wakita et al., 2003). Second generation neonicotinoids in the thianicotinyl subclass include thiamethoxam and clothianidin. Dinotefuran is the most recent neonicotinoid from the third generation with a proposed subclass, furanicotinyl.

Neonicotinoids are classified by the Insecticide Resistance Action Committee into Group 4A by mode of action as a nicotinic acetylcholine receptor (nAChR) agonist (Jeschke and Nauen, 2008) within the central nervous system (Tomizawa and Casida, 2003) and therefore neonicotinoids disrupt neurological signals. Insect nAChRs enable fast information transmission at synapses with the use of a neurotransmitter and receptor complex which occurs throughout the insect brain (Jeschke and Nauen, 2012). Acetylcholine (Ach) is the most common neurotransmitter found in insect brains and is confined to the central nervous system where it is the most abundant neurotransmitter (Dupuis et al., 2012). Neonicotinoids target and bind to the postsynaptic nAChR in insects (Jeschke and Nauen, 2012) which prevents ACh from being able to bind to nAChR and pass along the excitatory signal to the following neuron. This agonistic activity causes channel opening resulting in hyperexcitation and then paralysis. The nAChR is important to excitatory synaptic transmission in the insect nervous system that has been found on presynaptic and postsynaptic nerve terminals, motor neurons, sensory neurons, and cell bodies of interneurons.
Honey bees have eleven structurally diverse subunits which represent species-specific receptor subtypes resulting in species-specific responses to insecticides at different concentrations (Jescke and Nauen, 2012; Jones, 2006). The nAChR subunits are assembled in an array of formations that create channels in different nAChR subtypes which have different physiological properties (Jescke and Nauen, 2012). The different subtypes correspond to various locations within the insect central nervous system influencing specific functions. The eleven nAChR subunits found in honey bees are transcribed at all developmental stages including larval, pupal, and adult periods (Jones et al., 2006). In larval development, the nAChR is expressed in the esophageal ganglion which is involved in feeding senses and motor functions (Dupuis et al., 2012; Thany et al., 2003). In the adult however, nAChR expression occurs in in the dorsal lobe, optical lobes, antennal lobes, and mushroom bodies involved in the neurological processes which influence olfactory and visual processes, learning and memory, and antennal use (Dupuis et al., 2012; Jones et al., 2006; Thany et al., 2003). These are potential functions of the honey bee that may be affected by neonicotinoids.

The major pests targeted by neonicotinoids are sucking insects such as aphids, leafhoppers, and whiteflies (Tomizawa and Casida, 2003) as well as several coleopteran and a few micro lepidopteran insects (Elbert et al., 2008). The insecticide is evenly distributed throughout plants through the xylem via root uptake from seed or soil application or dispersion through the leaves from foliar application. The systemic activity and wide insect target range allows neonicotinoids to be useful on numerous plants and crops. Some of the crops neonicotinoids are listed for use on include vegetables, stone fruit, rice, cotton, citrus, potato, corn, and soybean. Neonicotinoids are replacing the use of many organophosphates, carbamates, and pyrethroids because of regulatory decisions, improved human safety, effectiveness, and resistance (Elbert et al., 1990; Tomizawa and Casida, 2003).

Neonicotinoids have several key characteristics that make them exceptional insecticides. Neonicotinoids are relatively safe to humans compared to previously used classes of insecticides because of their high selectivity for insects and low toxicity to humans (Liu and Casida, 1993; Tomizawa and Casida, 2003; Yamamoto et al., 1998; Yamamoto et al., 1995). Neonicotinoids bind to the nAChR specifically in insects and not mammals thus making them safer than previously used nicotinic insecticides (Tomizawa and Casida, 2003). Attributing to their safety, neonicotinoids are greater than 100 times more selective to insect nAChRs than mammalian
nAChRs although the reason for this is poorly understood (Jeschke and Nauen, 2012). The selection for insect versus mammalian nAChRs is thought to be due to their differing chemical features causing sensitivity differences of their respective nAChR subtypes (Tomizawa and Casida, 2009). Neonicotinoids, unlike nicotinoids, are not protonated which selects for the insect nAChR rather than the mammalian nAChR (Tomizawa and Casida, 2005).

Neonicotinoids are also versatile with foliar spray, soil applied drench, and seed treatment as the methods of application. Neonicotinoids move through the plant exceptionally well due to high water solubility (Tomizawa and Casida, 2003; Tomizawa and Casida, 2005) providing the entire plant with protection. Of the neonicotinoids assessed in this study, acetamiprid has the greatest water solubility at 4.25 g/L followed by thiamethoxam at 4.1 g/L, imidacloprid at 0.61 g/L, and clothianidin at 0.34 g/L (Tomizawa and Casida, 2005). These values are extremely high compared to the herbicide atrazine which has been an environmental concern due to its presence in surface water in the United States (Graziano et al., 2006) in part due to its water solubility at 0.03 g/L (US EPA, 2003b).

Neonicotinoids have been detected in surface waters at a higher frequency than previously used classes of insecticides which has been attributed to their high water solubility and persistence (Hladik et al., 2014). In 2013, 79 water samples were taken in Iowa from nine locations and tested for neonicotinoid presence (Hladik et al., 2014). Clothianidin was present in 75% of the water samples, thiamethoxam in 47%, and imidacloprid in 23%. Seed treatments of field crops were suggested as the main source of the neonicotinoids. In California, 75 samples were taken from the rivers, creeks, and agricultural drains at 23 sites encompassing three agricultural regions (Starner and Goh, 2012). Imidacloprid was present in 89% of the samples taken and attributed to its applications on lettuce, broccoli, cauliflower, and wine grape crops. In the Southern High Plains throughout Texas, New Mexico, and Oklahoma wetlands sampled near agricultural fields tested positive for acetamiprid and thiamethoxam in 17% and 31% of the samples, respectively (Anderson et al., 2013). The main crop produced in this area, cotton, was recognized as responsible for the majority of the pesticide content, yet wheat, sorghum, corn, and sunflowers are also grown in the area. High water solubility enhances the ability of neonicotinoids to protect plants from pests, yet also may result in their presence in surface or ground water due to runoff or leaching.
Neonicotinoids have a long residual activity (Elbert et al., 2008) which is an important aspect of effective insecticides. The rate of breakdown is reduced in neonicotinoids because the nitroimine or cyanoimine is replaced by nitromethylene which decreases the amount of sunlight absorbed by the insecticide (Tomizawa and Casida, 2003). Long residual time may be undesirable when considering impact on unintended targets and ultimate environmental fate. Neonicotinoid degradation, specifically in soil, depends on several environmental factors including texture and organic matter content of the soil, ultraviolet light exposure, moisture content, temperature, and pH (Bonmatin et al., 2014). Therefore, published half-lives of neonicotinoids in soil are quite variable. The influence of these factors on neonicotinoid degradation are described in Bonmatin et al. (2014). Drier soil results in slower degradation due to decreased microbial activity and reduced leaching. In addition, lower temperatures also decrease chemical breakdown due to slowed or halted microbial activity. Sunlight accelerates decomposition, yet only when the compound resides on the soil or water surface. In short, dry soil, low temperatures, and minimal sunlight exposure all increase the time neonicotinoids will remain within soil. Soil or seed applied neonicotinoids would be expected to persist longer than foliar applied sprays with minimal sunlight exposure if any. This is especially true for commercially grown cucurbits when black plastic mulch is used.

The time it takes for 50% of an applied insecticide to degrade into different compounds, or its half-life, provides insight as to the duration that it may reside in the environment. The half-life of imidacloprid is estimated to range from one month to over three years (Goulson, 2013). Thiamethoxam persists in the soil with a half-life of one week to almost 11 months. Clothianidin has the widest half-life range of just under five months to over 19 years (DeCant and Barrett, 2010). Acetamiprid’s half-life ranges from one month to almost 15 months (Goulson, 2013). Neonicotinoid persistence throughout the growing season is desired for extended insect control, yet long residual beyond targeted pest control may lead to unintended consequences especially since neonicotinoids are very mobile in water and readily taken up by plants.

The potential for exposure of neonicotinoids to honey bees is enhanced by their persistence in the environment and exceptional systemic activity leading to movement into the pollen of flowers. Studies have investigated honey bee toxicological sensitivity to neonicotinoids due to concern for pollinator health. Honey bees are a model species for toxicological analysis because their learning process for foraging activities is well understood (Desneux et al., 2007).
The chemical composition of neonicotinoids determines their individual magnitude of toxicity. Nitro-substituted compounds, or nitroguanidines, are the most toxic to honey bees and include imidacloprid and its metabolites, thiamethoxam, clothianidin, dinofuran, and nitenpyram (Decourtye and Devillers, 2010). The cyano-substituted compounds, or cyanoamidines, which include acetamiprid and thiacloprid are much less toxic to bees.

Lethal toxicity, sublethal effects, visual effects, and the no observed effect level (NOEL) are measures to evaluate the impact that neonicotinoids have on honey bees. Studies either assess toxicity with acute (limited one time exposure) or chronic exposure (exposure duration over a period of time). Chronic studies are more realistic assessments as typical exposure occurs in repeated small amounts rather than one intense administered dose. Unfortunately, most values in the literature represent acute exposure and few chronic studies have been performed. Additional chronic studies are needed to more realistically assess risks posed to honey bees as neonicotinoids present in pollen would be most likely be encountered at small, repeated doses. Toxicity studies refer to a concentration of the neonicotinoid within a food source as ng/g or as an administered dose presented as ng/bee. Conversions need to be made to compare concentrations in ng/g and doses in ng/bee to make use of the toxicity studies within the literature.

The contact dose of pollen can be converted to a concentration value based on the average weight of the honey bee by dividing ng/bee by the average bee weight of 0.1 grams as was done in Mullin et al. (2010). For example, a contact dose of 10 ng/bee would be equivalent to a pollen concentration of 100 ng/g (10 ng/bee x bee/0.1 g). Field realistic neonicotinoid concentrations in the pollen of flowers were determined in ng/g and therefore, this conversion enables the use of contact doses within the literature presented in ng/bee. The oral dose of pollen in ng/bee is no so readily converted to a concentration of ng/g as it is ultimately dependent on how much pollen a honey bee consumes, and several assumptions must be made.

The pollen consumption of the average adult bee is never the same in two different colonies, and can in fact vary greatly even with a comparable population structure (Crailsheim et al., 1992). Crailsheim et al. (1992) estimated the pollen consumption for adult bees at 3.82 mg per day. This information, coupled with known field concentrations of neonicotinoids will tell if the honey bees are consuming a lethal or a sublethal dose. For example, in Kasiotis et al. (2014),
pollen concentrations of imidacloprid were found to reach 74 ng/g imidacloprid. If an average adult honey bee that consumes 3.82 mg of pollen per day with a concentration of 74 ng/g of imidacloprid will have been exposed to 0.283 ng (3.82 mg x 74 ng/g) of imidacloprid per day (0.283 ng/bee). The connection between a field realistic neonicotinoid pollen concentration in ng/g to the oral dose that a honey bee may receive in ng/bee can only be made once it is known how much contaminated pollen a honey bee will consume. Crailsheim et al. (1992) and Rortais et al. (2005) are the only studies to have assessed honey bee pollen consumption. Further studies that focus on honey bee pollen consumption are needed to provide the information necessary to most accurately assess honey bee risk based on field concentrations and toxicological sensitivity presented in the literature as ng/bee. For the purposes of this study, only contact doses in ng/bee reported in the literature were converted to concentration values in ng/g. The oral dose in ng/bee was not converted to a concentration in ng/g so as to avoid extrapolation of the data since the results would have been based on many assumptions including the age and task being performed by the honey bee, the grams of pollen consumed, and the number of days of consumption.

