A Thesis Presented to
The Faculty of Alfred University

INVESTIGATION OF THE PRESENCE OF THE PARASITIC PHORID FLY *APOCEPHALIS BOREALIS*, DEFORMED WING VIRUS AND ISRAELI ACUTE PARALYSIS VIRUS IN HONEYBEE HIVES IN ALFRED, NY

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In Partial Fulfillment of
the Requirements for
The Alfred University Honors Program
Date: 2.May.2013

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ABSTRACT

The European honeybee (*Apis mellifera*) has a long history in the United States as a source of honey, beeswax and other products. In the last couple centuries the honeybee has become a key pollinator in world agriculture with an estimated 80% of the crops we produce relying on them for pollination. In 2006 a mysterious syndrome termed Colony Collapse Disorder (CCD) was first reported. CCD is characterized by the abandonment of the hive by all workers leaving behind healthy brood, food and the queen and the absence of dead bees within or around the hive. Many possible causes have been proposed through efforts to explain the disappearances. Some of these suspects include viruses, pesticides and parasites as well as longstanding pests such as *Varroa destructor*, tracheal mites, *Nosema ceranae* and *Nosema apis.* While previous studies have not revealed any direct correlations between these possible causes and the occurrence of CCD, they are detrimental to hive health on their own and could have more acute effects synergistically. This study focused on determining the presence or absence of three threats to honeybee health: the parasitic Phorid fly *Apocephalis borealis*, Deformed Wing Virus and Israeli Acute Paralysis Virus. Other potential causes are examined and discussed. There was no observed presence of *A. borealis* in Alfred hives. The presence of Deformed Wing Virus and Israeli Acute Paralysis Virus is still being determined. The results of this study will serve to evaluate honeybee hive health in Alfred, NY.

INTRODUCTION

Of the 20,000 bee species known, only seven are recognized as honeybees, with *Apis mellifera* (Western/European/Common honeybee) being the most well-known. Since its introduction to North America in 1622, the honeybee has proven its pricelessness as a producer of honey and beeswax, which have been used over the centuries as raw material for other products. Later, the honeybee was recognized for its role in pollination of agriculture and natural environments and became the major pollinator of crops throughout the world.

Biology and Social Network of Honeybees

Honeybees live in colonies with an average of 60,000 to 80,000 individuals, however some hives have over 100,000. The population consists of a fertile queen bee, a small number of drones (fertile male honeybees) and a majority of workers (infertile female honeybees); (Quint, 2010). This immense workforce, along with their extremely organized behavior and other physical qualities, make the honeybee an efficient and productive pollinator. The level of organization maintained in a colony of so many individuals is complex, the result of factors including chemicals secreted by the queen and seasonal change.

Once the laying season begins in early Spring, the queen goes on a mating flight to mate with drones from other hives. This is the honeybees way of avoiding inbreeding since drones are haploid and identical to the queen that laid them. Once they mate with the queen, the drones die and the sperm is stored by the queen to be used throughout the season. Brood, the honeybee larvae, are raised in the iconic hexagon shaped wax cells built by workers. A single egg is laid in each cell. Whether or not this egg is fertilized is dependent on the size of the cell. Larger cells are built for drones (the egg will remain unfertilized) and normal sized cells are meant for

workers (the egg will be fertilized). Queens are laid in the largest cells. Once the eggs hatch on the fourth day, young worker bees known as nurse bees feed them. All of the larvae are fed royal jelly produced by nurse bees from glands on the head for the first three days. They are then switched to beebread (a combination of honey and pollen) until they reach full size and pupate 6 days later. It is at this stage that the development of a queen is decided. Larvae that are destined for queendom are continuously fed on royal jelly, while those destined to be workers and drones are switched to beebread. This continual feeding of royal jelly allows the queen to grow to her large size as well as gives her the ability to reproduce. Right before a larvae pupates, the nurse bees seal off the cell with wax and after a week or so the new adult bee emerges. Drones do nothing and will remain mostly useless until they leave to mate. Workers on the other hand begin their busy lives immediately their first job being to clean their cell (Winston, 1987).

The jobs adopted by worker bees change throughout their lives and while it is not a fixed sequence (depends on hive needs) workers of a normal, unstressed hive usually follow a pattern. Once a new bee emerges she cleans her own cell. She then moves on to clean other cells and to remove debris from the hive. This is her job for the next few days after which she develops the ability to produce royal jelly. Once she has this ability she becomes a nurse bee, feeding new larvae and sealing up the cells of those ready to pupate. At 11 days of age, she will lose her ability to produce royal jelly and will begin to take on other jobs. These jobs include cell construction, honey sealing, pollen packing, fanning (to cool the hive and remove water from honey), propolizing (spreading propolis on the inside wall of the hive), attending the queen and receiving. As a receiver she will take pollen, propolis, nectar and water from the foragers as they return. These duties are performed until she is about 22 days old. At the age of 22 days she will become a forager and will remain in this occupation until she dies approximately three weeks

later). Aside from nectar and pollen, foragers are also responsible for obtaining water and propolis, a substance that is resin-like and has anti-bacterial and anti-fungal properties (Winston, 1987).

Communication in the hive is complicated and essential. Honeybees have evolved ways of communicating the whereabouts of food sources to other foragers. They communicate through a variety of dances that depict the distance and direction of food sources. One of these dances is the waggle dance. The waggle dance consists of a straight "waggle run" where the worker will rock back and forth as she moves in the direction of the source in relation to the sun. This is shown through angles, straight vertical is directly towards the sun and deviants from straight represents angles from the sun. The length of this waggle run is the distance to the food source. At the end of the waggle run the bee will circle to the right and return to the starting point. This is repeated, this time circling left and repeated ending in a right turn, then left turn until she stops. If the bee is very excited, she will waggle faster and attract more attention, recruiting more foragers to the source. The round dance is used for food sources in close proximity and is a circular pattern. The tremble dance is used to recruit receiver bees to accept materials from foragers (Winston, 1987).

Other communication within the hive is through pheromones released from both workers and the queen. Queen Mandibular pheromone is spread throughout the hive via food sharing and queen attendants. It suppresses ovary function in worker bees, attracts drones on mating flights and keeps bees together when swarming. The absence of this pheromone signals the workers to feed royal jelly to produce a new queen. When a bee stings something the bee releases pheromones which recruits other bees from the colony to protect the hive. (Winston, 1987).

