Emerging Honey Bee Susceptibility to Varroa Mites and Transmission of Deformed Wing Virus

Erica James

Briarcliff High School

Abstract

Various viruses and parasites threaten the health and vitality of honey bee populations. External parasitic mite, the Varroa Destructor, is considered one of the most serious threats to western honeybees because it serves as a vector for bee viruses such as Deformed Wing Virus, DWV. This research focuses on interactions between the Honey bee, *Apis mellifera*, and mites and viruses. Emerging bees and older bees from two different hives were introduced to mites. After recording the number of mites on each bee, to test whether or not emerging bees were more attractive to mites than non-emerging bees, honey bee and mite RNA were extracted and tested for Deformed Wing Virus using PCR. Bees that were younger when placed in the cup had significantly higher levels of mites and of DWV and the study showed a trend toward more DWV for bees with more mites on their body. This research proved that mites did, in fact, serve as a vector for the virus, and that emerging bees were more attractive to mites than older bees, and had higher levels of DWV. This study indicates an effect of Varroa mites on adult bees that might relate to the roles of these mites in Colony Collapse Disorder and bee declines generally.

Table of Contents

Review of Literature

Colony Collapse disorder, first reported by beekeepers in 2006, is an internationally recognized syndrome afflicting honey bees, *Apis mellifera* across the United States, and in countries throughout the world. Its symptoms involve a rapid and unexplainable loss of adult worker honeybees whose dead bodies are not found in or around the hive to which they belong, suggesting that they have left the hive and are unable to find their way back. Another distinguishing characteristic of this phenomenon is that supplies of brood and honey that would normally be taken by an invader to the hive are left untouched and intact, suggesting that the hive gives off some chemical signal to ward off predators. The end of a colony's collapse is marked by the presence of a queen with a severe shortage of worker bees attending her (Cox Foster, 2007). While many theories have been suggested to account for the cause of disorder, a single cause has been difficult to pinpoint because of the widespread nature of the disease. However, one hypothesis involves the possibility that a virus may be working in combination with various stress factors that weaken the bees' immune system making it susceptible to disease (Evans, 2009).

Viruses play a serious role in threatening the health and vitality of this countries primary agricultural pollinator, the honey bee. Viruses pose such a serious threat because without a metabolic system of their own, they are forced to live off a host and control its metabolism. To the day, 19 different viruses have been identified globally that negatively impact the wellbeing of the honeybee (Evans, 2009). The key to understanding the dynamics of these viruses is to understand the modes through which they are transmitted. Viruses are known to be transmitted through either a horizontal or vertical pathway. Horizontal transmission of a virus refers to transmission between members of the same generation. Vertical transmission refers to the

transmission of a disease from one generation to another. Vertical transmission poses a serious threat to the survival of honeybees because within a hive, the queen bee is the primary reproducer: she is the mother of all of the offspring in the hive. If the queen is able to vertically transmit a virus it would potentially be passed down to all of her offspring (Chen and Evans, 2006). In a recent study, transmission dynamics in honeybees were tested using six different honeybee viruses: acute bee paralysis virus, black queen cell virus, chronic bee paralysis virus, deformed wing virus, Kashmir bee virus and sac brood bee virus. Various tissues of individual queen bees and their offspring were examined for these viruses and confirming the potential for vertical transmission in honeybee hives (Chen, 2006).

One specific mode of horizontal transmission that has posed a serious threat to honeybees in the United States since 1987 is indirect transmission of viruses by the parasitic mite *Varroa destructor*. This mite has been detected primarily on adult bees, in sealed brood, and in hive debris. The presence of these mites is difficult to identify because they typically attach to the adults between abdominal segments. Infestation by parasitic mites can lead to a disease known as parasitic mite syndrome, which includes symptoms such as premature death of adult bees and evacuation of adult bees from the hive (Shimanuki, 2000). Kashmir Bee Virus (KBV), one of the most common bee viruses afflicting bees in the United States can be transmitted by the Varroa destructor to bee brood as well as to other uninfected mites. In a study by Chen et al. in 2004, *Varroa destructor* was identified as a key vector of KBV. Both Kashmir Bee Virus and sac brood virus have been detected in the bodies and saliva of mites, demonstrating the co-occurrence of viruses in individual Varroa mites. Multiple infection pathways exist that fall under the category of horizontal transmission (Cox-Foster, 2005).In a study conducted, mite samples were tested using real time PCR and five out of seven bee viruses were identified in the