LD₅₀ values for oral ingestion and contact application quantify the lethal toxicity of neonicotinoids. The LD₅₀ refers to a single dose of chemical that kills 50% of the individuals that are treated (Cresswell, 2011). The LD₅₀ values reported from several studies for various neonicotinoids and their metabolites are reported in Table 1. The US EPA (2012) describes compounds as highly toxic to bees based on LD₅₀ values that are less than 2000 ng/bee. All compounds in Table 1 reside in this highly toxic category aside from acetamiprid which is considered moderately toxic to bees. Variation of LD₅₀ values within the literature may be due to several factors. The number of colonies from which the bees were sampled, the time after treatment when the LD₅₀ was recorded (24 h, 48 h, etc.), the health of the bees, the quality of pollen or nutritional stress (Wahl and Ulm, 1983), and the starvation period preceding treatment all affect LD₅₀ values. In addition, the age of the bees (Guez et al., 2001) where older bees are more sensitive to pesticides (Wahl and Ulm, 1983) as well as the time of year the bees were sampled (Decourtye et al., 2003) may also lead to variation in LD₅₀ values.

A sublethal effect is a negative change in some aspect of the honey bee’s ability to survive and contribute to colony reproduction without resulting in mortality (Cresswell, 2011; Desneux et al., 2007). These include reduced memory, disrupted olfactory senses, feeding
changes, reduced motor function, and the inability to return to the hive (Decourtye et al., 2003, 2004a, 2004b, 2005; Guez et al., 2001, 2003; Hassani et al., 2008; Henry et al., 2012; Lambin et al., 2001). Visual effects are evaluated by simply observing the reaction of the honey bee to certain doses of insecticide. The no observed effect level (NOEL) represents the dose of pesticide that results in no observed effect yet should be used with caution. NOEL should not be interpreted as an overall safe level of insecticide as it is case specific, and studies cannot possibly account for all effects that a pesticide may inflict. For example, a study may determine that the NOEL for a honey bee is 10 ng/bee of an insecticide. Yet if the authors looked for effects on consumption rate, mortality, and learning ability but did not assess effects on foraging duration, then it cannot be said that this indeed is a NOEL. This NOEL level of 10 ng/bee must be specifically applied to no effects on mortality, learning ability, and consumption only. Due to the ambiguity of NOELs, this paper will instead refer to the lowest values of neonicotinoids published that cause any adverse effect. Collectively, these impact assessments are used to evaluate the negative impacts that several neonicotinoids have on honey bees. The neonicotinoids discussed in this report are those that are specifically recommended by the Midwest Vegetable Production Guide (Egel et al., 2013) for use on cucurbits in commercial production.

**Imidacloprid**

Imidacloprid was the first neonicotinoid sold in the insecticide market, first offered in 1991 by Bayer CropScience (Jeschke and Nauen, 2008). Imidacloprid has the highest sales of all insecticides worldwide and studies have evaluated its impact on honey bees more than any other neonicotinoid. This may be attributed in part to its wide spectrum of pest control on 140 different crops applied as a foliar spray, soil drench, or seed treatment (Jeschke et al., 2011). The estimated acute oral ingested LD\textsubscript{50} values for imidacloprid range from 3.7-81 ng/bee (Cresswell, 2011; Decourtye et al., 2003; Nauen et al., 2001; Schmuck et al., 2001; Suchail et al., 2001). Acute contact LD\textsubscript{50} values are estimated to be 18-243 ng/bee (Iwasa et al., 2004; Nauen et al., 2001; Schmuck et al., 2001; Stark et al., 1995). Chronic studies may demonstrate a more realistic exposure to neonicotinoids, yet few exist. After consumption of 0.1 ng/g imidacloprid concentrated sucrose solution for 8 days, equivalent to a dose of 0.01 ng/bee, 50% of the tested honey bees had died (Suchail et al., 2001). Another study found a significant increase in
mortality after feeding 11 days on 48 ng/g concentrated imidacloprid sucrose (Decourtye et al., 2003).

Negative sublethal effects include decreased feeding (Decourtye et al., 2003), decreased mobility (Lambin et al., 2001; Teeters et al., 2012) disrupted olfactory learning senses (Guez et al., 2001; Decourtye et al., 2003, 2004b; Lambin et al., 2001) which may alter foraging abilities (Decourtye et al., 2005), and reduced mid-term memory of 15 minutes to one hour (Decourtye et al., 2004a). Other studies demonstrated a reduced number in overall foraging trips (Schneider et al., 2012) along with an increased foraging duration determined by increased flight time to food source, duration spent at food source, and return flight time to the hive. The lowest level of imidacloprid reported resulting in adverse effects on honey bees is 0.01 ng/bee equivalent to 0.1 ng/g administered over 8 days (Suchail et al., 2001).

Aside from LD$_{50}$ values and sublethal responses, visual effects are also considered when evaluating insecticide applications. The initial response to oral treatment of imidacloprid to honey bees is hyperactivity and tremors (Suchail et al., 2003) as well as obvious mobility distress such as shaking, incoordination, and knock down (Decourtye and Devillers, 2010; Nauen et al., 2001; Suchail et al., 2001). These side effects slowly end and after several hours the bees become hypoactive (Suchail et al., 2001).

The metabolites of the parent compound must be explored when considering the toxicity of imidacloprid to honey bees. Imidacloprid metabolizes very quickly within the honey bee into its major metabolites 5-hydroxyimidacloprid and then olefin (Suchail et al., 2003) which are the only metabolites toxic to honey bees (Suchail et al., 2001). Olefin is more toxic than its parent compound with an acute oral LD$_{50}$ of 23 ng/bee (Suchail et al., 2001) and greater than 36 ng/bee (Nauen et al., 2001). Olefin has also inhibited the honey bee olfactory learning ability at a contact dose of 0.1 ng/bee (Guez et al., 2003). 5-hydroxyimidacloprid is less toxic than imidacloprid with an oral LD$_{50}$ ranging from 153.5-222 ng/bee (Decourtye et al., 2003; Nauen et al., 2001; Suchail et al., 2001). In a chronic study, when 5-hydroxyimidacloprid was fed upon for 11 days, increased morality occurred at 240 ng/g and decreased feeding was observed at 30 ng/g (Decourtye et al., 2003). Suchail et al. (2003) concluded imidacloprid is responsible for the initial neurological deterioration of the honey bee within the first 10 minutes of treatment and the metabolites cause mortality. Similarly, Guez et al. (2001) found that imidacloprid is responsible for initial sublethal effects and its metabolites are accountable for long term effects.
The toxicological effects of imidacloprid and its metabolites olefin and 5-hydroxyimidacloprid vary depending on the age of the honey bee (Guez et al., 2001, 2003) as well as the time of year (Decourtye et al., 2003). The lowest amounts reported to cause adverse effects on honey bees are an acute contact dose of 0.1 ng/bee olefin imidacloprid (Guez et al., 2003) and chronic exposure for 11 days of 30 ng/g 5-hydroxyimidacloprid (Decourtye et al., 2003).

Thiamethoxam

Thiamethoxam was first commercially sold in 1998 and is easily produced with high chemical yield relative to initially required input materials (Maienfisch et al., 2001). Syngenta Crop Protection, the patent holder of thiamethoxam, claims that thiamethoxam has greater defense for plants against chewing and sucking insect pests compared to imidacloprid. Applied as a foliar spray, soil drench, or seed treatment, thiamethoxam is registered for use on 115 crops (Jeschke, et al., 2011). The estimated acute oral LD$_{50}$ values for thiamethoxam are 4.3 ng/bee (Laurino et al., 2011) and 5 ng/bee (European Commission, 2006; Syngenta Group 2005). Acute contact LD$_{50}$ values include 24 ng/bee (European Commission, 2006; Syngenta Group 2005) and 29.9 ng/bee (Iwasa et al., 2004). In one chronic study, all honey bees allowed to feed upon 100 ng/g thiamethoxam concentrated sugar for one hour, died within 72 hours of exposure whereas no control bees died (Laurino et al., 2011). The only study to address sublethal effects of thiamethoxam demonstrated a significant reduction in the number of foraging honey bees that returned to their hive after being fed an acute dose of 1.34 ng/bee thiamethoxam which is the lowest observed adverse effect dose and equivalent to a concentration of 67 ng/g (Henry et al., 2012).

Thiamethoxam is quickly metabolized in plants and converted to clothianidin, another neonicotinoid (Nauen et al., 2003). Nauen et al. (2003) found the concentration of clothianidin in cotton to be double that of thiamethoxam when thiamethoxam was applied as a soil drench. This study demonstrated that thiamethoxam is quickly metabolized to clothianidin within cotton. Clothianidin affects the central nervous system at one hundred times the magnitude of thiamethoxam (Nauen et al., 2003) making it an important metabolite to consider when evaluating toxicity in residues (Tomizawa and Casida, 2005). The oral LD$_{50}$ for clothianidin on honey bees is estimated at 2.6 ng/bee (Laurino et al., 2011) and 3.8 ng/bee (European Commission, 2005), whereas the contact LD$_{50}$ dose ranges from 21.8-44.3 ng/bee (European
An increase in mortality occurred after an acute feeding of 75 ng/g clothianidin (Laurino et al., 2011). In a chronic exposure study conducted by Sandrock et al. (2014), honey bees were provided pollen patties placed within the nest containing a concentration of 5.3 ng/g thiamethoxam and 2.1 ng/g clothianidin for 46 days. The exposure to these compounds resulted in significantly less adult worker populations compared to the control populations after 1.5 months of feeding. The reduced number of adults was even more pronounced one year after the feeding trials. Other significant effects included reduced honey production, reduced pollen collection, and reduced long term colony growth measured one year after exposure. At the lowest dose causing adverse effects, honey bees demonstrated a reduced ability to fly back to their nest following a topically applied dose of clothianidin at 2.18 ng/bee (Matsumoto, 2013).

Acetamiprid

Acetamiprid was first patented in 1989 (Tomizawa and Casida, 2005) and is registered for use on 60 different crops applied as a foliar spray or soil drench (Jeschke et al., 2011). The acute oral LD$_{50}$ is 14,530 ng/bee (European Commission, 2004). The acute contact LD$_{50}$ of acetamiprid has been reported at a dose of 7,070 ng/bee (Iwasa et al., 2004) and 8,090 ng/bee (European Commission, 2004). Topically applied doses of 100, 500, and 1000 ng/bee of acetamiprid reduced responsiveness to water (Hassani et al., 2008). Reduced sucrose responsiveness occurred when acetamiprid was administered orally at 100 and 500 ng/bee yet not at 1000 ng/bee. Similarly reduced learning performance occurred at 100 ng/bee yet not at 500 or 1000 ng/bee when orally administered. A dose of acetamiprid at 100 ng/bee is the lowest level to cause adverse effects on honey bees. Acetamiprid is much less dangerous to honey bees than imidacloprid and its metabolites, thiamethoxam, and clothianidin.

Acetamiprid is quickly metabolized once ingested by honey bees (Brunet et al., 2005). Iwasa et al. (2004) found that the metabolites of acetamiprid IM-2-1, IM-O, and IC-O caused no mortality when applied at a contact dose of 50,000 ng/bee. In addition, the concentrations of acetamiprid metabolites in plants are insignificant when compared to parent concentrations (Roberts et al., 1999). Therefore, toxicological studies need only evaluate the parental compound acetamiprid and not its metabolites.
Honey Bees *Apis mellifera*

Honey bees are one of the most important pollinators in the world (National Research Council, 2007), therefore, there is great concern for the harm that neonicotinoids may cause. Honey bees originated in Africa and have since spread to many parts of the world (Whitfield, 2006). Linnaeus named them *Apis mellifera* (Whitfield, 2006) and as the only species of honey bee in North America (Calderone, 2012) they are referred to as the European or Western honey bee (Whitfield, 2006).