Honeybees in Agriculture

While there are other native pollinators such as butterflies, bumblebees and hoverflies, the sheer mass of a honeybee hive and its pollination abilities are difficult to beat. Since honeybees are easily managed, active earlier/longer in the season and the hives can be moved, their attractiveness for commercial beekeeping is much greater than that for other pollinators.

It is estimated that approximately 80% of food production relies on pollination by honeybees. As human-dominated habitat has spread and crop fields and orchards have increased in size, the habitat for native pollinators has been greatly diminished. This has increased the demand for honeybee hives in order to compensate for the pollination needs (Jacobsen, 2008). Along with a decrease in suitable and available habitat, there is a lack of diversity of plant species in agricultural areas due to the monoculture style production. This situation is detrimental to wild pollinators because the crops will only be available as a source of nutrition during the blooming period. Once the bloom is over, the pollinators must find other sources of food which may not be possible due to the size of the fields and dearth of other plant species (McGregor, 1976).

To fill the pollination demand, many beekeepers have switched over from honey production to migration services. Hives are over-wintered in the South and then transported hundreds to thousands of miles to follow the blooms around the country. The destinations for these hives stretch from the blueberry fields in Maine, to apple orchards through New York and Washington to the almond fields in California with many hives visiting multiple places throughout the country. The almond fields of California produce the majority of the world's almonds and rely so heavily on honeybee pollination (about two hives per acre) that blooming

draws nearly 60% of the migratory hives in the US every February (Jacobsen, 2008). It is worrisome then that there have been mysterious, serious declines of the honeybee population in recent years - a condition known as Colony Collapse Disorder (CCD). Many people not involved in apiculture are aware that there is a problem with the honeybees, but there are few who know much about the problem or the significance of CCD. The real value of the honey bee is that it is irreplaceable. There is no mechanical replacement, and there is no other insect that can compare for its foraging and pollination abilities. If the decline continues as is, there is little to counter the predictions of food shortages, the increased price of food and the decreased biodiversity and productivity of wild plants.

Colony Collapse Disorder

Colony collapse disorder (CCD) is a mysterious syndrome afflicting a large number of honeybee hives throughout the Northern hemisphere. It is characterized by 3 criteria including the sudden disappearance of worker bees from a seemingly healthy hive, leaving behind all food, brood and the queen, the lack of dead bees within or around the hive and the delayed scavenging of the abandoned hive by wax moths and small hive beetles (vanEnglesdorp *et al*, 2009).

These odd disappearances were first reported in 2006 in the US and by 2007 one quarter of the honeybee population was reported to be gone. CCD is believed to have begun prior to these reports, although on a smaller scale, and discussed under different names such as Spring dwindle disease, Fall dwindle disease, or disappearing disease. All of these names are misleading as CCD occurs abruptly, at any time of the year and the involvement of a pathogen has not yet been determined. There have been many theories put forward as to what the cause of CCD is

including cell phones, parasites, viruses, bacteria, fungus, pesticides and stress. Some theories, such as the electromagnetic radiation caused by cellphones, were put to rest quickly - but many possibilities remain. Traditional pests such as the parasitic mite *Varroa destructor*, *Nosema ceranae* and *Nosema apis* were originally the most suspected culprits, but due to their long-term infestations of hives without CCD symptoms attention has been shifted elsewhere to include pesticides, other parasites and viruses. While all of these suspects lead to a decline in colony health and numbers, very few of them have been shown to produce CCD-like symptoms (Watanabe, 2008). One parasite, *Apocephalis borealis,* was discovered accidentally in hives in California and upon investigation was shown to produce hive abandonment behavior in bees, disorientation and lack of equilibrium which fit well in the description of CCD symptoms (Core *et al*, 2012).

Phorid fly mechanism of parasitism

Andrew Core *et al*, (2012) discovered the parasitism of honey bees by the phorid fly *Apocephalis borealis*. The parasitism begins with the female fly depositing her eggs into the abdomen of the host insect. The larvae hatch within the host and feed on it. During this time, the host begins to show abnormal and inactive behavior. In studies done with fire ants, the ants would become antisocial, hiding out in sparsely populated tunnels. After some time, the host would exit the nest or hive after dark and wander away to die moving in a disoriented and unbalanced way. The fly larvae then emerge from the host between the head and the thorax (Henne and Johnson, 2007). This behavior is what earned the Phorid fly their other name – the decapitating fly. The larvae pupate and emerge as adult flies to continue the cycle. These

symptoms associated with *A. borealis* parasitism show a pattern that seemingly explains the CCD hive abandonment behavior.

Apocephalis borealis

A. borealis is a native species to the U.S. known to be a parasitoid of bumble bees and paper wasps; the honey bee not a normal host to this fly, leading to the suspicion that it is a recent host shift. As the honeybee is a highly studied organism, it is unlikely that the presence of this parasitic infestation would go unnoticed if it has been occurring for some time. The Core study revealed the presence of *A. borealis* infestation in hives in California and South Dakota. Fly range extends into the Northeast with reports of fly presence in Ontario, Canada, Maine and Albany, NY, but there are no reports of infestations of honeybees by the fly in these areas (Core *et al*, 2012). Following the conclusion of this study the citizen science project ZomBee Watch was launched in an effort to track *A. borealis* parasitism of honeybees. Infected hives have been found in Washington and Oregon with South Dakota remaining the only inland region infested. Sampling is being carried out in most other states. The reasons for this host shift may be due to the declining bumblebee populations pushing the fly to change preference to a new species they were always capable of parasitizing. Flies that possessed mutations which allowed them to infect honeybees survived, passing the mutation along and increasing the amount of flies able to infect honeybees and increasing the overall visible infestation of hives (Poullain and Nuismer, 2012).

