THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENTS OF BIOLOGY AND ENTOMOLOGY

THE EFFECTS OF *BT* CORN POLLEN AND CLOTHIANIDIN ON HONEY BEES (APIS MELLIFERA)

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Spring 2011

A thesis
submitted in partial fulfillment
of the requirements
for a baccalaureate degree
in BIOLOGY
with interdisciplinary honors in BIOLOGY AND ENTOMOLOGY

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ABSTRACT

Currently, 92 million acres of *Bt* corn are grown throughout the United States. Much of the corn crop is protected from chewing and sucking insects by the systemic neonicotinoid, clothianidin, making the exposure of honey bees (*Apis mellifera*) to the combination of *Bt* corn pollen and clothianidin a very likely scenario. Although corn is a wind pollinated crop, honey bees typically collect this abundant source of pollen in mid-summer. The objective of this study was to determine if a synergistic interaction occurs when bees consume *Bt* corn pollen and clothianidin, resulting in increased mortality. Two preliminary experiments were conducted that led to the use of artificial queen rearing cups as the ideal pollen feeding delivery method. Initially, newly-emerged caged bees were fed one of three pollen diets (mixed pollen, non-*Bt* corn pollen, and *Bt* corn pollen). After feeding on the pollen for nine days, each group received a 0.3ppm dose of clothianidin fed in a 50% sugar solution. The average pollen consumed per treatment group and average weight gain per treatment group were recorded throughout the first nine days of the experiment and the mortality was recorded at regular intervals throughout the clothianidin treatment period. Chi-square tests were performed between the different treatment groups to determine if the mortality was significant. Adding clothianidin to the diet significantly affected the survival of the bees independent of which pollen diet they received (Figures 11-13). No synergistic effect between *Bt* corn pollen and clothianidin was observed.

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ACKNOWLEDGEMENTS

I am sincerely thankful to my thesis supervisor, Maryann Frazier, for the extreme guidance, support, and assistance she provided throughout the entire process of my honors work.

I would like to show my sincere gratitude to Sara Ashcraft and Jim Frazier for their guidance, advice, and willingness to help in any way throughout the entire duration of my honors work.

I am extremely thankful to Wanyi Zhu for her huge assistance in performing the statistical analysis of my data and for her patience when answering my many questions.

I am grateful to Chris Mullin and Jeff Pettis for their advice and interest in helping me develop my experimental design.

I am thankful to Stephanie Mellott and Lauren Rusert for any assistance that they provided me in the lab when I was carrying out my study.

Introduction

Long-term population trends for many North American pollinators have been declining. Although it is difficult to pinpoint the exact cause, potential contributing factors range from parasites and disease to habitat loss and climate change to competition with other species. For most pollinators, the lack of long-term population data and knowledge of basic ecology has resulted in the inability to definitively recognize the status of these pollinator species (1). However, evidence exists for the past and continuing decline in honey bee (*Apis mellifera*) populations. Possible factors for this species decline include pesticide use, pathogens, parasites, and encroachment of Africanized honey bees (*Apis mellifera scutellata)* in the southeastern United States (2).

Since the 1980s, honey bee health has been in decline and this rate of decline seems to be accelerating. In the United States during the winter of 2006-2007, there was a large-scale loss of honey bee (*Apis mellifera*) colonies where an unusual 30-90% of beekeepers' hives were lost (3). These losses were witnessed again in the winter of 2007-2008. These colonies were identified based on three distinct symptoms: [1] excess brood populations relative to adult honey bee populations, indicating an accelerated loss of worker bees; [2] no visible masses of dead worker bees in or around the hives; and [3] the delayed attack of hive pests and kleptoparasitism from neighboring colonies (4). This unexplained syndrome has been labeled Colony Collapse Disorder (CCD).

There has long been a concern among beekeepers and growers of bee-pollinated crops regarding the exposure of honey bees to chemical pesticides. Pesticides are used to control pest insects, weeds, and diseases; they are even utilized within the hive to control Varroa mites, parasites of honey bees. In the past, toxic exposures of honey bee colonies to certain pesticides have resulted in a lethal kill, which was easy to diagnose due to the large number of poisoned bees that made it back to the hive and died around the hive entrance. Studies have also shown that pesticides at sublethal levels impair the learning abilities of honey bees and have repressed the performance of their immune systems. In a report by Frazier, et al, pollen and wax were analyzed for pesticide residues; large numbers of multiple types of pesticides were found and it is believed that interactions of these pesticides could result in increased toxicity to honey bees (5). In a more recent and detailed paper by Mullin, et al. 887 wax, pollen, bee and hive samples were screened for 171 pesticides and metabolites in which 121 different pesticides were detected.

In samples of trapped pollen and beebread, a major component of food for the developing brood and young adult bees, an average of seven pesticides per pollen sample per hive was found. Many of the toxins detected are known to have acute sublethal impacts on honey bees. Pesticides found most frequently and at the highest levels include fluvalinate, coumaphos, chloropyifos, and chlorothalonil (6).

Neonicotinoids are a class of insecticides that have a structure similar to that of nicotine (7). Most pesticides within the class are considered toxic to bees. They disrupt the central nervous system of insects by inducing paralysis and ultimately death within a few hours and have a decreased toxicity to mammals (8). Neonicotinoids are systemic pesticides that can remain in the lymph of a plant for up to 85 days (9). The neonicotinoid imidacloprid, however, was found to stay in the foliage of woody plants for more than two years and in the soil for more than a year after application (10). This long duration of residues allows for potential contamination of the nectar and pollen in the plant, causing chronic exposure to pollinators.

Neonicotinoids are widely used and many conflicting studies exist regarding the effect of these insecticides on non-target species. For example, results from the European Draft Assessment Report determined that there was no impact of the neonicotinoid, imidacloprid, on honey bees when used as a seed treatment whereas other studies have found that imidacloprid causes sub-lethal effects on honey bees when they are exposed to relevant environmental levels (pollen- 10 μg/kg; nectar-6 μg/kg) (11).