Pollinators are imperative for agricultural production worldwide (National Research Council, 2007). The economic importance of honey bees as agricultural insect pollinators has been well documented. The worldwide economic value of pollinator insects in 2005 for all agricultural crops used directly for food was estimated just under $200 billion (Gallai et al., 2009). North America constitutes over 10% of this estimate at almost $20 billion worth of estimated economic pollinator value. Honey bees provide pollination services to many agricultural crops valued at an estimated $17.07 billion for the United States in 2009 (Calderone, 2012).

**Foraging**

Honey bees are social insects and rely on well-defined roles of individuals in cooperation to maintain a successful and productive colony. There is a wide range of tasks that are carried out by individuals within a colony. Workers carry out the majority of the non-reproductive activities within the hive including cleaning, building, venting and guarding the hive, brood and queen caring, foraging, and handling food (Winston, 1987). One of the most important activities performed by workers is foraging for nectar and pollen. Communication between the nest mates is key to everyday activities and most important to foraging. When a worker honey bee flies out and finds a plentiful resource, it returns to the hive and performs a dance to inform the other worker bees (Dyer and Could, 1983). The various dances correspond to distance and direction of the resource. The colony is able to thrive and gather the necessary resources by using these dance communications and working together. Foraging is the last task that a honey bee worker performs which most often begins between 2 and 3 weeks of age (Winston, 1987). The duration of time that a honey bee spends foraging ranges from 2 to 17 days with an average duration of 7.7 days and is dependent upon the time spent foraging, days of foraging activity, or
the number of foraging trips (Visscher and Dukas, 1997). Honey bee foraging distance ranges from 0.67 km to greater than 9.5 km with a median distance of 6.1 km and is dependent upon colony size, time of year, patch size, and patch quality (Beekman and Ratnieks, 2000; Beekman et al., 2004). Motor function disruption and uncoordinated movement caused by neonicotinoid exposure (Decourtye and Devillers, 2010; Nauen et al., 2001; Suchail et al., 2001) may negatively impact the ability to forage as well as communicate information vital to foraging success. A foraging honey bee that is not able to return to its nest should be considered dead since they cannot survive independent of their colony.

Honey Bee Reliance on Flowering Plants

Honey bees rely on the nectar and pollen they receive from flowers in exchange for the pollination of the plant. Pollen collection is the primary reason for honey bee foraging (Simpson and Neff, 1981). They rely on pollen for protein, lipids, vitamins, and minerals (Winston, 1987). Larvae are highly dependent on nutrients and proteins supplied by the nurse bees who feed them (Haydak, 1970). Pollen and nectar are the primary components of jelly fed to all larvae by nurse bees (Haydak, 1970) and royal jelly fed to queen larvae (Chen and Chen, 1995). Pollen is also processed by the worker bees for long term storage in the hive as “bee bread” (Winston, 1987). Pollen is important to the development of the hypopharyngeal gland and ovaries in honey bees (Pernal and Currie, 2000). When pollen supplies are limited and do not meet colony needs, brood rearing ceases subsequently leading to a decrease in worker bees that would have otherwise developed from the larvae (Imdorf et al., 1998).

Nectar is just as essential to honey bee survival as pollen. This sugary resource meets the energy requirements of the colony by providing the necessary carbohydrates (Winston, 1987). Foragers, large larvae, and nurse bees either translocate or consume nectar which is distributed throughout the entire bee hive (Nixon and Ribbands, 1952; Rortais et al., 2005). Honey is produced in part by the evaporation of water from nectar collected by foragers during foraging activities, in flight back to the colony, and within the hive (Nicolson and Human, 2008). Honey is stored in the hive and used for adult and larva feeding (Winston, 1987).

Possible modes of exposure to neonicotinoids are through contact or consumption of pollen or ingestion of nectar (Krupke et al., 2012; Rortais et al., 2005). Clothianidin and thiamethoxam were found in pollen samples from two different hives at the same apiary, yet
neither of the neonicotinoids was detected in nectar (Krupke et al., 2012). In addition, the most frequently detected pesticides in bee bread sampled from various honey bee hives in France were neonicotinoids and included imidacloprid, thiamethoxam, and acetamiprid (Giroud et al., 2013). Kasiotis et al. (2014) sampled bees, pollen, and honey from various colonies throughout Greece where colony loss or bee deaths had been reported. The three year study found pollen concentrations ranging from 72 to 74 ng/g imidacloprid and 6 to 1273 ng/g clothianidin. Honey bees were found to contain neonicotinoids concentrations of up to 5.74 ng/g imidacloprid, 50 ng/g thiamethoxam, and 40 ng/g clothianidin. In a similar study, neonicotinoids were found in wax and pollen samples obtained in Florida and California from 13 apiaries owned by 11 different bee keepers (Mullin et al., 2010). Two samples of wax contained imidacloprid at an average concentration of 8 ng/g. Of the pollen samples with detected neonicotinoids, the average concentrations were 21 ng/g imidacloprid, 554 ng/g imidacloprid olefin, 152 ng/g 5-hydroxyimidacloprid, 53 ng/g thiamethoxam, and 57 ng/g acetamiprid. Neonicotinoids were not detected within any of the sampled bees. Brood combs used to rear honey bees and study sublethal effects were collected in the Pacific Northwest where deaths were suspected from colony collapse disorder (Wu et al., 2011). Bees reared in contaminated brood combs which contained on average 45 ng/g imidacloprid, 38 ng/g thiamethoxam, and 35 ng/g clothianidin incurred a significant decrease in life span as well as delayed development. The continual need for immediate use and storage of pollen and nectar within the hive presents a danger if contaminated by neonicotinoid pesticides.

Multiple Stressors Enhance Neonicotinoid Impact

Several factors including parasites, pathogens, Africanized honey bees, and pesticides (Robinson et al., 1989) may influence the declining population of commercially managed honey bee colonies which has decreased in North America from 5.9 million colonies in 1947 to 2.6 million in 1996-2004 (National Research Council, 2007) and continues to decline (vanEngelsdorp and Meixner, 2010). This report focuses on the impact that neonicotinoid pesticides have on honey bee health, yet the extent to which honey bees are affected is synergistically effected by many factors. Nosema ceranae is a parasite that weakens honey bee energy stores by decreasing nutrients within the honey bee midgut (Fries et al., 1996). The individual and interactive effects of Nosema microspores and concentrations as low as 0.7 ng/g imidacloprid
were assessed in a chronic, 10 day exposure study on honey bees (Alaux et al., 2010). *Nosema* and imidacloprid treated bees had significantly lower protein concentration in their heads than control bees. *Nosema* infected bees consumed more sucrose than imidacloprid treated and untreated bees. In addition, all treatments resulted in significantly higher deaths than in the control with *Nosema* and imidacloprid exposed bees having the highest mortality rate. The increased consumption of sucrose due to *Nosema* infection increased the dose of imidacloprid received. This study demonstrated just one example in which multiple factors influence honey bee health. In another chronic study, bees were exposed to 5 and 20 ng/g imidacloprid concentrated pollen patties for 5 to 8 weeks (Pettis et al., 2012). Emerging adult bees were then fed *Nosema* pathogenic spores and then sacrificed for analysis. Bees exposed to imidacloprid and dosed with *Nosema* spores had significantly higher levels of *Nosema* infection than bees dosed with *Nosema* originating from control colonies. Di Prisco et al. (2013) observed clothianidin disrupt honey bee immune response at a contact dose of 21 ng/bee. In addition, clothianidin and imidacloprid applied topically at a dose of 0.02 ng/bee promoted replication of the viral pathogen deformed wing virus. These studies demonstrate increased susceptibility to disease following sublethal exposure to neonicotinoids. Neonicotinoid impact may be more pronounced when colonies are exposed to these compounding factors.

**Cucurbit Pollination and Production**

Cucurbit production is highly reliant on pollinators for fruit development (McGregor, 1976) with the most abundant contributor being the honey bee (Mann, 1953; McGregor and Todd, 1952; McGregor et al., 1965). Cucurbits are vine plants within the family *Cucurbitacea* and include *Cucumis melo* L., muskmelon, which has vines with bright yellow flowers (Lerner and Dana, 2001). Most cucurbits are monoecious yet still require fertilization by pollinating insects (Free, 1970). The male reproductive part of a flower is the stamen containing anthers with pollen, and the female reproductive organ is the pistil which comprises the ovary that produces the fruit and the seeds or ovules (Lerner and Dana, 2001). Male (staminate) flowers occur near the tops of the vine leaves, and female (pistillate) flowers occur under leaves nearer to the ground (Free, 1970). Bees see the male flowers more readily due to their location and therefore are more inclined to visit them initially. All early flowers of melon are male with short stems in
clusters of three to five (Johnson, 2011). Female flowers have a small round ovary directly beneath the flower, that when pollinated, sets fruit.

Once pollination has been initiated, the pollen grains germinate within a few hours (Mann and Robinson, 1950). Each developing seed requires at least one pollen grain to reach the stigma (McGregor and Todd, 1952). For fruit to set, pollen from the male flower must be transferred to the female flower (Johnson, 2011). In the act of pollination, the bee proboscis rubs the stamens and accumulates pollen which is then rubbed onto the pistil of a female visited flower (McGregor and Todd, 1952). Insect pollinated muskmelon produce larger fruit than those that rely on hand-pollination (Mann, 1953). The more a flower is visited by a honey bee, the greater the quality of the melon fruit (McGregor et al., 1965; Taylor, 1955). There is a significant correlation between the number of times a flower was visited by a honey bee and the number of seeds produced as well as between the seed number and melon size (McGregor et al., 1965).

Natural pollinators are less abundant and less diverse in agriculturally isolated fields far from natural habitats (Garibaldi et al., 2011). Monoculture fields frequently seen in agricultural production including cucurbit crops are often isolated from these important natural biomes. Reduced wild pollinator availability in cucurbit fields may, in part, have increased the need for domesticated honey bee use for commercial production. Commercial growers need 1-2 honey bee colonies per acre to ensure pollination for fruit set (Spivak, 2011). Colonies are rented prior to the first bloom of flowers in cucurbit fields and remain in the field for a duration of 2-4 weeks.

Muskmelon is a European variety of *Cucumis melo* L. and is monoecious with staminate and pistillate flowers (Free, 1970). Each muskmelon flower opens after sunrise for one day and closes that afternoon (McGregor and Todd, 1952). In California, pollination begins soon after the flowers open around 8 am with peak activity around 11 am followed by decreasing numbers until their absence around 6 pm (Mann, 1953). Similarly the duration of pollination activity was observed from 9 am until noon in Arizona (McGregor and Todd, 1952; McGregor, 1965) and Free (1970) confirmed peak foraging at 11 am.

The melon variety ‘Athena’ was used to determine the amount of combined pollen grain removal by honey bees and the bumble bee (Stanghellini et al., 2002). Staminate flowers produced a total of 11,176 pollen grains per staminate flower of which 44-62% was removed by the foraging honey or bumble bees. Most pollen (57%) was removed in the first two hours.
‘Athena’ pollen grains were measured to be on average 27 micrometers in diameter. In North Carolina, the ‘Athena’ variety flowers opened at 7 am at 21˚C and closed around 1:30 pm at 32˚C. Bumble bee foraging initiated at 7 am followed by honey bee foraging at 7:15 am. The ratio of staminate to pistillate flowers was 5.3 to 0.4 flowers per plant per day. The phenological information derived from this study is important to proposed project methods. The variety ‘Athena’ was used in this research project as it is recommended for use in the Midwest by Egel et al. (2013).