The hive abandonment behavior could be the result of a few possibilities. As with some other species, the bee that knows it is going to die will exhibit altruistic behavior. By removing itself from the hive it also reduces the risk of passing along the infection to its hive mates. The afflicted bee may be pushed out by others who detect something to be wrong with that bee. A

third option is parasitic host control (Core *et al*, 2012). This interaction is seen in multiple other parasite-host interactions where the parasite will cause behavioral changes in the host that function to improve the survival of the parasite. An example of parasitic host control is the behavioral changes exhibited by the spider *Plesiometa argyra* when it is parasitized by the wasp *Hymenoepimecis argryraphage*. The larvae within the spider will release a chemical that causes the spider to build a web completely different from the usual structure. When the time comes for larvae emergence this web will serve to support the pupated larvae (Eberhard, 2001). In the same sense, the fly larvae may release some kind of chemical which directs the bee to leave the hive at night. While it seems like it would be more beneficial for the parasite to remain within the hive, if the fly larvae were to emerge from its host within the honeybee hive the larvae would be killed and removed immediately. Even before emergence the carcass of the host would be removed from the hive by cleaning bees. It is uncertain where exactly the parasitized bee would end up if lights being observed were not present in the surrounding area, but regardless it would die there never to return to the hive. This behavior replicates the lack of dead bees around or in the hive characteristic of CCD. The disorientation and lack of equilibrium may simply be caused by the effects of the parasitic larvae pushing against nerves and causing other problems as it moves within its host.

Viruses

The opportunity arose to look into other potential health afflictions in Alfred hives. Since the hives I was sampling have history with *Varroa destructor* infestation, I investigated the presence or absence of the Deformed Wing Virus (DWV) and Israeli Acute Paralysis Virus

(IAPV), both of which are associated with *Varroa* mite infestation and are suspects in the cause of CCD.

DWV is a positive, single stranded RNA virus of the iflaviridae family. It can be transmitted to bees via *Varroa destructor* or through contact with other infected bees via food sharing or the removal of excrement. When this virus is present in a hive without *Varroa* infestation, prevalence is low and the virus does not generally manifest any noticeable symptoms in the bees although some will have learning defects and reduced longevity. However, in the presence of *Varroa* mites, the virus is transmitted directly to larval bees by attachment of the mite itself. Transmission of DWV to larval bees causes them to either die before emergence or to emerge as adult bees with short, bloated abdomens, discoloration and the trademark shriveled wings. They will die shortly after emergence (deMiranda and Genersch, 2010). DWV is distributed globally through the mite and can also infect bumblebees. While DWV is not considered a marker for CCD hives, it is associated with excessive winter die-offs which undermine hive survival in future foraging seasons (Highfield *et al*, 2009). Also as there is a possibility that CCD is a result of synergistic effects of multiple causes, the presence of DWV may weaken the hive enough to make it susceptible to CCD in the future.

The other virus, IAPV, is also transmitted through *Varroa* mites and is the only virus to have been shown to have a strong correlation between its presence and the occurrence of CCD. It is a single-stranded RNA dicistrovirus closely related to other paralysis-causing bee viruses. Bees infected by IAPV exhibit symptoms including shivering, progression into paralysis and death outside of the hive (Cox-Foster *et al*, 2007).

It is logical that these two viruses are being investigated in this study due to their unique associations with honeybee health. DWV infection is widespread and is associated with die-off

and thus compromised hive vitality. IAPV infection may be a marker of CCD due to its high prevalence in affected hives and near absence in healthy hives. Based on hive histories and knowledge of viral incidence, it is likely that DWV will be present and IAPV will be absent from the hives tested.

METHODS: Phorid Fly Investigation

Location

For the field portion of this research project, I was looking for the presence or absence of activity of the parasitic Phorid fly in honeybee colonies of Alfred, NY. I identified the colonies to be used: two hives on Reynolds Road in a small garden next to a garage with lights of windows and streetlights at night, seven hives on Moland Road in a clearing surrounded by woods away from all lights at night, and one hive on Waterwells Road surrounded by a large garden at the rear of a farmhouse with no lights at night. The hives all varied in the number of stacked boxes, most having two or three with one hive having one box and one hive having four boxes.

Bee Trapping

The attraction of potentially affected honey bees was done in accordance with the protocol set forth by Andrew Core *et al*, (2012). An elevated light in the form of a 16 W fluorescent lamp was hung on a trellis in the center of a tarp and plugged into the nearest outlet via extension cords. One of these set-ups was placed at each site. The set-ups are pictured in Figure 1.

The distances from the hives could not be equalized due to geography, location of outlets and hive arrangement. The distances from each hive ranged from 25 feet to 60 feet but each was in clear sight of the hive entrance.