Legislation exists in Europe for the purpose of regulating plant protection products. This legislation requires that all products available in the European Union Member States must undergo a two-stage approval process. In the first stage, the active substances in the products are assessed based on their acute and chronic toxicity as well as on their sub-lethal effects. In the second stage, the products must be approved at the national level. In the UK, The Chemicals Regulation Directorate is the body of government that tests and ensures the safety of pesticides on the environment. Before approving the pesticide, certain requirements must be met. One such requirement is that the use of a pesticide must not have a hazardous influence on the environment, or on the non-target species (11).

Incidents involving the acute poisoning of honey bees by neonicotinoids have led several European countries to suspend the use of these systemic pesticides. In France, certain treatments containing imidacloprid have been suspended. In Italy and Slovenia, certain treatments

containing imidacloprid and other neonicotinoids have been suspended as the losses of bees correlate with the application of pesticides containing these compounds (12). In Germany, the neonicotinoid clothianidin is suspended as a seed treatment for corn following an incident in May 2008 in which bees exposed to clothianidin were subsequently poisoned. At the time of the corn planting, dry and windy conditions caused clothianidin-laden dust from the seeds to be blown into the foraging grounds of the bees, which led to the poisoning (13).

In the United States, over one billion tons of pesticide products are utilized each year (14) and it is the U.S. Environmental Protection Agency (EPA) that regulates the use of these pesticides by registering new pesticides before they can be marketed and by re-registering older pesticides (registered before November 1984) to ensure that they meet current safety standards. Hundreds of tests are performed on the pesticides to ensure that the use of these products pose no risks to human health or to the environment (14). However, in February 2003, it was brought to the EPA's attention that "[Clothianidin] is toxic to honey bees. The persistence of residues and the expression of clothianidin in nectar and pollen suggests the possibility of chronic toxic risk to honey bee larvae and the eventual stability of the hive." (15) Yet, in April 2003, the EPA gave the pesticide conditional registration under the agreement that a life cycle study of clothianidin on corn was completed by December 2004; the study was not completed until August 2007 and not reviewed by the EPA until November 2007. The EPA found the study to be "scientifically sound" and promoted clothianidin to full registration, although many beekeepers and scientists found the study to be flawed (16). It should be noted that clothianidin was on the market since spring 2003 even though its effects on the environment were not fully understood and its toxicity to honey bees was known.

Clothianidin (trade name: Poncho 600) is a neonicotinoid produced by the Bayer Corporation that is utilized as an insecticide against chewing and sucking insects that are pests of corn and canola (17). In 2009, $262 million worth of clothianidin was purchased by farmers (18). It was chosen for this experiment due to its high toxicity, systemic-nature, and its wide use on *Bt* corn plants. Although corn is a wind pollinated crop, honey bees typically collect this abundant source of pollen in mid-summer. These factors make the exposure to the combination of clothianidin and *Bt* corn pollen to honey bees a likely and possibly destructive scenario.

Bacillus thuringiensis (*Bt*) is a naturally-occurring soil bacterium that produces insecticidal toxins. By inserting a single modified gene from *Bt* that controls the production of

the toxins into corn, these crops have the ability to produce these insecticidal toxins, making them resistant to certain pests. When some groups of insects ingest the *Bt* toxins, known as delta endotoxins, a reaction occurs between the alkaline midgut of the insect and the toxins; the toxins cause a lysis of the midgut epithelial cells leading to total paralysis of the pest's digestive system. This occurs almost instantaneously causing the damage to the plant to be ceased after the insect is exposed to the *Bt* (19).

Bt insecticides have been used in agricultural crop production and commercially for more than 30 years. There are approximately 280 strains of *Bt* that produce many different types of delta endotoxins that are toxic to a variety of different pests (19). There are 92 million acres of *Bt* corn crops planted throughout the United States that are protected against many chewing and sucking pests. Although *Bt* crops are intended for specific pests, studies have shown that non-target species are affected as well. For example, in a study conducted by Sears, et al. it was found that *Bt* corn pollen is toxic to monarch butterflies, a non-target species (20). Han, et al. found that transgenic Cry1Ac+CpTI cotton pollen had an anti-feedant effect on honey bees (21) and Ramirez-Romero, et al. demonstrated that honey bees exposed to the Cry1Ab protein at 5000 ppb expressed negative effects in their feeding behaviors and learning processes (22). Conversely, Rose, et al. (2007) found that honey bee brood development was not affected by exposure to *Bt* pollen (23) and a meta-analysis performed by Duan, et al. concluded that *Bt* crops are not harmful to honey bee larvae or adults (24).

At the USDA Agriculture Research Service (ARS) and around the world, scientists are investigating four areas as potential causes of Colony Collapse Disorder (CCD): pathogens, parasites, environmental stresses such as pesticides, and management stresses such as poor nutrition of colonies when pollinating certain crops. The research leader of the USDA ARS Bee Research Laboratory in Beltsville, Maryland, Jeff Pettis, is looking at the effects that combinations of these potential causes have on honey bees. One study looks at the combination of either the Israeli acute paralysis virus (IAPV) or *Nosema* and pesticides on bees and another examines the effects of the combination of Varroa mites and pesticides on bees (3). As shown by Mullin, et al. honey bee exposure to combinations of pesticides is common.

The following experiments were designed to study the effects of the combination of the insecticide, clothianidin, and *Bt* corn pollen on honey bee mortality. Initial preliminary experiments were conducted to identify suitable pollen and sucrose syrup feeding techniques.

The experiment was set up to first determine the effects of *Bt* corn pollen on honey bees and then to determine the effects of *Bt* corn pollen combined with clothianidin on honey bees. My hypothesis is that when the bees are fed mixed pollen, non-*Bt* corn pollen, and *Bt* corn pollen, the bees fed the *Bt* corn pollen will have a high rate of mortality. My second hypothesis is that when the bees are fed clothianidin, the bees fed *Bt* corn pollen and clothianidin will have the highest rate of mortality when compared to the bees fed mixed pollen and non-*Bt* corn pollen with the clothianidin.

Materials and Methods

Two preliminary experiments were performed to determine the best pollen feeding methods for honey bees held in cages in the lab. For the following experiments, newly emerged worker bees from a single source colony were obtained from the PSU Wiley apiary. Frames of capped brood with all adult bees removed were selected and placed in an incubator and held at 25°C ± 1.5°C under 24 hour darkness with 70-80% humidity controlled with a saturated salt solution [Figure 1]. After 24 hours in the incubator, newly-emerged bees, less than 24 hours old, were removed for use in the experiments.