Cucurbits are often planted in rows of covered plastic mulch and drip irrigated. According to the USDA (2010), in 2004-2006, the standard planting dates for muskmelon in Indiana are between April 15th and May 15th. Harvest often begins June 20 and ends August 15 with the highest activity occurring between July 5th and July 31st.

Cucurbits are grown throughout the United States and contribute greatly to agricultural production. In North and Central America, cucurbit production was estimated for melon at 2,440 thousand metric tons in 2004 (Nunez-Palenius et al., 2008). In the year 2011, the land area planted for muskmelon in the United States was 72,590 acres which produced 854,560 metric tons of fruit worth $349,725,000 (USDA, 2012). Indiana was the fourth highest muskmelon producing state valued at $12,698,000. As a widely produced crop, cucurbit management using neonicotinoids may have great impact on honey bee survival. Management of cucurbit pests is vital to production success.

Cucurbit Pests and Management

The pests of cucurbits and management strategies presented here have been obtained from chapter twelve of Vegetable Insect Management (Foster and Flood, 2005) unless otherwise stated. The major insects that threaten muskmelon produced in the Midwest are Delia platura Meigen (seedcorn maggots), Diabrotica undecimpunctata hovardi Barber (striped cucumber beetles), Acalymma vittatum Fabr. (spotted cucumber beetles), Aphis spp. (aphids), and Tetranychus urticae Koch (twospotted spider mites). Many neonicotinoids have been recommended to commercial growers for pest management.

Aphids and mites are rare muskmelon pests. Outbreaks may occur when frequent insecticide sprays are made that kill their naturally occurring predators. If these pests become a problem, spot treatment is often used for control. Foliarly applied thiamethoxam (Actara®) and
soil applied imidacloprid (Admire Pro™) and thiamethoxam (Platinum™) are recommended for aphid control (Egel et al., 2013). Seed maggots are early season pests, and the first generation is the only one that is economically significant. Maggot damage is favored in cool, wet conditions when they feed on and bore into the seedlings causing the plant to wilt and die or inhibit germination altogether. Management practices include plowing the soil and planting in warm, dry soil. Seed treatment or systemic insecticide soil application at planting is recommended with the use of imidacloprid (Admire Pro™) or thiamethoxam (Platinum™). These neonicotinoid compounds also control another major pest, cucumber beetles.

The spotted cucumber beetle is a problem early in the season when plants are small and must migrate annually into Midwestern fields from Southern states. Conversely, striped cucumber beetles overwinter in sheltered areas near fields such as fence rows, buildings, or wooded areas. Adults enter fields, begin feeding, and lay eggs in the soil near host plants when temperatures rise in mid spring. The hatched larvae then feed on cucurbit roots, pupate, and become the second generation of adults which continue to feed on plant vines, leaves, and the fruit throughout the season. Striped cucumber beetles are the primary pest around which the integrated pest management program is built. They are most concerning to growers because they transmit the bacterium *Erwinia tracheiphila* which causes bacterial wilt. Bacterial wilt is detrimental to cucurbits as there is no rescue treatment to save the plant once infected. The transmission process requires the beetle to feed upon the vines and then excrete frass in the plant wound infecting the cucurbit with the bacterium. The bacterium then multiplies within the plant preventing nutrients, food and water movement through the xylem and phloem causing leaves to wilt. The integrated pest management economic threshold to begin applying insecticides for striped cucumber beetles was set by Brust and Foster (1999) at one beetle per plant. In addition to applying systemic insecticides to the soil at planting which provides control for 2-6 weeks, foliar sprays are necessary for continual management. The neonicotinoids recommended are soil applied imidacloprid (Admire Pro™) and thiamethoxam (Platinum™) and the foliar spray acetamiprid (Assail™) (Egel et al., 2013).

**Alternative Pollinators**

There are few known pollinators for muskmelon other than honey bees. McGregor and Todd (1952) investigated viable pollinators for commercial muskmelon production in California
and concluded that thrips, beetles, and native bees were ineffective and unable to provide adequate pollination. During two days spent observing all bee visits to flowers, researchers recorded 586 honey bee visits and only four native bees such as the bumble bee, *Bombus* spp., and solitary bees were seen. Furthermore, the honey bee visited successive flowers while the native bees only visited staminate flowers and just one flower before flying away.

Limited studies have looked toward alternative pollinators to replace or buffer the declining honey bee populations. Bumble bees have been the focus of this research. The literature does not readily support that bumble bees will be able to replace the diminishing honey bee pollinators. Studies have shown that bumble bees are just as efficient if not more so than honey bees at pollinating the cucurbit *Citrullus lanatus* (Thunb.), watermelon in the field (Stanghellini et al., 1998). Bumble bee pollination of melon results in significantly higher average fruit weight than those pollinated by honey bees in a greenhouse (Dasgan et al., 1999). The problem is that bumble bee populations are also declining and little is known about their susceptibility to neonicotinoids.

Half of all bumble bee (*Bombus*) species have been eradicated or have demonstrated population decline in studies conducted in Illinois (Grixti et al., 2009). This trending decline in overall relative abundance may represent the status of bumble bee population decline for the Midwest which is in line with the overall declines of bumble bee populations in North America and worldwide (Goulson et al., 2008).

Cresswell et al. (2012) found bumble bees to be more sensitive to imidacloprid than honey bees suggesting the dangers that neonicotinoids pose to wild and domesticated bumble bee populations. In addition, neonicotinoids have been found to decrease feeding (Cresswell et al., 2012; Laycock et al., 2012), foraging (Mommaerts et al., 2010), worker survival rate, brood production (Laycock et al., 2012; Tasei et al., 2000), and queen production (Whitehorn et al., 2012). Bumble bee exposure to pesticides is also estimated to be comparable if not more than that of honey bees (Thompson and Hunt, 1999).

The viability of the bumble bee as a replacement of honey bees is unknown. Thus, honey bee health remains a high priority among researchers and growers alike. This study will focus on the honey bee, which has considerably more toxicological data in the literature. There is a need for more research to investigate the sensitivity of wild pollinators and bumble bees to...
neonicotinoid insecticides. The honey bee may demonstrate potential impacts of neonicotinoids that face other, less understood pollinators such as the bumble bee.

Current Field Residue Concentrations

Neonicotinoids have been detected in the pollen, wax, bee bread, and brood combs within honey bee colonies (Giroud et al., 2013; Kasiotis et al., 2014; Krupke et al., 2012; Mullin et al., 2010; Wu et al., 2011). The negative impacts of exposure to these insecticides has been evaluated in many toxicity studies on lethal, sublethal, and visual effects. Few studies have been published regarding the realistic field concentrations of these toxins within flowers. Cresswell (2011) recognized the need for more studies to determine field realistic concentrations of neonicotinoids in pollen and nectar. Only two studies were found that evaluated the concentration of neonicotinoids in cucurbits.

Dively and Kamel (2012) studied the pollen concentrations of imidacloprid, dinotefuran, and thiamethoxam with 9 treatments applied to pumpkins, *Cucurbita pepo* L., with different methods at different times. Treatments were applied in bedding-tray drench, transplant water, drip irrigation, foliar spray, and seed treatment in various combinations. Three general observations were made. First, the lowest levels of insecticides detected for individual treatments were seed treatment, bedding tray drench, and transplant water treatment. Second, higher residues were found when applications were made closer to the pumpkin flowering stage. Finally, the highest residues were found when foliar sprays and when drip irrigation were applied during flowering.

The highest levels of imidacloprid averaged 80.2 ng/g in pollen for the split treatment in transplant water and drip irrigation. This concentration is above the levels in which, imidacloprid has been shown to increased mortality, disrupted olfactory learning senses, decreased feeding, mobility, and mid-term memory (Decourtye et al., 2003, 2004a, 2004b; Guez et al., 2001; Lambin et al., 2001; Suchail et al., 2001; Teeters et al., 2012). The only metabolites of imidacloprid detected above trace concentrations were olefin and the hydroxy metabolites. Thiamethoxam average levels in pollen reached 68.0 ng/g and 95.2 ng/g in the transplant-drip irrigation split treatment and in the treatment of two foliar sprays, respectively. These values observed in 2009 were significantly higher than the residue concentrations detected in 2010 in which the differences were attributed to heat and water stress in 2010. Thiamethoxam at these
concentrations is above the threshold in which honey bees have been shown to have a reduced ability to return the hive after foraging (Henry et al., 2012). A FarMore® seed treatment was added in 2010 and neither thiamethoxam nor clothianidin were detected. Pollen alone was suggested to be used in future research to estimate pollinator exposure risk since nectar residues were consistently 73.5-88.8% lower than pollen residues and difficult to obtain. The author also noted that melons with fewer and smaller flowers and less plant biomass may have different residue concentrations. This emphasizes the need for more studies to focus on pollen analysis on other cucurbits such as muskmelon.

Stoner and Eitzer (2012) studied neonicotinoid concentrations in pollen and nectar of summer and winter squash, *Cucurbita pepo* L. The 5 treatments included field applications of imidacloprid and thiamethoxam applied in drip irrigation, soil drench, and foliar spray. All pollen and nectar samples from treated plants in the study had imidacloprid and thiamethoxam concentrations above 4 ng/g. To have enough nectar for analysis in both 2009 and 2010, the nectar from all three replications had to be combined. The range of concentrations was between 5-35 ng/g in pollen and 5-20 ng/g in nectar, encompassing all residue concentrations from treated plants. The average concentrations of both insecticides used at label rates over the course of the study for pollen were 14±8 ng/g imidacloprid and 12±9 ng/g thiamethoxam. Nectar concentrations were 10±3 ng/g imidacloprid and 11±6 ng/g thiamethoxam. Imidacloprid at concentrations as low as 1 ng/g have been shown to have the ability to increase mortality and disrupt olfactory learning senses, (Guez et al., 2001; Suchail et al., 2001).

These studies provide information for their respective commodities. Although all cucurbits have similar properties, one key difference between muskmelon and pumpkin or squash is that muskmelon have smaller flowers. No studies have been conducted to determine the content of neonicotinoids in muskmelon. In the year 2011, the land area planted for muskmelon in the United States was 72,590 acres worth $349,725,000 (USDA, 2012). In Indiana alone, muskmelon production was valued at $12,698,000 in 2011. Based on the recommended pesticides in the Midwest Vegetable Production guide (Egel et al., 2013) as well as interactions with growers, neonicotinoids are often applied in commercial muskmelon production. Muskmelon growers rely on renting honey bee colonies for sufficient pollination and proper fruit set. The potential impact of neonicotinoids on honey bees determined by this study could greatly impact grower pesticide application decisions and ultimately honey bee colony health.
MATERIALS AND METHODS

The research goals were to first determine the potential impact that neonicotinoids have on honey bees in muskmelon production and to secondly determine the extent and duration of striped cucumber beetle control among treatments. Quantifying the potential neonicotinoid impact on honey bees was accomplished in two steps. First, the residue concentrations in muskmelon pollen were determined for several recommended neonicotinoid insecticides applied at high and low label rates. The delivery methods for the neonicotinoids included bedding tray applications, transplant soil drenches, foliar sprays, and seed treatments. Secondly, the field residue concentrations were coupled with known honey bee toxicological sensitivity to determine the health risk associated with such neonicotinoids and delivery methods. The neonicotinoids evaluated were imidacloprid, thiamethoxam, and acetamiprid and their corresponding metabolites imidacloprid olefin, 5-hydroxyimidacloprid, and clothianidin. Neonicotinoids studied were based on IPM recommendations for muskmelon production (Foster and Flood, 2005; Egel et al., 2013). Personal conversations with melon growers from Indiana and Illinois showed their interest in neonicotinoids especially Admire Pro™ suggesting that growers are using these products in commercial production. The metabolites tested are toxic to honey bees and have been previously found in significant field concentrations (Nauen et al., 2003; Suchail et al., 2001; Suchail et al., 2003; Tomizawa and Casida, 2005). The residue concentrations found in this study were categorized as lethal, sublethal, or causing no effect. The results led to the modification of neonicotinoid product and application method recommendations to growers to maximize insecticide efficacy while minimizing honey bee health risks.