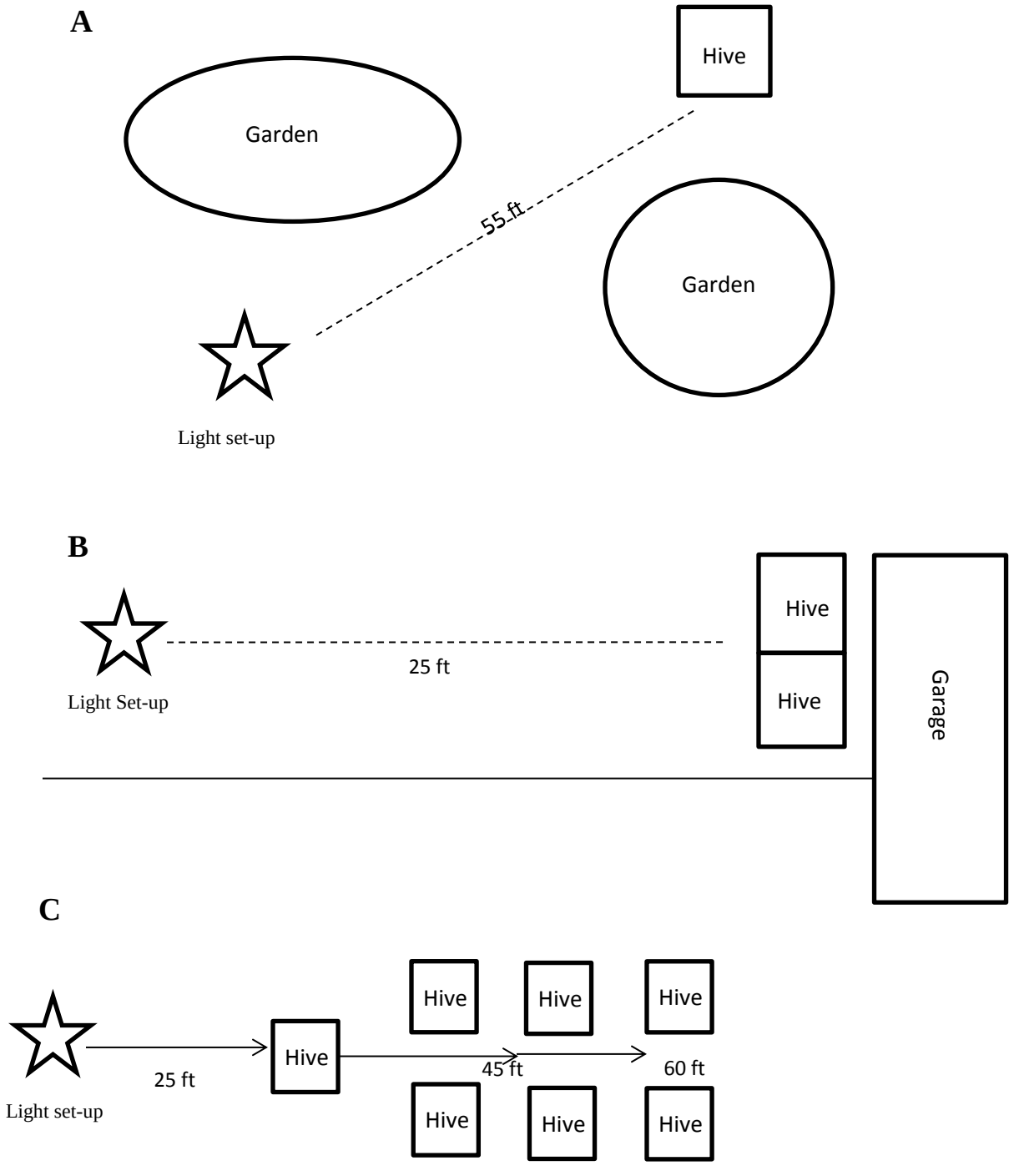


Figure 1. Diagrams of the set-up of the light traps in relation to each of the hives of the hive of the test site. Depicted are Waterwells (A), Reynolds (B) and Moland (C). Lights from streetlamps and houses were present at the Reynolds (B) site.

The lights were turned on as close to sundown as possible every night prior to each collection day and turned off every morning as close to sunup as possible (on average within 24 minutes). This was done for 26 days between the period of August 28th and October 3rd, 2012.

Collection and Observation

Any bees that left the hive during the night and became stranded were found on the tarp the next morning. Those that were dead or in a state of low activity were collected individually into glass vials. Others that just seemed cold and were actively trying to defend themselves when approached were not collected. The vials were labeled, and taken back to the lab for observation. The samples were kept at room temperature and each vial was observed daily for any sign of maggot emergence which would have occurred between 7 and 10 days after collection.

Hive History

The hive histories were taken to determine the age of the hives, any die-offs, re-queenings, and any known previous or current pest infestations.

The hives at the Reynolds and Moland sites shared a similar history. Both were finishing their second summer when sampling began in August. One of the Reynolds hives had been re-queened following a bad winter. Both have had *Varroa* mite infestations in the past and are treated for it twice per year.

The hive at the Waterwells site was completing its first year when sampling began in August. The bees came from a colony in Belfast. There is no history of Varroa mites or other pests but they are still preventatively treated for mites twice per year.

Data Analysis

Data will be analyzed using the Pearson correlation test to determine strengths of relationships and significance of these correlations between variables.

RESULTS: Phorid Fly Investigation

A total of 57 bees in total were collected from the Reynolds and Moland hives. No bees were collected from the Waterwells hive. After observation, which was a minimum of two weeks and up to three weeks for the older samples, there was no sign of maggot emergence. After observation, the bee-containing vials were placed in the freezer at -80 ◦C. These samples were later used to look for the presence of DWV and IAPV.

While no bees were collected from the Waterwells hive, there was one bee found on the trellis one morning. However she only seemed cold as she was able to cling and defend herself. She was not collected and after a few minutes she was able to fly away. This bee was notably different in appearance than the bees collected from the Moland and Reynolds hives as she was fluffier, seemed bigger and was brighter in color.

On two consecutive mornings, 16 and 20 bees respectively were found on the tarp at the Moland site. These occurrences do not correlate with any of the other categories of data that were collected. The relationships between the number of bees observed at the light traps at the time of collection and night duration (Figure 2), night low temperature (Figure 3) and day high temperature (Figure 5) are shown below. Additional data that was collected has been summarized in tables 1 and 2. Table 1 shows the number of times bees were present or absent at the light trap concurring with the previous day's weather conditions. Table 2 shows the change in the time of sunrise and sunset and the average temperature at the time of collection.

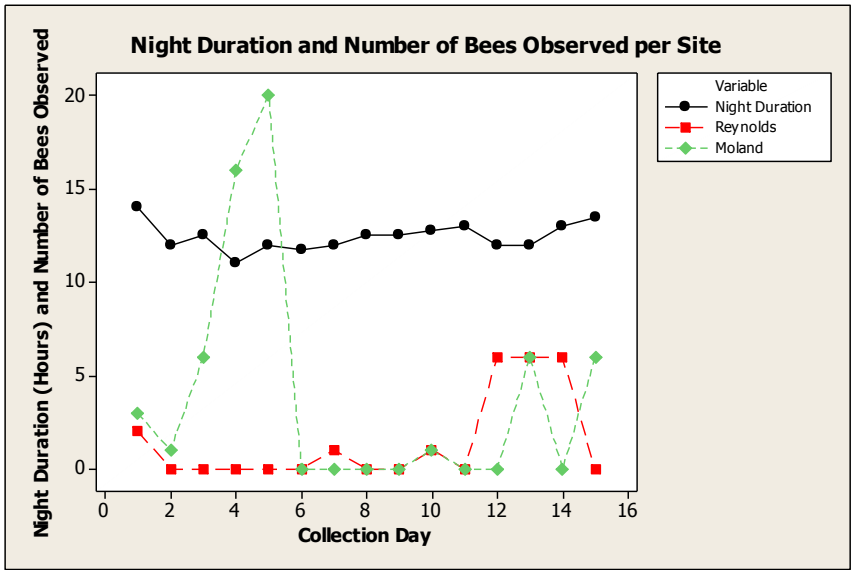


Figure 2. Graph showing the night duration (aka the time the lights were on) for each night of collection and the number of bees observed each collection day at the Reynolds and Moland sites. There is a positive weak relationship between the observed bees at the Reynolds site and duration (0.241) p=0.386. There is a weak negative relationship observed between the Moland site and duration (-0.267) p=0.336.