Figure 1 – Saturated salt solution used to regulate humidity between 70-80%

Feeding Delivery Methods; Experiment One

In April 2010, three pollen delivery methods were tested: queen cups, small Petri dishes, and 1.5-mL micro-centrifuge tubes. Six pieces of screen-mesh were cut (0.3cm by 0.3cm) to form the body of each cage. Disposable Petri dishes (15mm x 100mm) were fitted to the screen

cylinders as tops and bottoms. A heated metal cork borer (#7) was utilized to create three holes in the tops of the Petri dishes. A probe was used to poke three holes each in the bottom of 12 1.5-mL micro-centrifuge tubes, which were used to deliver water and sucrose solution. Six of the tubes were filled to the top with distilled water and the remaining six were filled to the top with a 50% sucrose solution. One tube filled with water and one tube filled with the sucrose solution each were hung in the top of the Petri dish lids fitted to the screen-mesh cages. The pollen mixture was prepared by mixing 10 grams of the respective pollen treatment (mixed pollen, non-*Bt* corn pollen, or *Bt* corn pollen) with 9.5 grams of honey until the pollen was finely grounded and evenly distributed within the honey.

The weight of three queen cups, three 1.5-mL micro-centrifuge tubes, and three Petri dishes (50mm x 9mm) were recorded. The queen cups were filled to slightly overflowing with the pollen mixture. The micro-centrifuge tubes were filled to the top with the pollen mixture and a little over one gram was put into the Petri dishes. Each container was weighed again. One of each container was kept aside to control for moisture loss [Figure 2]. Fifteen of the newly-emerged honey bees were placed into each cage and the cages divided into three treatment groups [Table 1].

Table 1: Pollen Feeding Methods for Preliminary Experiment 1

Cage Numbers	Pollen Feeding Method
1,2	queen cups
3,4	1.5-mL micro-centrifuge tubes with bottom tips cut off
5,6	Petri dishes

The six cages and moisture controls were placed into a plastic bin with a damp paper towel on the bottom and a lid on top [Figure 3]. A saturated salt solution in a test tube was also put into the bin to help keep the humidity between 70-80% at 25°C ± 1.5°C.

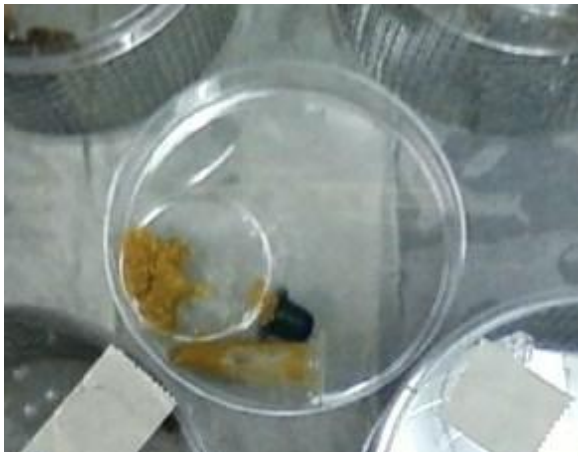

Figure 2 – Moisture controls for each of the three possible pollen delivery methods

Figure 3 – Preliminary experiment 1 experimental set-up

Observations of the bees were recorded once daily Monday through Friday for 12 days. The moisture controls were weighed again and disposed of after seven days.

Feeding Delivery Methods; Experiment Two

The second preliminary experiment began in April 2010, set up as previously described, except scintillation vial feeders were used to supply the bees with distilled water and sugar solution and the Petri dish method was eliminated [Figure 4]. A cork borer (#15) was used to create two holes in the top of each Petri dish lid to hold these vials. A hole was then punctured in the center of the lid of each scintillation vial feeder using a drill bit (0.2mm). Next, 18 of the vial feeders were filled with distilled water and another 18 of the vial feeders were filled with the 50:50 sucrose solution. After filling each feeder, a piece of mesh (100µ with 48% open area and 51µ thread diameter) was placed between each lid and vial.

The weight of two queen cups and two 1.5-mL micro-centrifuge tubes were recorded. The queen cups were filled to slightly overflowing with the pollen mixture and the micro-centrifuge tubes were filled to the top with the pollen mixture. Each container was weighed again. Fifteen of the newly-emerged honey bees were placed into each cage and the cages were divided into three treatment groups [Table 2].

Table 2: Pollen Feeding Methods for Preliminary Experiment 2

Cage Numbers	Pollen Feeding Method
1,2	queen cups
3,4	1.5-mL micro-centrifuge tubes with bottom tips cut off
5,6	None - control

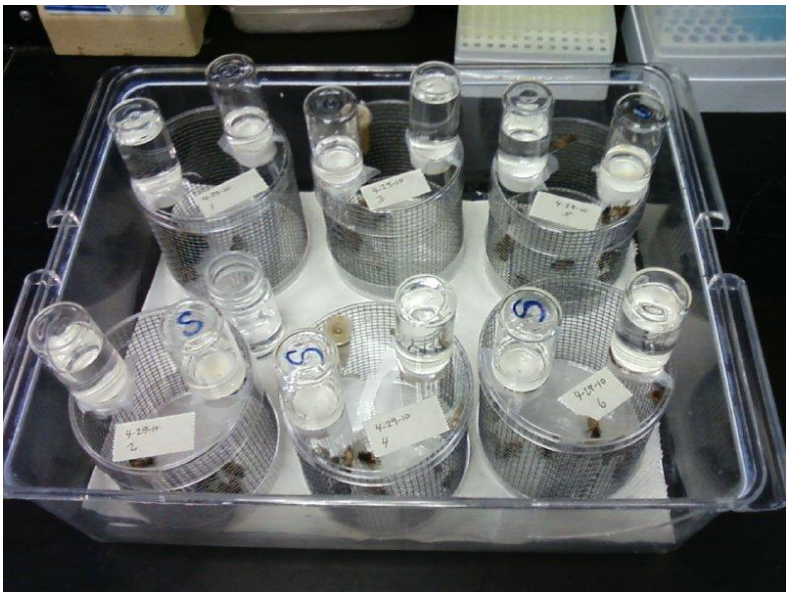

Figure 4 – Preliminary experiment 2 experimental setup

Observations of the bees were recorded once daily for 22 days.