Experimental Field Design

The field portion of this study was conducted during the summers of 2013 and 2014 at Throckmorton Purdue Agricultural Center near Lafayette, IN. The fields had silt loam soils and
were previously used for research on cucurbits and other fruiting vegetables, soybean, and corn. Muskemelon, *Cucumis melo* L., (‘Athena’), seeds were planted 17 April in both years and grown in potting mix in 72-count plastic bedding trays in a greenhouse then hardened off for several days prior to being transplanted into the field on 13 May, 2013 and 23 May, 2014. Transplanting in 2014 was delayed because of wet field conditions. Transplants were planted into raised beds covered with black plastic mulch and irrigated with drip irrigation. Fertilizer water was released into each transplant hole at planting.

The study was arranged in a randomized completed block design with 4 replications. Each experimental unit consisted of three rows, each 12.2 meter long with rows 2.4 meters apart and plants spaced every 1.2 meters within the row, for a total of 30 plants. Experimental units were spaced 4.6 meters apart and a barrier of six rows of field corn was planted between experimental units to reduce cross contamination of foliar treatments. Fertilizers and herbicides were used according to normal growing practices.

**Soil Sampling**

Soil samples were taken to determine background concentrations of neonicotinoids prior to transplanting. Soil samples of 1.6 cm diameter cores 15.2 cm deep were taken using the Wintex 1000 attached to a Kubota all-terrain vehicle. Two cores were taken 20 feet apart from one another in each treatment plot. Subsamples from all treatments within a replication were combined and mixed together in a soil sampling bag into 1 compiled sample for each replication. The four final replication compilations were used for analysis. Soil samples were stored at -20°C until 3 g of soil from each replication were used for chemical analysis.

**Insecticide Treatments**

Neonicotinoid insecticide treatments and application methods were based on recommendations in the Midwest Vegetable Production Guide (Egel et al., 2013) to represent neonicotinoids muskmelon producers may be using. The seven treatments in 2013 were:

1. Untreated control
2. Imidacloprid (Admire Pro™, 42.8% AI, Bayer CropSciences, Research Triangle Park, NC) applied as a bedding tray treatment 3 days prior to transplanting and as a soil drench treatment
at transplanting (high label rates 0.44 fl oz per 10,000 plants and 10.5 fl oz per acre, respectively)

3. Thiamethoxam (Platinum®, 75% AI, Syngenta Crop Protection, Greensboro, NC) applied as a soil drench treatment at transplanting (high label rate 3.67 oz per acre)

4. Imidacloprid (Admire Pro™) applied as a soil drench treatment at transplanting (high label rate 10.5 fl oz per acre)

5. Thiamethoxam (Actara®, 25% AI, Syngenta Crop Protection, Greensboro, NC) applied as a foliar spray (high label rate 5.5 oz per acre)

6. Acetamiprid (Assail® 30 SG Insecticide, 30% AI, Nippon Soda Co. Ltd., Chiyoda-Ku Tokyo, Japan) applied as a foliar spray (high label rate 5.3 oz per acre)

7. Thiamethoxam (FarMore®, Syngenta Crop Protection, Greensboro, NC) as a seed coated treatment

In 2014, the treatment of imidacloprid applied as a bedding tray drench combined with a soil drench at transplanting was omitted due to no significant differences with solely applying imidacloprid as a soil drench treatment at transplanting. In 2014, four new treatments were added to evaluate low label rate applications as well as early emergence flowers collected for residue analysis. The pesticide labels recommend the use of the high label rate for heavy pest pressure and longer residual control and the low label rate for short residual control. The ten treatments in 2014 were:

1. Untreated control

2. Imidacloprid (Admire Pro™) applied as a soil drench treatment at transplanting (low label rate 7.0 fl oz per acre)

3. Thiamethoxam (Platinum®) applied as a soil drench treatment at transplanting (low label rate 1.66 oz per acre)

4. Imidacloprid (Admire Pro™) applied as a soil drench treatment at transplanting (high label rate 10.5 fl oz per acre)

5. Thiamethoxam (Platinum®) applied as a soil drench treatment at transplanting (high label rate 3.67 oz per acre)

6. Thiamethoxam (Actara®) applied as a foliar spray (low label rate 3.0 oz per acre)

7. Thiamethoxam (Actara®) applied as a foliar spray (high label rate 5.5 oz per acre)

8. Acetamiprid (Assail® 30 SG Insecticide) applied as a foliar spray (high label rate 5.3 oz per acre)
9. Thiamethoxam (FarMore®) as a seed coated treatment for early emergence flower collection
10. Thiamethoxam (FarMore®) as a seed coated treatment for late flower collection

The bedding tray drench treatment of Admire Pro™ was mixed with water in a beaker, and 4 mL of the solution was applied on 10 May, 2013 to each plant with a syringe within the greenhouse resulting in nearly saturated soil media in the bedding trays without gravitational loss of liquid from the bottom of the tray. All transplant soil drench treatments were mixed in a bucket and then applied as 500 mL of solution to the base of each plant shortly after transplanting. All foliar treatments were applied with a carbon dioxide powered backpack sprayer midday on 24 June, 2013 and 7 July, 2014 when enough flowers had bloomed to collect for chemical analysis. To prevent spray drift, a plastic sheet 15.2 meters long and 1.5 meters high was stretched between 2 poles downwind of the plot being sprayed. In addition, 3 water sensitive yellow cards were placed in each adjoining plot to verify no spray drift occurred. One card was also placed in each row being sprayed to ensure even foliar coverage.

Pollen Sample Collection

Flowers were collected three days following foliar applications on 27 June, 2013 and 10 July, 2014 from 6 am until 10 am before most pollinators entered the fields to ensure the majority of pollen was still present on the anthers of collected flowers. The three day interval between spraying and flower collection was chosen to represent the half-way point between foliar applications under normal conditions as commercial growers often spray weekly. An additional flower collection in 2014 was conducted on 23 June to determine if seed treatments resulted in different pollen concentration earlier in the season. Early morning collection was important because 44-62% of pollen grains from ‘Athena’ staminate flowers are removed by honey bees or bumble bees, and the majority of collected pollen (57%) is removed within the first 2 hours of foraging (Stanghellini et al., 2002). The flowers were placed into 1 gallon Ziploc® bags and stored in coolers until brought back to the lab immediately after collection where they were stored in a refrigerator at 4°C. In the lab, the pollen and anther complex were removed from the stamen of staminate flowers with forceps and put into 15 mL centrifuge tubes until 6 g was collected. Centrifuge tubes with the pollen and anther complex were then stored at -80°C until chemical analysis. All materials, surfaces, and hands, were cleaned with methanol between treatments to prevent contamination.
Sample Preparation and Chemical Analysis

The chemical analysis portion of this study was conducted at the Bindley Bioscience Center at Discovery Park on the Purdue University campus in West Lafayette, IN. The internal analytical standards imidacloprid-d₄, thiamethoxam-d₃, clothianidin-d₃, and acetamiprid-d₃ were obtained from Sigma Aldrich. Imidacloprid olefin and 5-hydroxyimidacloprid were gifts from Dr. Brian Eitzer from the Department of Analytical Chemistry of the Connecticut Agricultural Experiment Station. All pollen and soil samples were extracted separately using a refined method of QuEChERS (Stoner and Eitzer, 2012).

Imidacloprid samples required 3 g of pollen/anther complex rather than 1 g for analysis to enable quantification of its metabolites imidacloprid olefin and 5-hydroxyimidacloprid. Soil samples were also run using the 3 g analysis due to the need to look for imidacloprid and its metabolites in addition to thiamethoxam, clothianidin, and acetamiprid. The 3 g samples were homogenized in 50 mL centrifuge tubes with a KIMAX USA glass pestle. Then, 15 mL of water, 100 ng of respective isotopically labeled internal standards, and 15 mL of acetonitrile were added to the tube. The QuEChERS salts of 6 g magnesium sulfate and 1.5 g sodium acetate were added to the tubes. The samples were then vortexed for 1 minute with the S8220 Deluxe Mixer and rocked for 10 minutes on the VWR W-150 Waver at speed 10. Samples were then centrifuged with the Eppendorf Centrifuge 5810 R, 15 amp version, at 4°C and 2500RPM for 10 minutes. Then, 10 mL of the supernatant was pipetted into 15 mL BondElut Agilent Sample Prep Solutions Dispersive SPE tubes (ingredients: 400 mg PSA, 400 mg C18EC, Magnesium Sulfate 1200 mg; part no: 5982-5158). The samples were again vortexed for 1 minute with the S8220 Deluxe Mixer, rocked on the VWR W-150 Waver at speed 10 for 5 minutes, and centrifuged with the Eppendorf Centrifuge 5810 R, 15 amp version, at 4°C and 4000RPM for 5 minutes. Then 6 mL of the supernatant was pipetted into a 15 mL centrifuge tube which was then dried in a Savant Automatic Environmental SpeedVac System AES2010 and concentrated to 1 mL solution for instrumental analysis.

All other samples required only 1 g of pollen/anther complex for chemical analysis and prepared for instrumental analysis similarly to 3 g samples with minimal differences. The 1 g samples were homogenized in Precellys 7 mL tubes with 2.8 mm ceramic beads using Bertin Technologies Precellys 24 lysis and homogenization and were run twice on setting 2: 5000 rpm, 2 cycles 25 seconds long with 90 second break times. The samples were then transferred to 15
mL centrifuge tubes and extracted with 3 mL water, 100 ng of respective isotopically labeled internal standards, 3 mL acetonitrile, 1.2 g magnesium sulfate, and 0.3 g sodium acetate. Samples were then vortexed, rocked, and centrifuged just as the 3 g samples were. Then, 1 mL of the supernatant was pipetted into 2 mL BondElut Agilent Sample Prep Solutions Dispersive SPE tubes (ingredients: 25 mg PSA, 7.5 mg GCB, 150 mg MgSO4; part no: 5982-5321). Samples were then vortexed for 10 minutes at maximum speed with the Labnet VX100 and then centrifuged at 15,000 rpm for 5 minutes with the Eppendorf Centrifuge 5424. Finally, 100 µL of supernatant was transferred to the LC/MS/MS autosampler vial to be analyzed using liquid chromatography/ mass spectrometry/ mass spectrometry (LC/MS/MS) as described in Stoner and Eitzer (2012).

The settings for the LC/MS/MS were set and reported by Amber Jannasch (Jannasch, 2014). An Agilent 1200 Rapid Resolution liquid chromatography (LC) system coupled to an Agilent 6460 series QQQ mass spectrometer (MS) was used to analyze pesticides in each sample. An Agilent Zorbax SB-Phenyl 4.6 mm x 150 mm, 5 µm column was used for LC separation. The buffers were (A) water + 5mM ammonium acetate + 0.1 % formic acid and (B) acetonitrile (90) + 5mM ammonium acetate (10) + 0.1% formic acid. The linear LC gradient was as follows: time 0 minutes, 5% B; time 0.5 minute, 5% B; time 8 minutes, 100% B; time 10 minutes, 100% B; time 11 minutes, 5% B; time 15 minutes, 5% B. Multiple reaction monitoring was used for MS analysis. The data were acquired in positive electrospray ionization (ESI) mode by monitoring the transitions in Table 2. The jet stream ESI interface had a gas temperature of 330°C, gas flow rate of 10 L/minute, nebulizer pressure of 35 psi, sheath gas temperature of 250°C, sheath gas flow rate of 7 L/minute, capillary voltage of 4000 V, and nozzle voltage of 1000 V. All data were acquired and analyzed with Agilent Masshunter software (version B.06.00).