mites. Varroa mites from developing honeybee pupae and RNA were extracted and examined. This study concluded that several viruses can exist at once in individual mites leading to multiple infections for bee colonies (Chantawannakul, 2006). Multiple virus infections were found in 93% of queen bees when tested for multiple virus infections suggesting the existence of vertical transmission pathways between a queen and her offspring (Chen, Y.P, 2005).

It is extremely challenging to diagnose honeybee viral infections because not only do infections not typically show signs of disease, but honeybees can be the host of multiple viral infections at once. Chen investigated the coexistence of infections in individual bees, testing for the presence of BQCV, DWV, SBV, and KBV. This investigation was the first to prove that a single honeybee can serve as the host of up to four viruses at once (Chen, 2004). This method of quantitative PCR array has proven to be an effective mechanism for investigating various honeybee threats including parasites, pathogens, and viruses. One specific PCR array was developed to transcript levels for honeybee and pathogen genes side by side. This method is used to validate honeybee immune response by assessing genetic aspects to bee immunity. Since honeybees serve as a model for studying disease transmission and immunity in invertebrates, this study has provided insight into the immunity of various other social insect species (Evans, 2006.).

One virus that has been established as closely related to Colony Collapse Disorder is Israeli Acute Paralysis Virus (IAPV). In a Metagenomic survey of microbes in collapsed colonies, and different candidate pathogens were screened for correlation with colony collapse disorder, and IAPV had the strongest correlation with CCD (Cox-Foster, 2007), suggesting a link between viruses and CCD.

One of the most common honeybee viruses in the United States, Deformed Wing Viruses (DWV) is closely associated with colony collapse. It is characterized by wing deformities, bloating of the abdominal region, body paralysis, quickened death of adult bees and is frequently transmitted by the Varroa destructor. It is one of 6 viruses whose genes have been sequenced (Lanzi, 2006). In a study done by Fievant et al, DWV was identified in high concentrations in queen reproductive organs suggesting DWV negatively affects ability of queens to produce offspring, and can enter into eggs before laying. Strands of DWV were also detected in sperm confirming the potential for a sexually transmitted disease (Fievent, 2006). The two most common honeybee viruses, Deformed Wing Virus and black queen cell virus can be accurately and efficiently detected using a one-step real time RT-PCR (Kukiejka, 2008). Kakugo Virus is closely related to Varroa destructor Virus and deformed wing virus and causes aggressive behavior in worker bee populations (Fujiyuki, 2006). This study focuses on the transmission of Deformed Wing Virus by Varroa mites, concentrating on honey bee attractiveness to mites and subsequent transmission of DWV to those bees that were most attractive.

Research Questions

1) Are some bees more attractive than others to mites (and therefore more often exposed to viruses)? Is there any correlation between honey bee age and their attractiveness to Varroa mites?

2) Do mites serve as vectors for Deformed Wing Virus, DWV? Do honey bees with higher levels of mites have higher level of virus exposures than honey bees with lower levels of mites?

3) Do bees with mites on them as adults live a shorter time?

Hypotheses

1. Emerging honey bees are more attractive to Varroa mites than non-emerging honey bees already working in the hive are.

2. Varroa Mites carry Deformed Wing Virus and serve as vectors for DWV. Exposure to honey bees by Varroa mites on adult honeybees leads to higher virus exposure. If hypothesis 1 is proven true, and emerging honey bees are more attractive to mites, they will have higher levels of DWV than non-emerging bees.

Null-Hypothesis: Non-emerging honey bees will be less exposed to Varroa mites and will have a lower level of Deformed Wing Virus.