Comparison of Three Pollen Treatments With and Without the Addition of the Pesticide Clothianidin

The experiment began in October 2010. Each pollen treatment was prepared by mixing 10 grams of the respective pollen type (mixed corn pollen, non-*Bt* corn pollen, or Cry3b *Bt* corn

pollen) with 9.5 grams of honey until the pollen was finely grounded and evenly distributed within the honey and it was delivered in plastic artificial queen cups.

The cages and scintillation vial feeders were set up as previously described except 18 scintillation vial feeders were filled with distilled water and another 18 scintillation vial feeders were filled with a 50:50 sucrose solution [Figure 5]. Thirty-nine queen cups were then numbered and their weights recorded. Seven cups each were filled with each treatment until the pollen mixture in each cap was slightly overflowing. The weights of the queen cups were recorded again. One queen cup from each treatment was used to measure the moisture loss. A water feeder and a sucrose feeder were put in the lid of each cage. Next, the cages with newly-emerged honey bees were divided into three mixed groups [Table 3] and placed in their own plastic bin. A vial filled with a saturated salt solution was put into each bin to regulate the humidity to be between 70-80% and the control queen cups were put into the bins [Figure 6]; lids were then put on the bins. The bins were put in an incubator with 24 hour darkness with an approximate temperature of 25°C ± 1.5°C.

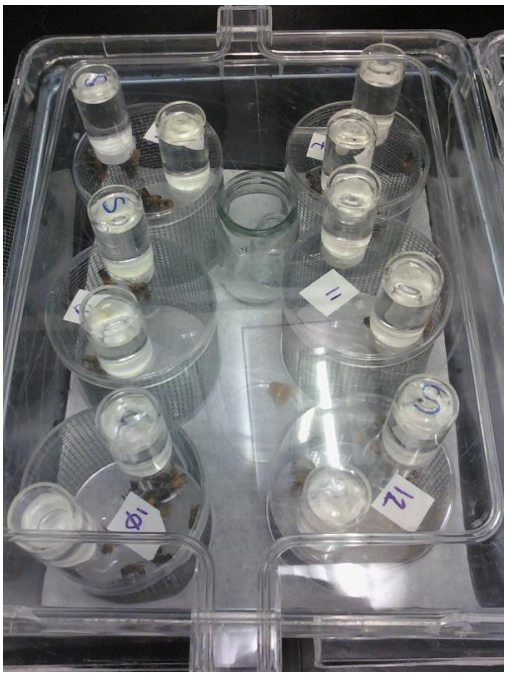

Figure 5 – Experimental setup for testing the comparison of the different pollen diets

Figure 6 – (Top) Bee feeding from queen cup; (Bottom) All bees feeding from queen cups

The mortality and any additional observations were recorded for each cage for the following ten days. The bees had pollen, sugar solution, and distilled water at all times. At the end of the ten

days, each queen cap was removed and weighed. The cages were then cooled until the bees were lethargic and then they were placed in a container and weighed together for each cage.

Clothianidin (99% Purity from ChemService) solutions were prepared at 0.3ppm. A micropipette was utilized to fill six 0.7-mL scintillation vials with the clothianidin solution and six tubes with a 50% sucrose solution. A small piece of mesh (100µ with 48% open area and 51µ thread diameter) was placed over the open top of the tube and then secured in place by wrapping parafilm around it. A ridge was created around the center of the tube using parafilm for the purpose of keeping the tube in place while positioned in the Petri dish lids. The original Petri dish lids were replaced with the Petri dish lids intended for the small tubes.

Due to excessive mortality in three of the cages, the bees had to be rearranged into four cages [Figure 7] and then into six treatment groups [Table 3].

Table 3: Bee Rearrangements and Treatment Groups

Cage #	Rearrangements	Ending Number of Bees	Initial Pollen Treatment	Second Treatment
1		Removed	Mixed	
2		12	Mixed	SS
3		12	Mixed	SS
4		13	Mixed	CL
5		Removed	Mixed	
6		13	Mixed	CL
7		12	Non-BT Corn	SS
8		Removed	Non-BT Corn	
9	1 bee received from cage 10	12	Non-BT Corn	SS
10	1 bee moved to cage 9; 1 bee moved to cage 12	13	Non-BT Corn	CL
11		Removed	Non-BT Corn	
12	1 bee received from cage 10	13	Non-BT Corn	CL
13		12	BT Corn	SS
14	2 bees received from cage 17; 2 bees received from cage 18; 1 bee moved back to cage 17	Removed	BT Corn	
15		12	BT Corn	SS
16		Removed	BT Corn	
17	2 bees moved to cage 14; 1 bee received back from cage 14	13	BT Corn	CL
18	2 bees moved to cage 14	13	BT Corn	CL

SS = 50% Sucrose Solution Only

CL = Clothianidin Solution Added to Sucrose Solution

Tape was then placed over the holes in the Petri lid dishes and the bees were starved for 24 hours. After 24 hours, each cage was given their specified second treatments. The honey bees were allowed to feed on the solution for 24 hours [Figure 8].

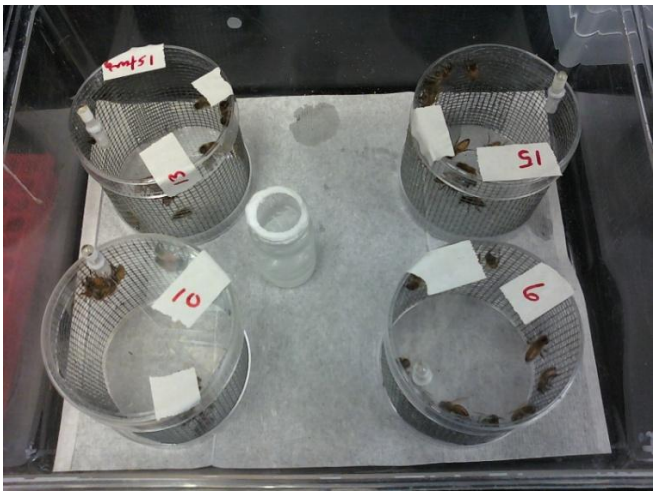

Figure 7 – Experimental setup for testing the effects of the pollen diets with clothianidin

Figure 8 – Bees feeding on the clothianidin solution from 0.7-µL scintillation vials

The mortality and any additional observations were recorded for each cage for the hours following the introduction of clothianidin to the honey bees: 3.5, 20, 25.5, 48, 72, and 94.5 hours.