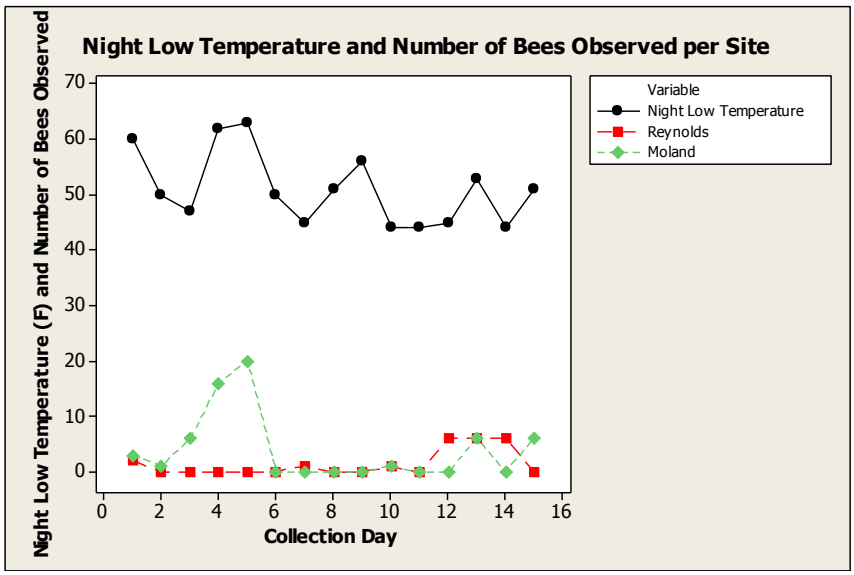
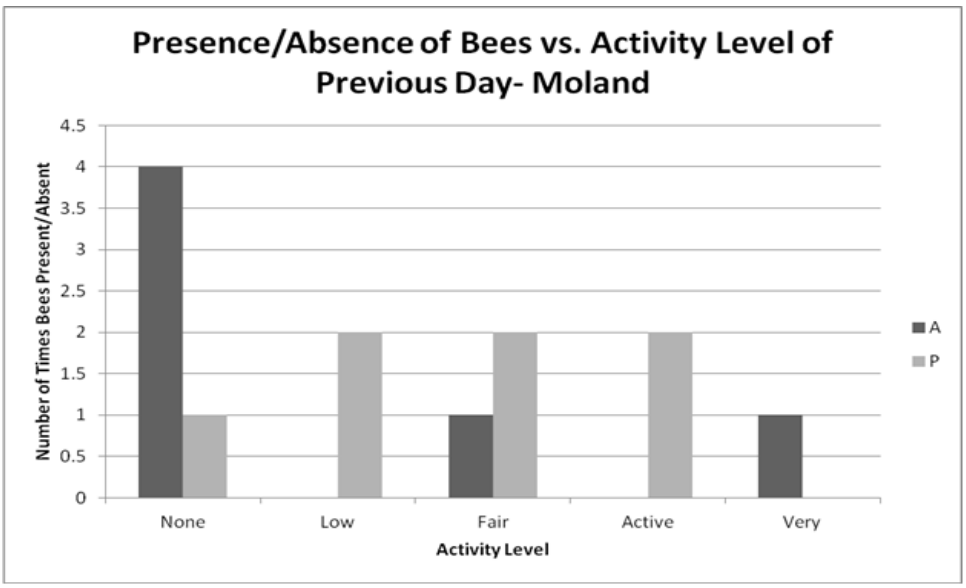


Figure 3. The number of bees observed at the Reynolds and Moland sites plotted against the nighttime low temperature of the night prior to collection. The relationship between the temperature and observed bees for each site was tested for significance. The Reynolds site showed a weak negative relationship (-0.247) (p = 0.375). The Moland site showed a strong positive relationship (0.449), p = 0.093.



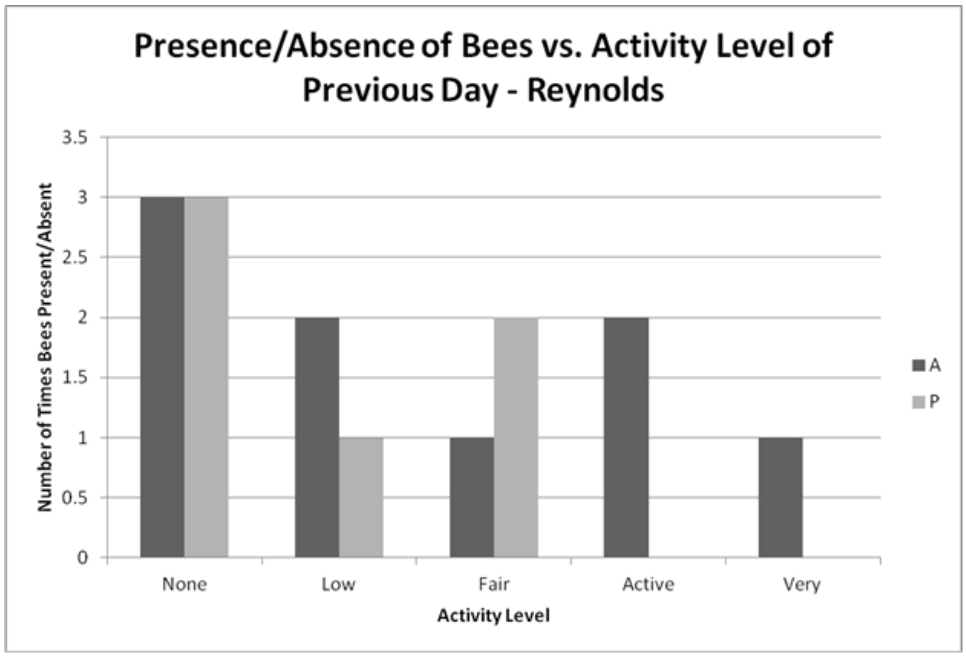


Figure 4. The presence (P) or absence (A) of bees at each site at collection following the recorded hive activity of the previous day. The activity level was determined through visual observation of the hives during the day previous to the morning of collection. "None" refers to no bees seen leaving or entering the hive," Low" refers to a few, "Fair" refers to obvious activity, "Active" refers to audible buzzing and obvious entering/leaving of hive and "Very" refers to extreme activity that stood out from normal hive activity.