Experimental Methods

Bee Collection

Honeybees, *Apis mellifera*, kept at the USDA Bee Research Laboratory Apiary in Beltsville, Maryland were used for this experiment. Both emerging honeybees and old honeybees were collected from two different colonies, colony 308 (c308) and colony 316 (c316). Honeybees were marked with the colors designating their colony source and age and both groups of old honeybees were placed in distinct, labeled cups and placed in a freezer to cool down so that they could be marked without stinging the handler. Mites for the experiment were collected

using the powdered sugar method (Shimanuki et. Al, 2000). Hives were sampled for mites and colonies with ample supplies of mites were used. A shake cup was filled with powdered sugar and bees from hives heavily infested by Varroa mites were placed in the cup and shaken over water. Mites in the water were dried, counted, and sorted into Petri dishes, each containing the same number of mites (14 mites for each cage that was being exposed to mites). Cup cages were set up [4 cups with 32 bees each: 8 from c316 emerging, 8 from c308 emerging, 8 from c316 old and 8 from c308 old].

After this initial set up, 14 mites were placed in three out of the four cages; those that were exposed to mites were marked with plus signs. All of the bee cup cages received a vial of sugar water (2:1 sucrose:water w/vol). Cages were placed on a tray and put in an incubator; along with the set up described above, any extra emerging or old bees from either of the colonies were placed in the incubator as well. After one week, bees were observed and the bee, their color, and the number of mites if any on the body were recorded. From each of the set ups 6 bees were chosen. bees of different colors with and without mites were selected from each for an accurate sampling. Tubes were labeled 1 through 48, one tube for each of the bees selected. After selecting the bees, each one was carefully removed from the tube that it was in and checked for mites before being placed into the new tubes that just labeled. If the bee was reported as having no mites, bees were examined for mites again and if the bee was supposed to have mites, they were checked and each mite was found and removed. The removed mites stayed in the original tube while the bee was removed and placed in the new container (this had to be done one set at a time because the samples needed to stay cold to preserve them)

RNA Isolation and Extraction

RNA was extracted from whole adult bees using TRIzol (Invitrogen), with 1 ml of TRIzol added to each bee in individual micro centrifuge tubes, DNA was removed from all extracts, then first-strand cDNA was synthesized as described by (Evans, 2004). Viral and control-gene transcript abundances for these cDNA's were assayed by quantitative real-time PCR with an Icycler real-time PCR machine (Bio-Rad). Primer pairs were designed to amplify 120-300 bp sections of the honey bee 'actin' gene (used as an internal standard for RNA amount) and two honey bee viruses, Deformed wing virus and Kashmir bee virus. Primer sequences were as described in Evans, 2006 and assays were run with a fixed thermal protocol consisting of 5 min at 95^{o}C, then 40 cycles of a four-step protocol consisting of 94^{o}C 20 s, 60^{o}C 30 s, 72^{o}C 1 min, and 78^{o}C 20s was used. Reactions were carried out on 0.5-2 ug cDNA along with 1 U Taq, the provided PCR buffer (Roche Applied Sciences), 1 mM dNTP mix, 2 mM added $MgCl_2$, 0.2 uM each primer, 1X concentration SYBR-Green I dye (Applied Biosystems), and 10 nM fluorescein in a 25 ul reaction volume. Amplification was followed by a melt-curve dissociation program in order to confirm expected product size. Relative viral loads were calculated using the 'dCT' method, by subtracting the fluorescence threshold value for the viral assays from that for the internal control gene (actin).

Statistical Analysis

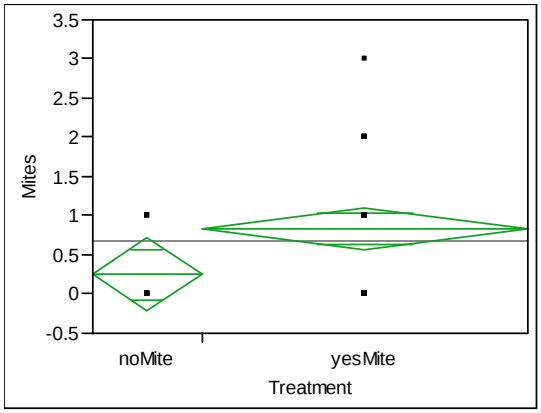
Viral loads were compared across treatments, source bees, and mite presence using the statistical program SAS JMP 7 to invoke analyses of variance (ANOVA) and non-parametric tests (Wilcoxon rank-sum) as appropriate.