Pollen Information

The pollen was not freshly collected and was stored in the freezer at -20°C.
The pollen was acquired from the Dekalb company.

<u>*Bt* Corn Pollen</u>

FC Cry 3Bb1
3C 153µm
8/14/03 8/18/03 (IA)

<u>Non-*Bt* Corn Pollen</u>

FC Iso – Genetically identical to *Bt* corn pollen except missing the Cry 3Bb1 gene
1B, 2D 153µm
8/18/03 8/11/03

Mixed Pollen

Collected from beekeeper Shan Ruoss from Winthrop, WA; Spring 2009
Results of pesticide test:

Pendimethalin 1.6 - 2.1ppb
Carbaryl 4.5 – 4.9ppb

Calculation of Clothianidin Solutions

0.3 ppm

Stock 1: 1 mg clothianidin + 1 mL Methanol = 1000 ppm made 9-17-10; used 10-13-10

Stock 2: 3 µL Stock 1 + 10 mL 50% SS = 0.3 ppm made and used 10-13-10

One mg of clothianidin (99% Purity from ChemService) was added to 1 mL methanol and dissolved using a Vortex. Three µL of stock solution was then mixed into 10 mL of 50% sugar solution using a Vortex. Using a pipet, 400 µL of sugar solution was put into six 7 µL scintillation vials and 400 µL of clothianidin/sugar solution was put into six 7 µL scintillation vials. The vials were wrapped with mesh (100µm with 48% open area and 51µm thread diameter) with parafilm holding the mesh. Each vial was given a rim made of parafilm in order to hold the vial in the lid of the cage.

Statistical Analysis of Mortality Data

Figures 10-13 use an exponential plot which charts the cumulative exponential failure probability by time for each group. Lines that are approximately linear empirically indicate the appropriateness of using an exponential model for further analysis.

$$S(t) = \exp\left(-\int_0^t \lambda du \right) = \exp(-\lambda t)$$

The exponential distribution is the simplest, with only one parameter-,λ-which is the hazard rate per individual per time interval. It assumes there is no memory of how long each individual has survived to affect how likely an event (death event for here) is going to happen. Therefore, λ is the instantaneous failure rate and is independent of t so that the conditional chance of failure

does not depend on how long the individual has been on the trial. This is referred to as the memory-less property of the exponential distribution. The exponential cumulative plot is based on this assumption and the cumulative value is Sum (λ_i), where i is instantaneous time. For instance, if we want to know the cumulative mortality until the second day, we need to estimate the λ_1 ,λ_2 by using the above function. S(t=1), S(t=2) are the actual data from the experiment, which is the surviving probability in the first and second days.

Results

Feeding Delivery Methods; Experiment One

The first preliminary experiment was designed to compare three potential pollen delivery systems: artificial queen-rearing cups, 1.5-mL micro-centrifuge tubes, and Petri dishes (50mm x 9mm). Based on the results of the first experiment, it was decided to eliminate the Petri dish method for feeding pollen to the bees and use the queen cups and 1.5-mL micro-centrifuge tubes for the second preliminary experiment [Table 4]. The pollen mixture in the Petri dishes had extensive mold growth and many of the bees became hopelessly stuck in the mixture and were unable to get free. The 1.5-mL micro-centrifuge tubes had no mold growth and the bees were able to feed on the pollen up to a point in which the pollen would have to be plunged down into the tip. The queen cups also had no mold growth and the bees were able to feed on the pollen. The issue with the queen cups was that they did not provide as much pollen mixture to the bees as the 1.5-mL micro-centrifuge tubes. It was decided to compare these two methods in a second experiment.

The first experiment also demonstrated that the micro-centrifuge tubes were not an efficient way to provide the bees with sugar solution and water since the water frequently needed refilled and dried sugar solution clogged the holes of the tubes, not allowing the bees to feed. For the second experiment, scintillation vials were used to supply the bees with water and sugar solution.

Table 4: Preliminary Experiment One Results

Pollen Feeding Method	Observations	Total Pollen Consumed* (g)	Possible Method?
Queen cups	Bees able to feed; no mold growth	0.452	Yes
Micro-centrifuge Tubes	Bees able to feed, but need way to make pollen mixture accessible at all times; no mold growth	0.249	Yes
Petri Dishes	Excessive mold growth; many bees became stuck in pollen mixture and died	Unable to be determined due to mold growth	No

*Moisture loss accounted for

Feeding Delivery Methods; Experiment Two

The second preliminary experiment was designed to compare queen cups, which could not hold as much pollen mixture, with the 1.5-mL micro-centrifuge tubes, which would need an effective plunging method. Based on the results, it was decided to utilize the queen cups for feeding the pollen mixture to the bees in the main experiment [Table 5]. No effective plunging method was discovered when using the 1.5-mL micro-centrifuge tubes. For the main experiment, it was decided that two queen cups would be sufficient for each cage.

Using the scintillation vials also proved to be a very effective method for providing the bees with distilled water and sugar solution and would be utilized for the main experiment.

Table 5: Preliminary Experiment Two Results

Pollen Feeding Method	Observations	Total Pollen Consumed (g)	Possible Method?
Queen cups	Bees able to feed; no mold growth	0.762	Yes
Micro-centrifuge Tubes	Bees able to feed, but unable to access remainder of pollen - would be too time-consuming to make pollen accessible	0.705	No

Comparison of Mixed Pollen, Non-Bt Corn Pollen, and Bt Corn Pollen

The average weight gain of bees for each pollen treatment was nearly equal, indicating that no pollen treatment had a nutritional advantage over another [Table 6]. The average pollen consumed by each treatment group was corrected for moisture loss. The bees receiving the mixed pollen diet consumed the most pollen, an average of 0.458 grams per cage, while those receiving the *Bt* corn pollen consumed the least, an average of 0.241 grams per cage. Bees in cages fed the non-*Bt* corn pollen consumed an intermediate amount of pollen or an average of 0.39 grams per cage [Table 6].