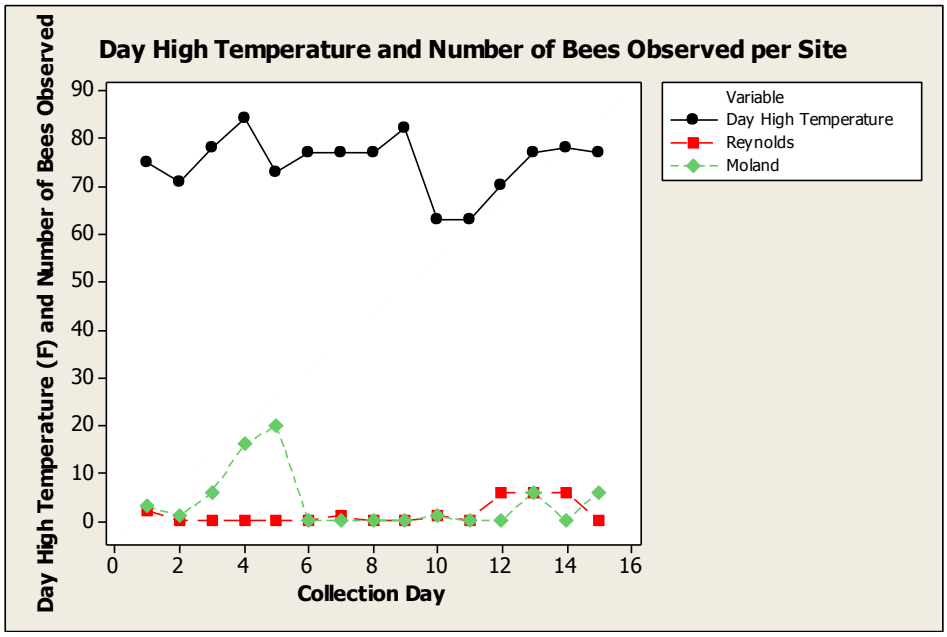


Figure 5. The high temperature of the day previous to collection and the number of bees collected at the Reynolds and Moland sites for each day. The Pearson correlation coefficient of the Reynolds site is -0.028 indicating a weak negative relationship. The p-value is 0.922 which is no significance. The Pearson correlation coefficient of the Moland site is 0.272 indicating a weak positive relationship but by the p-value is insignificant (0.327).

Table 1. Number of times bees were present or absent at time of collection depending on the previous day's weather conditions.

Weather Conditions (n=frequency of occurrence)	Number of Times Absent		Number of Times Present	
	Reynolds	Moland	Reynolds	Moland
Sunny (n=2)	2	1	0	1
Partly Cloudy (n=5)	4	2	1	3
Cloudy (n=7*)	4	2	3	4
Rain (n=2)	0	2	2	0

* Due to electrical problems the Moland site had 15 days of collection while Reynolds had 16. This lost day was cloudy. N=6 for the Moland site.

Table 2. Summary of the rest of the variables recorded. The time of sunup increased by 38 minutes by the end of the study and the time of sundown increased by 63 minutes by the end of the study thus decreasing the length of day (and foraging time) by 101 minutes.

Variable	Notes
Sunup Time	Began at 06:33 and ended at 07:11
Sundown Time	Began at 19:51 and ended at 18:48
Temperature at collection	Average = 59 ˚F

CONCLUSIONS: Phorid Fly Investigation

There were no significant correlations found between the number of bees found and night duration, night low temperature and day high temperature. There does not seem to be a correlation between the presence of bees and the previous day's activity levels or weather conditions.

No bees were found to be parasitized by *A. borealis* following observations determined by the lack of emergence of fly larvae from bees. The following portion of the study focuses on determining the presence or absence of two viruses within the honeybees collected from the phorid fly investigation portion of this study.

METHODS: Virus Investigation

Samples

This portion of the study was carried out to detect the presence or absence of deformed wing virus and Israeli acute paralysis virus in the bees collected from the field portion of this study. The samples were only from the Reynolds and Moland hives as no bees were collected from the Waterwells hive. After collection and observation the samples were stored at -80 C.

RNA isolation

Since these are both RNA viruses, RNA isolation was carried out using a whole bee and TRIzol reagent as per manufacturer's instructions with the following modifications. A high salt solution of 0.8M sodium citrate and 1.2 M sodium chloride was used in a 1:1 ratio with the 100% isopropanol in lieu of the original volume of isopropanol required. This modification was made after it was determined that the first RNA isolation was contaminated with extracellular material and leftover phenol from the isolation. The high salt solution was tested using a cricket as a sample and yielded pure RNA so it was used for all other bee RNA isolations. The bees were weighed prior to homogenization and many were quite a bit below the 80 mg average (30.4 mg) which was likely due to mild desiccation in some samples. The bees were also inspected for any signs of *Varroa* mites and general appearance. No mites were found on the bees or within the vials. Successful isolation of RNA was determined by using a NanoDrop Spectrophotometer. The majority of the samples taken had a 260/280 absorbance ratio within 0.10 of 2.00. 260/280 is the ratio of the absorbance of a sample at each the 260 and 280 nm wavelengths and is used to determine nucleic acid purity. A ratio of approximately 2.00 indicates pure RNA. Absorbance at

the 230 nm wavelength will indicate contamination by either phenol leftover from the isolation or extracellular materials/proteins (Thermo Fischer-Scientific, 2011).

Reverse Transcriptase – Polymerase Chain Reaction

The RNA will be used in Reverse Transcriptase - PCR (RT-PCR) in order to determine the presence of DWV and IAPV. While RT-PCR is usually used to determine what is being transcribed within a genome. This study required the use of RT-PCR to stabilize the viral RNA as cDNA to determine viral presence. The following primers will be used in the reaction:

Table 3. Primers used for detection of DWV and IAPV in bees collected from the Reynolds Rd. and Moland Rd. sites. These primers will be used in the RT-PCR reaction to determine the presence of either of these viruses within the honeybee samples.

Target	Primer Name	Sequence 5'-3'	Source
DWV	DWVf	CTTACTCTGCCGTCGCCCA	(Welch *et al*, 2009)
DWV	DWVr	CCGTTAGGAACTCATTATCGCG	(Welch *et al*, 2009)
IAPV	IAPVf	GCGGAGAATATAAGGCTCAG	(Welch *et al*, 2009)
IAPV	IAPVr	CTTGCAAGATAAGAAAGGGGG	(Welch *et al*, 2009)

The control will be made by using random oligonucleotides instead of a specific honeybee gene primer.