Striped Cucumber Beetle Counts

In addition to the pollen analysis in 2014, striped cucumber beetle counts were conducted to determine the extent and duration of striped cucumber beetle control among treatments. Striped cucumber beetles were manually counted using visual observation on and beneath plants while turning over vines and leaves. Insect counts were conducted 1 to 2 times per week from 29 May, 2014 through 21 July, 2014. Four plants per row, or 12 plants per
subplot were used for insect counts from 29 May, 2014 until June 23, 2014 whereas the remainder of the counts from 25 June, 2014 through 21 July, 2014 was conducted on 3 plants per row, or 9 plants per subplot. The number of plants used for counts was reduced when they became very large to limit the time required to conduct insect counts and maximize efficiency. The observational area for beetle counts was 0.6 meters wide by 0.9 meters long, perpendicular and parallel to the rows, respectively. This estimate for differentiation between plants was used as plants matured and the melon vines became intertwined and grew together. When striped cucumber beetle counts exceeded the economic threshold of 1 beetle per plant on any treatment, a half rate of carbaryl (Sevin®XLR Plus) was applied to all treatments to reduce beetle populations to levels that would not compromise the pollen residue analysis study. Carbaryl treatments were applied on 29 May and on 6 June.

Statistical Analysis

Neonicotinoid treatment effects were analyzed separately for each group of parent compounds and respective metabolites, and striped cucumber beetle count treatment effects were analyzed with a two-way analysis of variance followed by the post-hoc Fishers Least Significant Difference (LSD) test by using the main effects ANOVA in the program STATISTICA (StatSoft Inc., 2013). No data was transformed when the homogeneity of variance or normality of the residual error was violated due to the robustness of the ANOVA to these violations (Box, 1953, 1954).
RESULTS AND DISCUSSION

Soil Residue of Neonicotinoids

Soil collected within the field site revealed residues of all investigated neonicotinoids in all samples tested (Table 3). In 2013, mean soil concentrations were 10.43 ng/g imidacloprid and 21 ng/g 5-hydroxyimidacloprid while olefin imidacloprid soil residue was only 1.41 ng/g. The following year, imidacloprid and metabolites olefin imidacloprid and 5-hydroxyimidacloprid mean concentrations were all less than 4 ng/g. Soil samples were taken 3 weeks later in 2014 than 2013, which may have provided enough time in more ideal weather conditions for further compound degradation explaining the lower concentrations detected in 2014. In addition, 2013 and 2014 plots were located in different fields within the Throckmorton Purdue Agricultural Center having similar, yet not exact field crop rotation histories. Minimal mean concentrations of less than 1 ng/g were detected in soil in both years for thiamethoxam and acetamiprid. In 2014, clothianidin ranged from 3.4 to 6.8 ng/g in the soil which was expected due to crop rotation between cucurbits and field corn with seed treated with thiamethoxam, the precursor to clothianidin. These findings are in line with those concentrations found in soil samples taken at a nearby location in a previous study within corn and soy production fields ranging from 2.1 to 9.6 ng/g (Krupke et al., 2012). Clothianidin data from 2013 was not used because the compound adhered to the column within the LC/MS/MS which resulted in carryover between samples making it difficult to decipher between background noise and a real response in the compound peaks. This issue was resolved in 2014 as new software provided a different way to wash the machine column in between samples. These results show the residual concentrations of neonicotinoids in the soil from previous growing seasons. Annual applications of neonicotinoids may result in continued neonicotinoid presence in the soil.
Pollen Residue of Neonicotinoids

In 2013, pollen concentrations following imidacloprid applied as Admire Pro™ at the high label rate to the bedding tray and soil drench at transplanting and as a transplant soil drench alone, ranged from 55 to 127 ng/g and 44 to 141 ng/g, respectively (Fig.1). These values were not significantly different from one another (p>0.05). The resulting mean olefin imidacloprid concentration for both treatments was 0.003 ng/g while the resulting 5-hydroxyimidacloprid concentrations were 0.013 ng/g and 0.014 ng/g for the bedding tray soil drench and soil drench only, respectively (Fig 3, 5). In 2013, concentrations of imidacloprid following both treatments were at levels that may cause decreased olfactory learning and memory, reflex response, mobility, and increased mortality (Decourtye et al., 2003, 2004b; Guez et al., 2001; Lambin et al., 2001; Teeters et al., 2012). Olefin and 5-hydroxyimidacloprid were however, below concentrations that threaten honey bee health.

In 2014, the Admire Pro™ low label rate transplant soil drench resulted in a mean imidacloprid pollen concentration of 1.49±0.2 ng/g, while the high label rate transplant soil drench resulted in a mean concentration of 3.31±0.7 ng/g (Fig 2). Olefin imidacloprid mean residue concentrations were 6.51 and 5.38 ng/g following low and high soil drench applications, respectively (Fig 4). The resulting mean residue concentrations of 5-hydroxyimidacloprid following low and high soil drench applications were 0.50 and 1.82 ng/g (Fig 6). The resulting imidacloprid values following the high rate Admire Pro™ soil drenches were significantly less in 2014 than the previous year (p<0.0001) yet both concentrations reached values that may result in increased mortality and disrupted olfactory learning senses (Guez et al. 2001; Suchail et al. 2001. In addition, olefin imidacloprid at this concentration can potentially inhibit honey bee olfactory learning demonstrated at levels as low as 1 ng/g (Guez et al., 2003), yet 5-hydroxyimidacloprid is present below concentrations that threaten honey bee health.

As imidacloprid degrades and diminishes in concentration, one would expect to find increasing metabolite concentrations. Olefin imidacloprid and 5-hydroxyimidacloprid metabolite concentrations were significantly higher in 2014 than 2013 (p<0.0001), the opposite of their parent compound imidacloprid. The varied time from which flowers were collected between years may explain the difference in concentrations in 2013 and 2014 (Fig 1-6). Regardless of the time during the season in which flowers were collected, in both years, pollen residues following Admire Pro™ application reached levels that may harm honey bees.
In 2013, thiamethoxam applied as a high rate Platinum® transplant soil drench resulted in pollen concentrations ranging from 31 to 95 ng/g (Fig. 7). These values are sufficiently high to reduce foragers’ ability return to their colony after foraging (Henry et al., 2012). In 2014, the low rate Platinum® soil drench of thiamethoxam resulted in a mean residue concentration of 3.75±0.6 ng/g and was not significantly different than the high rate soil drench (p>0.05) which resulted in a mean concentration of 9.25±0.9 ng/g (Fig. 8). The high rate soil drench in 2014 was significantly lower than the residue concentrations following the high rate soil drench treatment in 2013 (p<0.0001). Low and high rate soil drenches of thiamethoxam in 2014 resulted in clothianidin mean residue concentrations of 0.72 and 1.38 ng/g, respectively (Fig. 9). Although 2013 residue values following soil drench applications reached concentrations that may cause harm to honey bees, thiamethoxam and clothianidin residues were both below levels that may cause harm to honey bees following soil drenches in 2014.

In 2013, thiamethoxam applied as an Actara® foliar spray resulted in pollen concentrations ranging from and 63 to 104 ng/g (Fig. 7). In 2014, the range of Actara® foliar spray high label rate residues from 117 to 157 ng/g was significantly higher than the low label rate residues (p<0.0001) ranging from 74 to 95 ng/g (Fig. 8). The resulting foliar spray concentrations following the application at the high label rate in 2014 were significantly higher than in 2013 (p<0.0001). In both years, the resulting thiamethoxam residues following low and high label rate Actara® applications were sufficient to reduce foragers’ ability to return to their hive (Henry et al., 2012). In addition, increased acute mortality may occur when thiamethoxam concentrations reach 100 ng/g, which occurred when the high label rate of Actara® was applied in both years (Laurino et al., 2011). In a previous study, pumpkin pollen resulted in similar concentrations of thiamethoxam ranging from 69 to 132 ng/g following transplant water combined with drip irrigation treatments and 71 to 162 ng/g following two applied foliar sprays (Dively et al., 2012). Additionally, in my study in 2014, the low and high foliar applications of Actara® resulted in average concentrations of 3.87±0.4 ng/g and 6.25±0.3 ng/g clothianidin which are levels that have been shown to negatively affect honey bees (Fig. 9). Clothianidin at a concentration as low as 2.1 ng/g when combined with thiamethoxam at 5.3 ng/g, resulted in decreased adult worker population as well as reduced honey production, pollen collection, and colony growth (Sandrock et al., 2014).
The thiamethoxam FarMore® seed treatment resulted in thiamethoxam residue below levels that have been shown to cause harm to honey bees in both years with means of 0.67±0.34 ng/g in 2013 and 1.75±0.2 ng/g in 2014. There was also no significant difference in the pollen concentration in flowers collected 17 days earlier (p>0.05) which averaged 1.98±0.1 ng/g in 2014. The early and late flower collections of the FarMore® seed treatment in 2014 resulted in mean clothianidin concentrations of 5.14±0.63 ng/g and 6.48±1.51 ng/g, respectively. These values are both above the threshold in which decreased adult worker population as well as reduced honey production, pollen collection, and colony growth have been demonstrated (Sandrock et al., 2014).

Acetamiprid resulted in an average of 151±31 ng/g and 82±6 ng/g following the high rate Assail® foliar spray in 2013 and 2014, respectively (Fig 10, 11). Acetamiprid is less toxic than imidacloprid and thiamethoxam and was present below those concentrations that have been shown to negatively impact honey bees in both years (European Commission, 2004; Hassani et al., 2008; Iwasa et al., 2004).

Overall, soil drench treatments resulted in parent compound pollen residues that were significantly higher in 2013 than 2014 (Fig 1, 2, 7, 8). The differences in concentrations may have been due to varied flower collection dates between years. Flowers were collected 3 days following the foliar spray applications in both years which explains why similar residue concentrations were detected following the foliar spray as compared to soil drench concentrations. In 2014, the spray and collection dates were 2 weeks later than anticipated due to inclement weather conditions. On 27 June, 2013, no fruit were present on vines at the time of flower collection, while on 10 July, 2014 several melons were present on more mature and longer vines at the time of flower collection. This may have provided a longer time for compound metabolism within the plant as well as more plant matter for insecticide distribution, diluting their concentrations within the pollen. This would explain the lower concentrations of residue in the pollen following transplant water applications in 2014.

The potential toxicity of Admire Pro™ to honey bees was due to imidacloprid and its metabolite olefin imidacloprid residues rather than its metabolite 5-hydroxyimidacloprid. Similarly, the potential toxicity of Platinum® was due to thiamethoxam residues rather than its metabolite clothianidin. However, when the Actara® foliar spray was applied, clothianidin also reached toxic levels. The FarMore® seed treatment toxicity was due to the presence of
clothianidin. The treatments that pose health risks to honey bees include Admire Pro™ and Platinum® soil drenches, the Actara® foliar spray, and the FarMore® seed treatment. The Assail® foliar spray does not threaten honey bee health.