Table 6: Weight Gain and Pollen Consumed for Treatment Groups

	Mixed Pollen (Cages 1-6)	Non-BT Corn Pollen (Cages 7-12)	BT Corn Pollen (13-18)
Average Weight of Bee per Treatment Group (g) Day 1	0.082	0.087	0.088
Average Weight of Bee per Treatment Group (g) Day 10	0.108	0.102	0.102
Average Weight Gain per Treatment Group (g)	0.095	0.094	0.095
Average Pollen Consumed per Treatment Group (g)	0.547	0.494	0.274
Average Pollen Consumed per Treatment Group Controlled for Moisture Loss (g)	0.485	0.390	0.241

Comparison of the Three Pollen Treatments with the Addition of the Pesticide Clothianidin

The purpose of this part of the experiment was to determine if a negative synergistic effect existed between *Bt* corn pollen and clothianidin when both were fed to honey bees. The clothianidin was expected to have a harmful effect on the honey bees since it is a highly toxic pesticide with an LD50 of 0.04ppm and the bees were administered a 0.3ppm clothianidin solution. Assuming each of the 13 bees received an equal share of the solution, they would each consume a dose of 0.02ppm of clothianidin. This represents a sublethal concentration. The concentration was expected to have a toxic effect, but below the LD50.

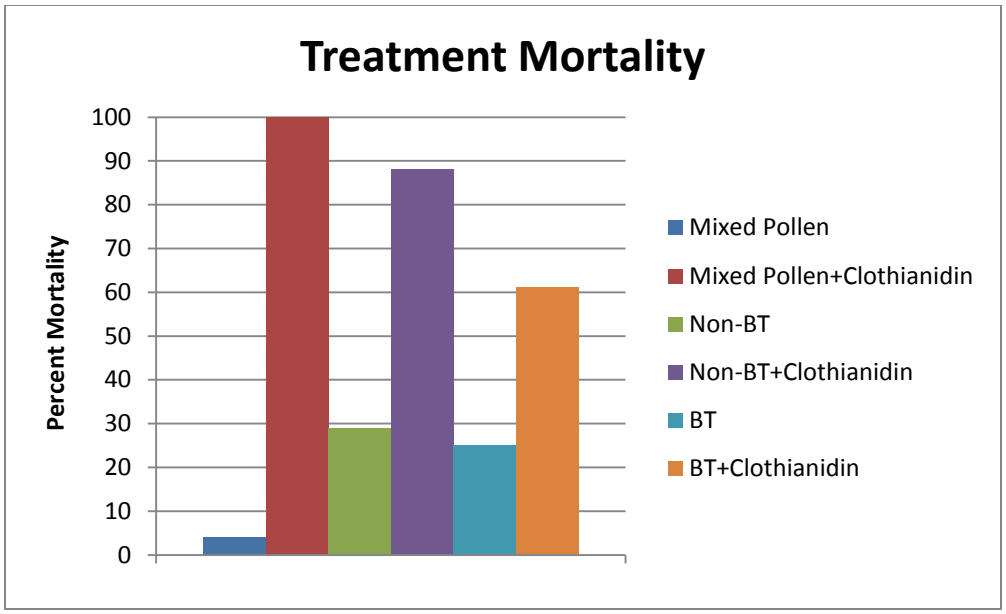

Figure 9 – Percent cumulative mortality for each treatment group

Figure 9 shows the average percent mortality for all six treatment groups at the end of the experiment. Bees consuming the mixed pollen diet had the lowest mortality (4%). Those consuming the non-*Bt* corn pollen (29% mortality) and *Bt* corn pollen (25% mortality) resulted in increased mortality compared to the mixed pollen diet with the highest mortality in bees fed the non-*Bt* corn pollen diet. However, adding clothianidin resulted in significant increased mortality in all pollen treatment groups with the greatest increase seen in the mixed pollen diet where the addition of clothianidin resulted in 100% mortality.

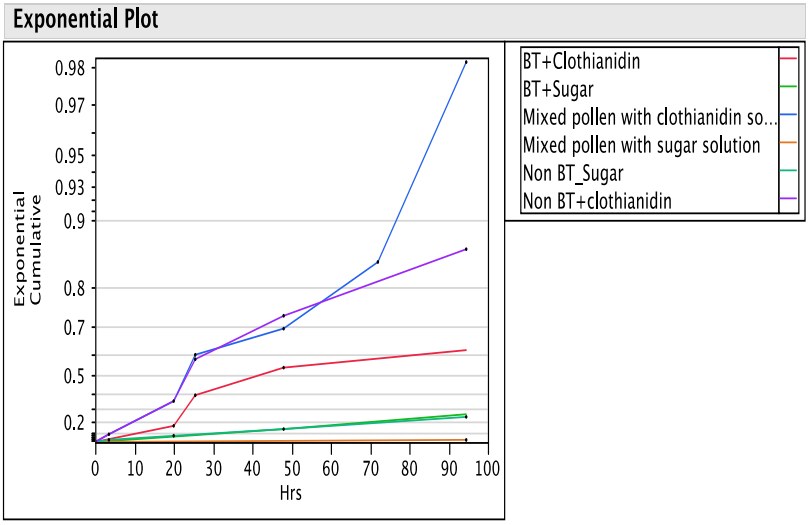

Figure 10 – Cumulative mortality plot for each group of bees after clothianidin treatment; $p<0.0001$

Figure 10 shows the cumulative mortality plot for the three pollen treatment groups with and without the addition clothianidin. A Chi-square test resulted in a significant increase in mortality in all three pollen treatment groups with the addition of clothianidin. The mortality plot for mixed pollen alone and with the addition of clothianidin is shown in Figure 11 ($p<0.0001$), non-*Bt* corn pollen alone and with clothianidin and *Bt* corn pollen alone and with clothianidin are shown in Figures 12 and 13 respectively ($p<0.0001$).