RESULTS: Virus Investigation

Of the 57 samples collected, 20 were removed from freezer storage and their RNA was isolated. The isolation from the first bee was very contaminated with chitin and other extracellular material. All isolations afterwards used the high salt solution so only 19 of the 20 samples will be discussed here. Only those that yielded a 260/280 absorbance ratio between 1.9 and over 2.0 nm will be used in the RT-PCR reaction. Samples with a 260/280 ratio over 2.0 will be considered because high 260/280 ratios are not indicative of an issue (Thermo Fischer-Scientific, 2011).

The 260/230 ratios for "pure" RNA are expected to be lower than the 260/280 nm ratios. Any samples with 260/230 ratios higher than the 260/280 ratios were omitted. This data is summarized in table 4 (Thermo Fischer-Scientific, 2011).

Low 260/230 ratios could be the result of phenol leftover in the sample from the isolation process. A high 260/230 was only seen in one sample and this could be the result of improper cleaning of the pedestal before sample measurement (Thermo Fischer-Scientific, 2011).

Four of the nineteen samples yielded 260/280 nm ratios around 1.8, which indicates DNA isolation. Contamination could have occurred during separation of the aqueous and phenol layers, the later which contains DNA and the former which contains RNA. The other option is that these low 260/230 ratios could be due to leftover phenol from the isolation procedure (Thermo Fischer-Scientific, 2011).

Table 4. The 260/280 and 260/230 ratios for each bee sample from which RNA was extracted. Whether or not the sample will be included in the RT-PCR reaction is noted.

Bee ID	260/280	260/230	Notes
Rey 5	0.15	-0.11	Omitted due to contamination – Pre use of high salt solution
	0.61	-0.77	
Mol 26	2.13	2.11	Included in RT-PCR
Rey 2	2.11	1.13	Included in RT-PCR
Rey 11	1.98	1.14	Included in RT-PCR
Mol 3	2.22	2.19	Included in RT-PCR
Rey 1	2.1	2.04	Included in RT-PCR
Mol 27	1.7	0.38	Trial one; omitted.
	2.09	1.74	Included in RT-PCR
Mol 24	1.98	1.69	Included in RT-PCR
Rey 9	2.08	1.52	Included in RT-PCR
Mol 7	2.03	1.9	Included in RT-PCR
Mol 2	2.13	1.77	Included in RT-PCR
Mol 5	2.05	1.77	Included in RT-PCR
Mol 3	2.22	2.19	Included in RT-PCR
Mol 23	1.8	1.88	260/280 too low. Omitted
Mol 25	2.04	0.63	Included in RT-PCR
Mol 8	1.88	1.73	260/280 too low. Omitted
Rey 12	2.11	2.19	260/230 higher than 260/280
Mol 9	1.86	0.65	260/280 too low. Omitted
Mol 11	2.09	2.25	260/230 higher than 260/280
Mol 10	1.92	1.82	Included in RT-PCR
Rey 13	1.9	1.84	Included in RT-PCR

CONCLUSION: Virus Investigation

This portion of the study is still in progress. At this point RNA has been successfully isolated from fifteen samples that will be used in the downstream RT-PCR reaction. Following this reaction it will be possible to determine whether DWV and IAPV are present or absent from the bees collected from the Moland and Reynolds sites.

DISCUSSION

This study revealed the absence of *A. borealis* infestation of honeybee hives in Alfred, NY. It is acknowledged that the sample sites were small in number (ten hives in three locations) and that the samples themselves only came from nine of these hives distributed across two of the locations. If this study were to be done again it would be necessary to expand the area being tested to surrounding regions of Western New York and thus increasing sample size.

The absence of *A. borealis* is a positive outcome of this study. The absence of *A. borealis* infestation means that there is one less possible threat to honeybee hives in at least this area of New York. If this proves to be consistently true throughout the state it would further support a regional host shift in flies along the West coast because of the infestations seen there and the uniform lack of parasitization of honeybees by *A. borealis* in the East.

For hypothetical purposes the possible implications of *A.borealis* infestation of honeybee hives should be discussed. What are the effects on a hive overall? Is there any way to prevent the spread of this parasite? What can be done about current infestations?

An interesting coincidence was the placement of the Core study. The Phorid fly infestation was discovered in the Central Valley of California – the same area in which the world supply of almonds is grown. These fields attract 60% of all the migratory hives in the U.S. during every pollination season. These same hives then leave California to visit Washington, Maine, the Dakotas, etc., for other blooms. It is completely plausible then that there is a chance the parasite can spread from the Central Valley to other regions through infestation of the hives that visit the almond fields. Current infestations are found along the West Coast and into the Dakotas which may be due to previously undetected parasitism of honeybees by *A. borealis*.

Noting this it is unlikely that migration of hives to CA for almond pollination will be stopped. Almonds are highly dependent on pollination, requiring about two hives per acre. If pollination from migratory hives is cut from areas currently lacking evidence of parasitism there would be a shortage of pollinators. Almond production would most likely drop and food prices would be driven up. There are few other barriers that can be set up to prevent spread. It would be difficult to test hives leaving an affected area but one possibility would be to quarantine trucks for one week after pollination, set up light traps similar to those used in the Core study and observe the bees that are collected. This would be an inefficient and timely process but could help in preventing the spread of this parasite to other regions not yet affected.

Luckily it seems that infestation of a hive alone is not enough to cause collapse of a hive. Core *et al*, (2012) set up a test hive to look at the effects of *A. borealis* on overall hive longevity and even with what seemed like quite a large number of bees collected, the hive remained productive and numerous. What is worrisome are the possible synergistic effects that may be seen if an infested hive is subjected to additional parasites, pesticides or pathogens in an increased state of susceptibility.

An additional implication of the shift from bumblebees to honeybees is significant in that the social structure of these bee species are much different; bumblebees live in small semi-social colonies while honeybees live in extremely large, dense and highly interactive colonies. The increase in potential hosts may lead to a large increase in *A.borealis* population. This coupled with the density of hives in an area, especially during blooms, provides ample opportunity for this parasitoid to disperse. A population boom may follow this dispersal which could lead to larger effects on hives than seen in the Core study (Core *et al*, 2012).