Results

Mite attractiveness to bees

This study tested whether certain factors such as Source Colony, or mite age has any factor on honey bee attractiveness to mites. While the study does suggest some correlation between source colony and honey bee attractiveness, as demonstrated by Chart 1, the cause for this difference is open for future research. This study did prove hypothesis 1 to be true by finding a correlation between honey bee age and attractiveness to mites. Younger, emerging bees from both colonies were had higher level of mites than non emerging bees, as demonstrated by Chart 2.

Chart 1: one-way analysis of mites by treatment



There were more mites on bees in cups with added mites (this gives a baseline mite level for 'control' bees).

Chart 1: one-way analysis of mite by source colony

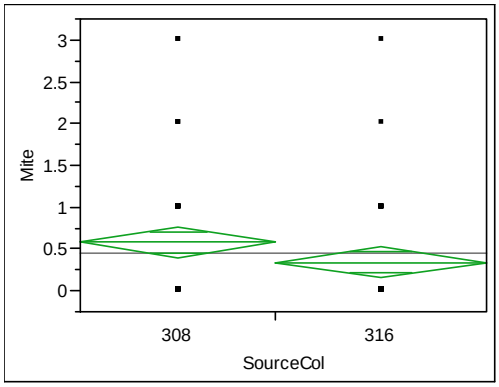


Chart 1 uses a one-way analysis of mite by source colony to show that bees from colony 308 tended to be more attractive to mites than bees from colony 316. This shows a correlation between colony source and mite attractiveness to bees but fails to explain why one colony is more attractive.

Chart 2: one-way analysis of mite by age (emerging vs. non-emerging)

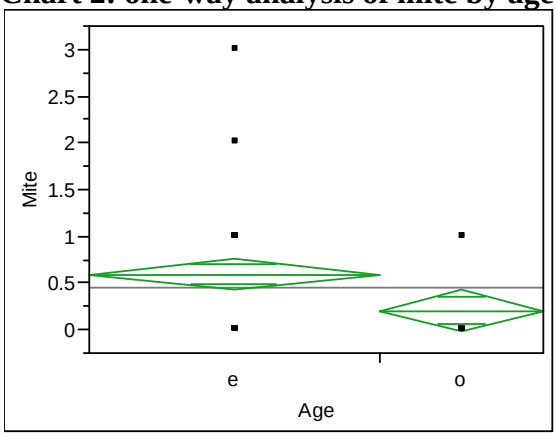
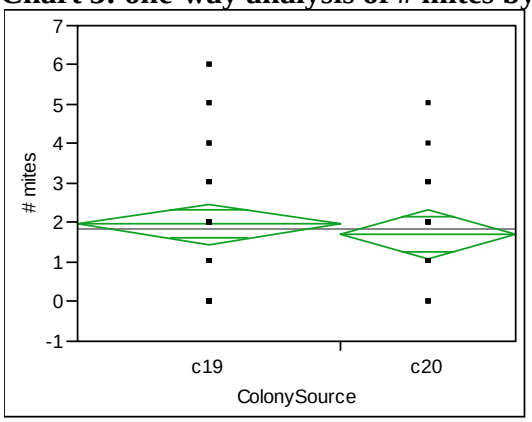


Chart 2 uses one-way Analysis of Mite by bee age to show that Emerging bees were more attractive to mites than older honeybees already in the hive suggesting correlation between Honey Bee age and attraction to mites and therefore susceptibility to honey bee viruses.