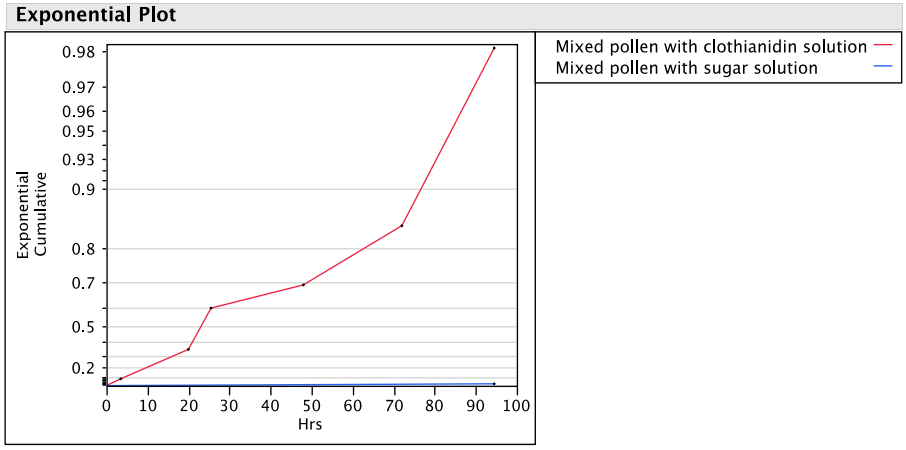

Figure 11 – Mortality plot for both mixed pollen treatments; $p<0.0001$

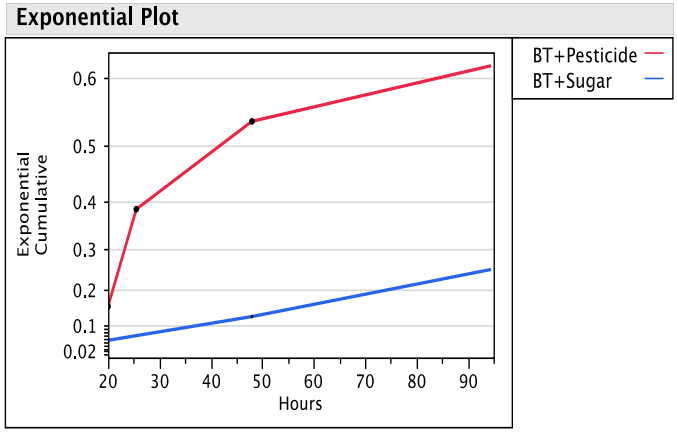

Figure 12 - Mortality plot for both non-*Bt* corn pollen treatments; $p<0.0001$

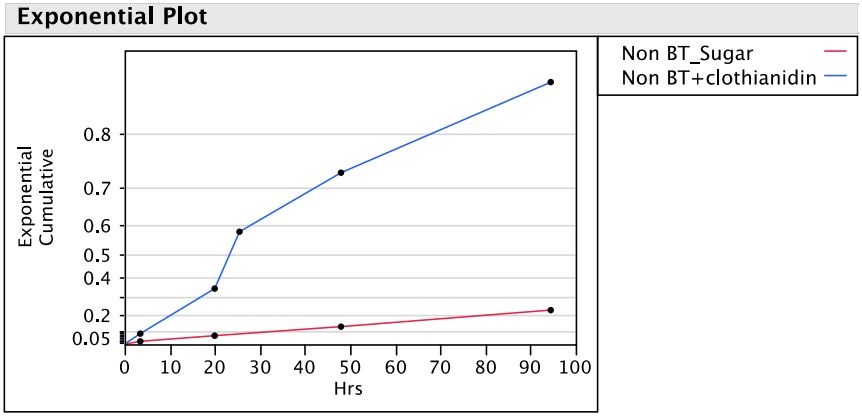

Figure 13 - Mortality plot for both *BT* corn pollen treatments; p<0.0001

Discussion

The objective of this study was to determine if a synergistic interaction results between clothianidin and *Bt* corn pollen when both are consumed by honey bees. Preliminary experiments clearly showed that artificial queen-rearing cups are an effective method for delivering pollen treatments to the bees. It was also determined that using scintillation vials was the most effective method to provide the bees with water and sugar syrup.

Comparison of the Three Pollen Treatments with the Addition of the Pesticide Clothianidin

In general, *Bt* Cry 3Bb1 is toxic to insects in the orders Coleoptera (beetles), Diptera (flies), and Lepidoptera (moths and butterflies) but not to those in the insect order Hymenoptera (bees, wasps, and ants). This would explain why many studies find no harmful effects of *Bt* corn pollen on honey bees and why the study by Sears, et al. referenced earlier, saw harmful effects on monarch caterpillars. It is believed that the pH of the midgut of honey bees is not optimal for activating the toxin while the targeted orders have an optimal midgut pH for activating the toxin. In a study by Broderick (2009), however, honey bees were found to be susceptible to the Cry1Ab toxin if they were infected with the microsporidia *Nosema apis* (25).

The treatments that received clothianidin all had a higher percent mortality when compared to the treatments that received the sugar solution alone as shown in Figure 9. Figures 11-13 illustrate that adding clothianidin significantly affected the survival of the bees independent of which pollen diet they received. Based upon these results, it seems apparent that

no synergistic effect exists between *Bt* corn pollen and clothianidin as was originally hypothesized.

Follow-up Experiments

The current study measured only potential toxic impacts of the combined Bt corn pollen and clothianidin. Possible follow-up experiments would examine potential sub-lethal affects such as longevity of honey bees when fed the different pollen diets with and without the addition of clothianidin. This would provide a better understanding of whether the varying pollen diets had any significant health effects on the bees not observed during this experiment.

If performing a repeat of this experiment, a new variable to be studied could be the hypopharyngeal gland development of the bees in each treatment group. Because the quality of pollen has an impact on the size of this gland, the weight of the gland would provide an indication as to how nutritious a certain pollen diet is. Another variable to study would be the Proboscis Extension Response (PER) to see if any significant adverse effects on memory were caused by the varying treatment groups.