Striped Cucumber Beetle Control

The mean number of striped cucumber beetles present when Admire Pro™ was applied at the low and high label rates were not significantly different from one another (May 29, June 6, June 12: p>0.05) and both controlled striped cucumber beetles better than the untreated control for 20 days after transplanting with significantly lower beetle counts 14 to 20 days after transplanting (Fig. 12). Platinum® applied at the low and high label rates were not significantly different from one another (May 29, June 6, June 12: p>0.05) and neither were significantly better at controlling striped cucumber beetles than the untreated control, although both kept the beetles below the economic threshold for 20 days after transplanting whereas the untreated control did not (Fig. 13). The lack of significance between Platinum® and the control may have resulted from the half rate applications of carbaryl made on 29 May and 6 June which were administered to keep beetles below the economic threshold to ensure enough flowers from all plots for collection, which meant that the control treatment was not an absolute control. Counts conducted 24 days after transplanting showed reduced striped cucumber beetle populations, assumed to be in between generations, with no significant differences among treatments (p>0.05). On 23 June, 31 days after transplanting, striped cucumber beetles, likely the second generation, exceeded the threshold for all treatments except Admire Pro™ applied at low and high rates. However, Admire Pro™ did not show significantly better control than the untreated plot (p>0.05). No treatments provided adequate control for striped cucumber beetles 33 days following transplanting, on June 25.

Foliar sprays were applied on 7 July, and counts taken two days later showed Actara® applied at the low and high rate as well as Assail® applied at the high rate resulted in significantly greater control than the untreated plots (Actara® low, p=0.006; Actara® high, p=0.003; Assail®, p=0.005) and reduced beetles below the economic threshold (Fig. 14). Both insecticides applied at the high label rates continued to perform significantly better than the untreated plots and maintained control at levels below the threshold 7 days after foliar application (Actara® high,
p=0.01; Assail®, p=0.004). The FarMore® seed treatment provided no control against striped cucumber beetles throughout the season (Fig. 15).

Overall, the low label rates for both soil drenches Admire Pro™ and Platinum® provided control comparable to the high label rates. Admire Pro™ provided the best protection, while both insecticides maintained populations below the economic threshold for 20 days after application at transplanting. Assail® and Actara® applied at high label rates both provided protection against striped cucumber beetles for 7 days after application. The FarMore® seed treatment did not provide protection against striped cucumber beetles.
CONCLUSIONS

Neonicotinoids have highly desirable properties making them the fastest growing class of insecticides in the world (Jeschke et al., 2011) often recommended for use in commercial muskmelon production (Egel et al., 2013). Neonicotinoids were found to be present in the pollen of muskmelon flowers in our study. These results in addition to those reported by Dively and Kamel (2012) and Stoner and Eitzer (2012) demonstrate that neonicotinoid use on cucurbits has the potential to reach concentration levels within the pollen and nectar that have been shown to negatively impact honey bees. Muskmelons and cucurbits in general, are another possible route of neonicotinoid exposure to honey bees in addition to their use as a seed treatment on almost all planted field corn (Krupke et al., 2012). Neonicotinoids have been detected in the pollen, wax, bee bread, and brood combs within honey bee colonies (Giroud et al., 2013; Kasiotis et al., 2014; Krupke et al., 2012; Mullin et al., 2010; Wu et al., 2011) as well as surface waters (Anderson et al., 2013; Hladik et al., 2014; Starner and Goh, 2012). The many possible routes of neonicotinoid exposure and their widespread presence in the environment makes them a target pesticide of concern in regards to their potential contribution to the alarming decline of commercial honey bee populations (vanEngelsdorp and Meixner, 2010). My results provide new information regarding the potential exposure and consequential impacts of neonicotinoids on honey bees when applied in muskmelon production. In addition, the effectiveness and duration of control against the most important pest of muskmelon, the striped cucumber beetle, was determined among various neonicotinoids. Commercial growers have a vested interest in the health of honey bees as they often rent honey bee hives to ensure pollination and fruit set of this commodity. Therefore, this study will be used to modify neonicotinoid product and application recommendations to growers.

The high and low label rates of Admire Pro™ and Platinum® provided control for striped cucumber beetles for 20 days based on one year’s data. Therefore, if these products are applied, the low label rate is recommended as to minimize risks posed to honey bees. These products
however, demonstrated the potential to negatively impact honey bees in two field seasons. Admire Pro™ resulted in imidacloprid concentrations that have been shown to cause decreased olfactory learning and memory, reflex response, mobility, and increased mortality (Decourtye et al., 2003, 2004b; Guez et al., 2001; Lambin et al., 2001; Teeters et al., 2012). Platinum* resulted in thiamethoxam concentrations that may hinder honey bees from being able to return to their hive (Henry et al., 2012). In addition, one research season demonstrated that Actara® and Assail® both controlled striped cucumber beetles for 7 days following application. In two field seasons only Actara® reached concentration levels in this study that have been shown to reduce the ability of foragers to return to the hive as well as increase mortality when fed to honey bees (Henry et al., 2012; Laurino et al., 2011). Therefore, Assail® foliar sprays instead of Actara® are recommended. The FarMore® seed treatment did not provide control for striped cucumber beetles throughout the season investigated and reached concentration levels that have been shown to decrease adult worker populations as well as reduce honey production, pollen collection, and colony growth (Sandrock et al., 2014). Therefore, the FarMore® seed treatment is not recommended for use when transplanting.

A follow up research project should repeat the comparison of striped cucumber beetle management using neonicotinoids to provide a second season of data to confirm which products are most effective. Neonicotinoids should be used in rotation with other insecticides recommended for use in the Midwest Vegetable Production Guide (Egel et al., 2013) such as carbaryl (Sevin® XLR Plus) and various pyrethroids such as permethrin (Pounce®) which have been shown to also be effective means of control (Brust and Foster, 1999). Alternating neonicotinoids with other insecticide modes of action will prevent resistance while an integrated pest management approach should be implemented using the established threshold of one striped cucumber beetle per plant.

In this study, pollen residue concentrations were determined following just one application of these neonicotinoids, yet the product labels allow multiple applications throughout the growing season with limitations. The maximum active ingredient allowed in one season for imidacloprid applied as Admire Pro™ permits two low label rate applications or one high label rate application. Similarly, the maximum number of applications of thiamethoxam permitted in a growing season applied as Platinum* are two low label rate or one high label rate application. Actara® may be applied three times at the low label rate or twice at the high label
rate so as to not exceed the maximum active ingredient of thiamethoxam allowed during a growing season. Assail® label directions allow no more than five applications during a growing season of the low or high label rates. Future research needs to determine the compounding concentrations that result from multiple neonicotinoid product applications in a growing season.

In addition, the time of flower collection may greatly impact results, and it would be beneficial to determine the concentrations of neonicotinoids in the pollen over time. All of these products are also long lasting in the environment and were detected in all soil samples at the test site. A future study is needed to determine the additive concentrations of neonicotinoids in the soil from year to year as carryover may occur in fields repeatedly receiving neonicotinoid applications. A study conducted within an area surrounded by neonicotinoid treated muskmelon fields as well as within a large scale organic production where neonicotinoids are not used would be beneficial to determine the pesticide pollen residues collected within bee hives present within those fields as well as the success and survival of those colonies.

Additional studies are needed to determine how much pollen is consumed by various ages of honey bees from multiple colonies as Crailsheim et al. (1992) and Rortais et al. (2005) are the only studies to have assessed honey bee pollen consumption, and the average adult bee is never the same in two different colonies. This information, coupled with known field concentrations of neonicotinoids will tell if honey bees may consume a lethal or a sublethal dose in ng/bee, and most oral toxicity studies present lethal and sublethal doses as ng/bee rather than as a concentration in ng/g. Further studies that focus on honey bee pollen consumption are needed to provide the information necessary to most accurately assess honey bee risk based on field concentrations and oral toxicological sensitivity.
TABLES
Table 1: Summary of published toxicity of neonicotinoids on honey bees: The metabolites of imidacloprid are olefin and 5-OH-imidacloprid. The metabolite of thiamethoxam is clothianidin. When multiple LD$_{50}$ values were reported at various times after treatment, the last observation time was used (e.g. When LD50 values were reported for 24, 48, and 72 hrs, only the 72 hr value is shown). Time after treatment is reported in hours (h). All exposure is acute unless denoted as chronic (C) followed by exposure duration in days (d).

Contact sublethal doses were converted to ng/g by dividing ng/bee by the average bee weight of 0.1 g (Mullin et al., 2010) and are displayed in italics.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Acute Oral LD$_{50}$</th>
<th>Acute Contact LD$_{50}$</th>
<th>Significant Mortality Increase</th>
<th>Oral Sublethal</th>
<th>Contact Sublethal</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chloronicotinyl Subclass</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>4.5 ng/bee</td>
<td>48 ng/g $^*$ (11d)</td>
<td>12 ng/g $^*$ (11d)</td>
<td>10 ng/bee, 24 ng/g</td>
<td>0.1 ng/bee (1 ng/g)</td>
<td>Decourtye et al. 2003</td>
</tr>
<tr>
<td></td>
<td>30.6 ng/bee (48h)</td>
<td></td>
<td>12 ng/bee</td>
<td>24 ng/g</td>
<td>2.5 ng/bee (25 ng/g)</td>
<td>Decourtye et al. 2004a</td>
</tr>
<tr>
<td></td>
<td>17.9 ng/bee (24h)</td>
<td></td>
<td></td>
<td>12 ng/bee</td>
<td>5 ng/bee</td>
<td>Decourtye et al. 2004b</td>
</tr>
<tr>
<td></td>
<td>41-81 ng/bee (48h)</td>
<td>42-104 ng/bee (48h)</td>
<td></td>
<td>0.1 ng/bee (8d)</td>
<td>1.5 ng/bee</td>
<td>Schmuck et al. 2011</td>
</tr>
<tr>
<td></td>
<td>37 ng/bee, 370 ng/g (96h)</td>
<td>0.01 ng/bee, 0.1 ng/g $^*$ (8d)</td>
<td></td>
<td>1.34 ng/bee, 67 ng/g</td>
<td>50 ng/g</td>
<td>Schmuck et al. 2001</td>
</tr>
<tr>
<td>Olefin</td>
<td>&gt; 36 ng/bee (48 h)</td>
<td></td>
<td></td>
<td>0.1 ng/bee (1 ng/g)</td>
<td>23 ng/bee, 230 ng/g (96h)</td>
<td>Nauen et al. 2001</td>
</tr>
<tr>
<td>5-OH-imidacloprid</td>
<td>153.5 ng/bee (48h)</td>
<td>240 ng/g $^*$ (11d)</td>
<td>30 ng/g $^*$ (11d)</td>
<td>222 ng/bee, 2220 ng/g (96h)</td>
<td>100 ng/bee</td>
<td>Suchail et al. 2001</td>
</tr>
<tr>
<td></td>
<td>159 ng/bee (48h)</td>
<td></td>
<td></td>
<td>29.9 ng/bee (24h)</td>
<td>100 ng/bee (1000 ng/g)</td>
<td>Nauen et al. 2001</td>
</tr>
<tr>
<td></td>
<td>222 ng/bee, 2220 ng/g (96h)</td>
<td>8090 ng/bee</td>
<td></td>
<td>4.3 ng/bee, 123 ng/g (72h)</td>
<td>100 ng/g (72h)</td>
<td>Suchail et al. 2001</td>
</tr>
<tr>
<td>Acetamiprid</td>
<td>14530 ng/bee</td>
<td>8090 ng/bee</td>
<td></td>
<td>7070 ng/bee (24h)</td>
<td>100 ng/bee (72h)</td>
<td>European Commission 2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>43.9 ng/bee</td>
<td>24 ng/bee</td>
<td>European Commission 2006</td>
</tr>
<tr>
<td>Thianicotinyl Subclass</td>
<td></td>
<td></td>
<td></td>
<td>2.18 ng/bee (21.8 ng/g)</td>
<td>43.9 ng/bee</td>
<td>European Commission 2005</td>
</tr>
<tr>
<td>Thiamethoxam</td>
<td>5 ng/bee</td>
<td>24 ng/bee</td>
<td></td>
<td>2.6 ng/bee, 75 ng/g (72h)</td>
<td>75 ng/g (72h)</td>
<td>European Commission 2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.0 ng/bee, 300 ng/g (72h)</td>
<td>100 ng/g (72h)</td>
<td>Nauen et al. 2001</td>
</tr>
<tr>
<td>Clothianidin</td>
<td>3.8 ng/bee</td>
<td>44.3 ng/bee</td>
<td></td>
<td>2.6 ng/bee, 75 ng/g (72h)</td>
<td>2.18 ng/bee (21.8 ng/g)</td>
<td>European Commission 2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.3 ng/bee, 300 ng/g (72h)</td>
<td>85 ng/g (72h)</td>
<td>Nauen et al. 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5 ng/bee</td>
<td>24 ng/bee</td>
<td>European Commission 2005</td>
</tr>
</tbody>
</table>

$^*$ Significant mortality increase, means at each time point are significantly different from those at other times (e.g., 72 h is significantly more toxic than 24 h).