DWV is commonly found in honeybee hives, transmitted initially by its vector *Varroa destructor* then vertically as well as horizontally throughout the hive via food sharing. DWV does not exhibit the characteristic symptoms in the absence of *V.destructor,* which earned it its name. In more severe cases of *Varroa* infestation, the transmission of DWV to larval bees will result in bees with bloated, discolored abdomens and deformed, shriveled wings. These bees die soon after emerging (deMiranda and Genersch, 2010). However, even in cases of low prevalence the virus may have minor effects on individual bees and overall hive health. DWV could be a factor in hive collapse by compromising the bees' immune systems, potentially increasing their susceptibility to other pathogens resulting in a synergistic effect and decline of hive health.

Because hives which have succumbed to CCD have been irradiated and successfully repopulated, it is insinuated that an infectious agent is the responsible party (Cox-Foster *et al*, 2007). This study has led to multiple studies looking at associations between CCD hives and prevalence of different viruses, bacteria and fungi. While some species of these pathogens are more commonly found in beehives with CCD, there are usually no significant correlations. The exception is Israeli Acute Paralysis Virus. IAPV is a virus of the dicistroviridae family that affects bees. Symptoms of infection are shivering wings with a progression into paralysis and eventual death outside the hive (Cox-Foster *et al*, 2007). The symptoms of this virus are not associated with CCD but IAPV could play a role in immunosuppression of the honeybee immune system making the honeybee susceptible to other stressors that in combination will overwhelm the bee. It should also be noted that American beekeepers have selected for strains of bees with less propolis in their hives to make honey more accessible. The problem with the removal of propolis is that with such weak immune systems, one of the hives best defenses has been removed. This opens the honeybee colony to invasion by pathogens (Watanabe, 2008).

While the hives in Alfred are not being menaced by *A. borealis* and do not seem to be compromised by these viruses (assuming presence until study completed), it remains to be noted that there were a considerable number of bees attracted to the light setups. Some mornings 16-20 bees were found on the tarp with no correlations found between the number of bees, temperature of previous days, nights or any other factor that I measured. This presence of a large number of bees could possibly be due to bloom elsewhere and a lot of bees were out that day. The presence could also potentially be due to the time of the year the study was carried out which was in the late Summer/ early Autumn when the foraging behavior of bees is at its highest. Additional possibilities include bees altruistically leaving to die or returning too late from a foraging flight. One specimen was noted to have pollen still attached to its legs during pre-homogenization inspection. This individual was also better-looking overall and weighed a substantial amount more than the majority of the other specimens (66.6 mg).

Additional threats

Outside from parasites and pathogens, pesticides and their implications of CCD are intensely studied. There are hundreds of different chemicals detected in pollen, honey and other substances collected and found in beehives that come from miteicides and agricultural sprayings. A number of studies (Alaux *et al*, 2010; Gregorc *et al*, 2012) have looked at the synergistic effects of pesticides in combination with an array of pathogens. The results of these studies ranged from no effect to colony collapse, colony health decline and loss of navigational ability. A lot of pesticide research focuses on neonicotinoids and formamidines. Use of neonicotinoids in agriculture has been followed with CCD-like disappearances over the years. France began using a neonicotinoid to coat sunflower seeds to protect against pests. This led to the drastic drop in

honeybee colonies referred to as “Mad Bee Disease” shortly after. Germany was using the same pesticide and also suffered major losses. Both countries banned the use of neonicotinoids. The U.S. brought in neonicotinoids in 1994. Corn seeds were treated with doses of the pesticide five times stronger beginning in 2004. In 2006 the first reported case of CCD in the U.S. occurred (Farooqui, 2013).

Neonicotinoid effects on honeybees include a decrease in communication, disorientation, disruption of learning, olfactory memory and navigational ability. This means that while foraging honeybees will not be able to remember flower locations or be able to inform the hive of the location of the food (Farooqui, 2013). It also means that with the loss of olfactory memory, the bee will lose its way back to the hive as they rely on smell to do so. The loss of navigation would explain the lack of dead bees around the hive and the sudden disappearance of only worker bees.

The honeybee immune system is lacking in detoxification enzymes. This susceptibility to the effects of pesticides even in small doses will lead to a weakening of the hive overall, allowing other pathogens and parasites to move in. These synergistic effects between pesticides, parasites and pathogens could, as seen in previous studies, lead to colony collapse (Farooqui, 2013).

Future of honeybees

In face of CCD there are measures being taken to alleviate the effects of known threats and to improve management of hives. Currently there are projects dedicated to developing bee stocks that are resistant to pathogens and parasites, especially *Varroa* mites (CCD Steering Committee, 2010). The honeybee has a weak immune system and while the bee is able to protect itself through individual and social defenses from a wide array of threats, it is hypothesized that due to coevolution between hosts and parasites the development of resistant bee stocks may

compromise bees' ability to defend against other threats (Evans and Spivak, 2010). In addition to breeding resistant stocks, other researchers are focused on improving bee diets, methods of limiting pests and pathogens and preventing the spread of new pathogens by placing guidelines on the importation and exportation of honeybees. There is also research in non-*Apis* pollinators in an effort to prepare for alternative pollinators to replace honeybees (CCD Steering Committee, 2010).

Some people are skeptical that CCD exists, associating the decline in colony numbers with a decline in beekeepers and a normal drop in numbers. While it is true that the number of colonies has dropped with the drop in beekeepers, the reality is that CCD is occurring. Beekeepers are losing massive portions (50-90%) of their hives in a way that is so far unexplainable and completely different than past losses (Farooqui, 2013). Honeybees are responsible for pollinating 60% of alfalfa, 100% of almonds and 90% of apples, grapefruit, cherries and oranges (CCD Steering Committee, 2010). With so many valuable agricultural products threatened by the loss of honeybees, it is imperative that research continues and action is taken. The general public's knowledge of CCD is growing with news frequenting radio talk shows and documentaries. This should prove to be beneficial to honeybee research since the more the public is enlightened on a subject, especially one that could affect their lives in such an impressive and negative way, the more they are willing to support finding ways of fixing the issue.

ACKNOWLEDGEMENTS

I would like to extend my thanks to the following people and organizations:

Dr. Jean A. Cardinale
Dr. Nathan Lamarre-Vincent
Alfred Research Grants for Undergraduate Students
Alfred University Biology Department
Dr. Cheryld Emmons
Dr. Gordon Godshalk
And Dr. Heather Zimbler-DeLorenzo
Douglas Clarke
Tom McDowell
Alec MacCrea and family

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