Bibliography

1. *Status of Pollinators in North America* [Report brief]. (2006, October). Retrieved from National Academy of Sciences website: http://www.xerces.org/wp-content/uploads/2008/11/nas_report_in_brief.pdf

2. Findings and Recommendations. (2007). In *Status of Pollinators in North America* (pp. 196-207). Retrieved from http://www.nap.edu/openbook.php?record_id=11761&page=197#

3. Kaplan, J. K. (2008, May 2). *Colony Collapse Disorder: A Complex Buzz*. Retrieved from United States Department of Agriculture: Agricultural Research Service website: http://www.ars.usda.gov/is/AR/archive/may08/colony0508.htm

4. VanEngelsdorp, D., Evans, J., Saegerman, C., Mullin, C., Haubruge, E., Nguyen, B.K., Frazier, M., Frazier, J., Cox-Foster, D., Chen, Y., Underwood, R., Tarpy, D., Pettis, J. (2009). Colony Collapse Disorder: a descriptive study. PloS ONE 4 e6481.

5. Frazier, M. T., C. Mullin, J. Frazier. 2008. What Have Pesticides Got to do with it? Amer. Bee J. 148:521-523.

6. Mullin C.A., Frazier M., Frazier J.L., Ashcraft S., Simonds R., VanEngelsdorp, D., Pettis, J. (2010) High Levels of Miticides and Agrochemicals in North American Apiaries: Implications for Honey Bee Health. PLoS ONE 5(3): e9754. doi:10.1371/journal.pone.0009754

7. *Neonicotinoid insecticides* [Fact sheet]. (n.d.). Retrieved from National Potato Council website: http://www.nationalpotatocouncil.org/NPC/p_documents/document_280607084102.pdf

8. *The story of neonicotinoid insecticides*. (2010). Retrieved from AgroNews website: http://news.agropages.com/WiKi/Detail---3189.htm

9. Greatti, M. (2009, May). *Bees go mad and become lost because of neonicotinoid dispersion*. Retrieved from European Media Research Center website: http://www.youris.com/Environment/Bees/Bees_go_mad_and_become_lost_because_of_neonicotinoid_dispersion.kl

10. Cowles RS, Montgomery ME and Cheah CAS-J (2006) Activity and residues of imidacloprid applied to soil and tree trunks to control hemlock woolly adelgid (Hemiptera: Adelgidae) in forests. *J Econ Entomol* 99:1258–1267.

11. Kindemba, V. (2009). *The impact of neonicotinoid insecticides on bumblebees, honey bees and other non-target invertebrates* [Pamphlet].

12. *Colony collapse disorder: European bans on neonicotinoid pesticides*. (2011, February 16). Retrieved from United States Environmental Protection Agency website: http://www.epa.gov/pesticides/about/intheworks/ccd-european-ban.html

13. *Pesticide news story: EPA acts to protect bees*. (2011, February 16). Retrieved from United States Environmental Protection Agency website: http://www.epa.gov/oppfead1/cb/csb_page/updates/2008/bees-act.htm

14. *The EPA and Food Security* [Fact sheet]. (2007, May). Retrieved from United States Environmental Protection Agency website: http://www.epa.gov/pesticides/factsheets/securty.htm#evaluation

15. Federoff, N., & Melendez, J. L. (2007, June 12). *EFED section 3 registration for a clothianidin and beta-cyfluthrin combination product for use on sugar beets as a seed treatment* [Memorandum]. Retrieved from http://www.grist.org/i/assets/Memo_1.pdf

16. Stevenson, H. (2010, December 19). *EPA may allow extinction of bees: Supports Bayer's profits from nerve poison*. Retrieved from Gaia Health website: http://www.gaia-health.com/articles351/000358-epa-allows-bee-extinction-supports-bayer-nerve-poison.shtml

17. *Clothianidin* [Fact sheet]. (2003, May 30). Retrieved from United States Environmental Protection Agency website: http://www.epa.gov/opprd001/factsheets/clothianidin.pdf

18. Philpott, T. (2010, December 10). Leaked document shows EPA allowed bee-toxic pesticide despite own scientists' red flags. *Grist Magazine*. Retrieved from http://www.grist.org/article/food-2010-12-10-leaked-documents-show-epa-allowed-bee-toxic-pesticide

19. Peairs, F. (2010, August). *Bt Corn: Health and the Environment* [Fact sheet]. Retrieved from Colorado State University Extension website: http://www.ext.colostate.edu/PUBS/CROPS/00707.html

20. Sears, M.K., Hellmich, R.L., Stanley-Horn, D.E., Oberhauser, K.S., Pleasants, J.M., Mattile, H.R., Siegfied, B.D. & Dively, G.P. (2001, September 14). Impact of Bt corn pollen on monarch butterfly populations: A risk assessment. *Proceedings of the National Academy of Sciences of the United States of America, 98*(21), 11937–11942. doi:10.1073/pnas.211329998

21. Han, P., Niu, C.-Y., Lei, C.-L., Cui, J.-J., & Desneux, N. (2010, August 11). Quantification of toxins in a Cry1Ac + CpTI cotton cultivar and its potential effects on the honey bee Apis mellifera L. *Ecotoxicology, 19*(8), 1452-1459. doi:10.1007/s10646-010-0530-z

22. Ramirez-Romero, R., Desneux, N., Decourtye, A., Chaffiol, A., & Pham-Delègue, M. (2008, June). Does Cry1Ab protein affect learning performances of the honey bee Apis mellifera L. (Hymenoptera, Apidae)? *Ecotoxicol Environ Saf, 70*(2), 327-333.

23. Rose R, Dively GP, Pettis J. (2007) Effects of *BT* corn pollen on honey bees: Emphasis on protocol development. Apidologie. 38:368–377.

24. Duan JJ, Marvier M, Huesing J, Dively G, Huang ZY. (2008) A meta-analysis of effects of *BT* crops on honey bees (Hymenoptera: Apidae). *PLoS ONE* 3:e1415.

25. Then, C. (2010) Risk assessment of toxins derived from Bacillus thuringiensis - synergism, efficacy, and selectivity. Environ Sci Pollut Res 16. 17:791-797.

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