References:
- Cresswell 2011
- Decourtye et al. 2003
- Decourtye et al. 2004a
- Decourtye et al. 2004b
- Decourtye et al. 2004
- Decourtye et al. 2001
- Lambin et al. 2001
- Nauen et al. 2001
- Suchail et al. 2003
- Guez et al. 2003
- Guez et al. 2001
- Iwasa et al. 2004
- Nauen et al. 2001
- Suchail et al. 2001
- Suchail et al. 2001
- European Commission 2004
- European Commission 2006
- Henry et al. 2012
- Iwasa et al. 2004
- Laurino et al. 2011
- Syngenta Group 2005
- U.S. EPA 2003
- Matthias et al. 2010
- Matsumoto 2013
- Teeters et al. 2012
### Table 2: Multiple reaction monitoring table with conditions for LC/MS/MS analysis of pesticides.

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Precursor Ion</th>
<th>MS1 Res</th>
<th>Product Ion</th>
<th>MS2 Res</th>
<th>Dwell</th>
<th>Fragmentor</th>
<th>Collision Energy</th>
<th>Cell Accelerator Voltage</th>
<th>Polarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>d3-thiamethoxam</td>
<td>294.9</td>
<td>Unit</td>
<td>213.9</td>
<td>Unit</td>
<td>50</td>
<td>60</td>
<td>10</td>
<td>7</td>
<td>Positive</td>
</tr>
<tr>
<td>thiamethoxam</td>
<td>291.9</td>
<td>Unit</td>
<td>210.9</td>
<td>Unit</td>
<td>50</td>
<td>60</td>
<td>10</td>
<td>7</td>
<td>Positive</td>
</tr>
<tr>
<td>thiamethoxam</td>
<td>291.9</td>
<td>Unit</td>
<td>180.9</td>
<td>Unit</td>
<td>50</td>
<td>60</td>
<td>20</td>
<td>7</td>
<td>Positive</td>
</tr>
<tr>
<td>5-OH-Imidacloprid</td>
<td>272</td>
<td>Unit</td>
<td>228</td>
<td>Unit</td>
<td>50</td>
<td>70</td>
<td>25</td>
<td>7</td>
<td>Positive</td>
</tr>
<tr>
<td>5-OH-Imidacloprid</td>
<td>272</td>
<td>Unit</td>
<td>191</td>
<td>Unit</td>
<td>50</td>
<td>70</td>
<td>15</td>
<td>7</td>
<td>Positive</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>256</td>
<td>Unit</td>
<td>209</td>
<td>Unit</td>
<td>50</td>
<td>70</td>
<td>20</td>
<td>7</td>
<td>Positive</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>256</td>
<td>Unit</td>
<td>175</td>
<td>Unit</td>
<td>50</td>
<td>70</td>
<td>20</td>
<td>7</td>
<td>Positive</td>
</tr>
<tr>
<td>Imidacloprid olefin</td>
<td>254</td>
<td>Unit</td>
<td>236</td>
<td>Unit</td>
<td>50</td>
<td>70</td>
<td>5</td>
<td>7</td>
<td>Positive</td>
</tr>
<tr>
<td>Imidacloprid olefin</td>
<td>254</td>
<td>Unit</td>
<td>205</td>
<td>Unit</td>
<td>50</td>
<td>70</td>
<td>15</td>
<td>7</td>
<td>Positive</td>
</tr>
<tr>
<td>d3-clothianidin</td>
<td>253</td>
<td>Unit</td>
<td>172.1</td>
<td>Unit</td>
<td>50</td>
<td>70</td>
<td>10</td>
<td>7</td>
<td>Positive</td>
</tr>
<tr>
<td>d3-clothianidin</td>
<td>253</td>
<td>Unit</td>
<td>131.9</td>
<td>Unit</td>
<td>50</td>
<td>70</td>
<td>15</td>
<td>7</td>
<td>Positive</td>
</tr>
<tr>
<td>clothianidin</td>
<td>249.9</td>
<td>Unit</td>
<td>169</td>
<td>Unit</td>
<td>50</td>
<td>70</td>
<td>10</td>
<td>7</td>
<td>Positive</td>
</tr>
<tr>
<td>clothianidin</td>
<td>249.9</td>
<td>Unit</td>
<td>131.9</td>
<td>Unit</td>
<td>50</td>
<td>70</td>
<td>15</td>
<td>7</td>
<td>Positive</td>
</tr>
<tr>
<td>d3-Acetamiprid</td>
<td>226.1</td>
<td>Unit</td>
<td>125.9</td>
<td>Unit</td>
<td>50</td>
<td>70</td>
<td>20</td>
<td>7</td>
<td>Positive</td>
</tr>
<tr>
<td>Acetamiprid</td>
<td>223.1</td>
<td>Unit</td>
<td>125.9</td>
<td>Unit</td>
<td>50</td>
<td>70</td>
<td>20</td>
<td>7</td>
<td>Positive</td>
</tr>
<tr>
<td>Acetamiprid</td>
<td>223.1</td>
<td>Unit</td>
<td>56.1</td>
<td>Unit</td>
<td>50</td>
<td>70</td>
<td>15</td>
<td>7</td>
<td>Positive</td>
</tr>
</tbody>
</table>

### Table 3: Mean soil concentrations for all replications of neonicotinoids and their metabolites sampled from muskmelon fields in the spring (ng/g).

<table>
<thead>
<tr>
<th>Compound</th>
<th>2013</th>
<th>2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imidacloprid</td>
<td>10.43</td>
<td>3.82</td>
</tr>
<tr>
<td>Olefin</td>
<td>1.41</td>
<td>1.37</td>
</tr>
<tr>
<td>5-OH-imidacloprid</td>
<td>20.83</td>
<td>1.27</td>
</tr>
<tr>
<td>Thiamethoxam</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>Clothianidin</td>
<td>no data</td>
<td>4.79</td>
</tr>
<tr>
<td>Acetamiprid</td>
<td>0.05</td>
<td>0.03</td>
</tr>
</tbody>
</table>
FIGURES
Figure 1: Pollen concentrations of Imidacloprid in ng/g following the high label rate application of Admire Pro as a bedding tray treatment 3 days prior to transplanting and as a soil drench treatment at transplanting, as well as a soil drench treatment at transplanting alone in 2013. Bars denote 95% confidence intervals.

Figure 2: Pollen concentrations of Imidacloprid in ng/g following the low and high label rate applications of Admire Pro as a soil drench treatment at transplanting in 2014. Bars denote 95% confidence intervals.
Figure 3: Pollen concentrations of olefin imidacloprid in ng/g following the high label rate application of Admire Pro as a bedding tray treatment 3 days prior to transplanting and as a soil drench treatment at transplanting, as well as a soil drench treatment at transplanting alone in 2013. Bars denote 95% confidence intervals.

Figure 4: Pollen concentrations of olefin imidacloprid in ng/g following the low and high label rate applications of Admire Pro as a soil drench treatment at transplanting in 2014. Bars denote 95% confidence intervals.
Figure 5: Pollen concentrations of 5-hydroxymidacloprid in ng/g following the high label rate application of Admire Pro as a bedding tray treatment 3 days prior to transplanting and as a soil drench treatment at transplanting, as well as a soil drench treatment at transplanting alone in 2013. Bars denote 95% confidence intervals.

Figure 6: Pollen concentrations of 5-hydroxymidacloprid in ng/g following the low and high label rate applications of Admire Pro as a soil drench treatment at transplanting in 2014. Bars denote 95% confidence intervals.
Figure 7: Pollen concentrations of thiamethoxam in ng/g following the high label rate applications of Platinum as a soil drench treatment at transplanting, Actara as a foliar spray 3 days prior to flower collection, and FarMore seed treatment in 2013. Bars denote 95% confidence intervals.

Figure 8: Pollen concentrations of thiamethoxam in ng/g following the low and high label rate applications of Platinum as a soil drench treatment at transplanting, Actara as a foliar spray 3 days prior to flower collection, and FarMore seed treatment in 2014. Bars denote 95% confidence intervals.
2.1 ng/g: Decreased adult worker populations when combined with 5.3 ppb thiamethoxam (Sandrock et al., 2014)

Figure 9: Pollen concentrations of clothianidin in ng/g following the low and high label rate applications of Platinum as a soil drench treatment at transplanting, Actara as a foliar spray 3 days prior to flower collection, and FarMore seed treatment in 2014. Bars denote 95% confidence intervals.
Figure 10: Pollen concentrations of acetamiprid in ng/g following the high label rate application of Assail as a foliar spray 3 days prior to flower collection in 2013. Bars denote 95% confidence intervals.

Figure 11: Pollen concentrations of acetamiprid in ng/g following the high label rate application of Assail as a foliar spray 3 days prior to flower collection in 2014. Bars denote 95% confidence intervals.
Figure 12: Striped cucumber beetles per plant were compared among imidacloprid treatments applied as an Admire Pro soil drench at the low and high label rates. The half rate of carbaryl (Sevin XLR Plus) was applied on 29 May and 6 June when beetle counts exceeded the economic threshold of 1 SCB per plant to ensure the pollen analysis was not compromised. Bars denote 95% confidence intervals.

Figure 13: Striped cucumber beetles per plant were compared among thiamethoxam treatments applied as a Platinum soil drench at the low and high label rates. The half rate of carbaryl (Sevin XLR Plus) was applied on 29 May and 6 June when beetle counts exceeded the economic threshold of 1 SCB per plant to ensure the pollen analysis was not compromised. Bars denote 95% confidence intervals.
Figure 14: Striped cucumber beetles per plant were compared among foliar spray treatments of thiamethoxam applied as Actara at the low and high label rates and acetamiprid applied as Assail at the high label rate. Foliar sprays were applied on 7 July. Bars denote 95% confidence intervals.

Figure 15: Striped cucumber beetles per plant were compared among the FarMore seed treatment and the control. The half rate of carbaryl (Sevin XLR Plus) was applied on 29 May and 6 June when beetle counts exceeded the economic threshold of 1 beetle per plant to ensure the pollen analysis was not compromised. Bars denote 95% confidence intervals.
REFERENCES
REFERENCES


Schneider, C., W., Tautz, J., Grunewald, B., and Fuchs, S. 2012. RFID tracking of sublethal effects of two neonicotinoids insecticides on the foraging behavior of Apis mellifera. PLOS ONE 7(1): e30023.


vanEngelsdorp, D. and Meixner, M. D. 2010. A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. Journal of Invertebrate Pathology 103: S80-S95.