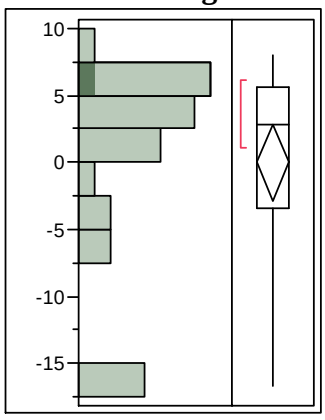
Chart 3: one-way analysis of # mites by colony source



This chart uses one way Analysis of number of mites by colony source to show that there is no significant correlation between colony source in this trial and mite levels. Mites from colony 19 showed relatively the same levels of mite numbers as Mites from colony 20.

Virus levels in mites

Chart 4: Distributions
Deformed Wing Virus



Quantiles

100.0%	max	8.06
99.5%		8.06
97.5%		8.06
90.0%		6.94
75.0%	quartile	5.60
50.0%	median	2.79
25.0%	quartile	-3.40
10.0%		-16.76

Quantiles

2.5%		-16.76
0.5%		-16.76
0.0%	minimum	-16.76

Moments

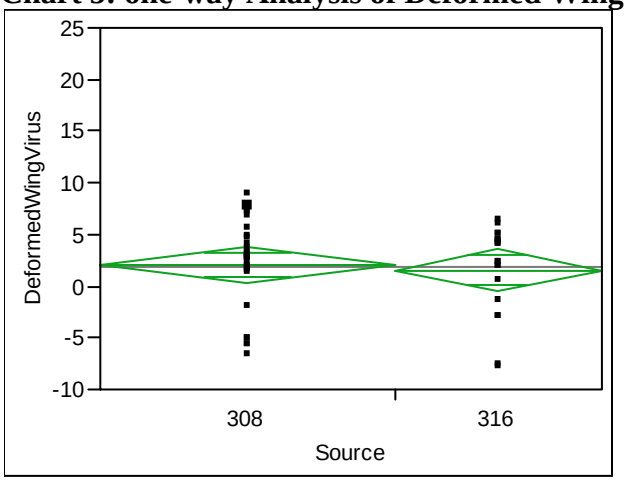
Mean	0.003
Std Dev	7.6648338
Std Err Mean	1.3994008
upper 95% Mean	2.865096
lower 95% Mean	-2.859096

The mites used for these experiments carried DWV; about 85% had at least some DWV, suggesting that they carried DWV and subsequently transmitted it to the honeybees that they were attracted to.

Virus levels in bees exposed to mites

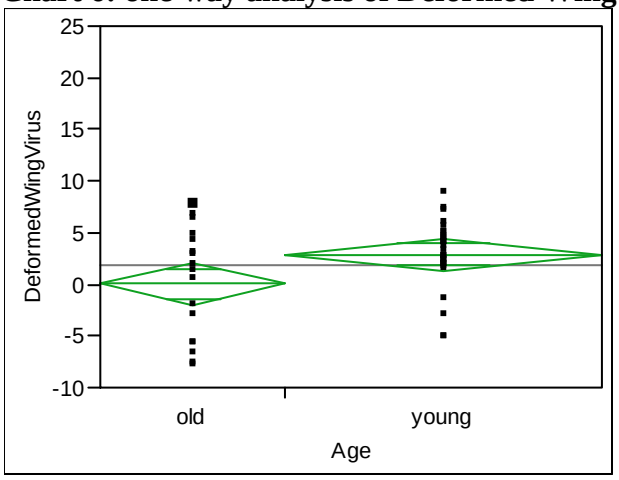
The charts above indicated that the mites did in fact carry high levels of deformed wing virus and subsequently served as a vector between DWV and honey bees, proving hypothesis 2 to be correct, and strengthening proof for the link between Varroa mites and virus transmission. These charts demonstrate whether this information would tend to affect certain bees above others. In a survey for the correlation between DWV levels and colony source, bees from different source colonies did not a show a statistically significant difference in DWV levels as demonstrated by Chart 5. However, Chart 6 shows that emerging bees showed a higher affinity for DWV along with the higher attractiveness to Varroa mites demonstrated above. Chart 7 links all of these results together by confirming the trend that bees with more mites also had more DWV.

Chart 5: one-way Analysis of Deformed Wing Virus by Source



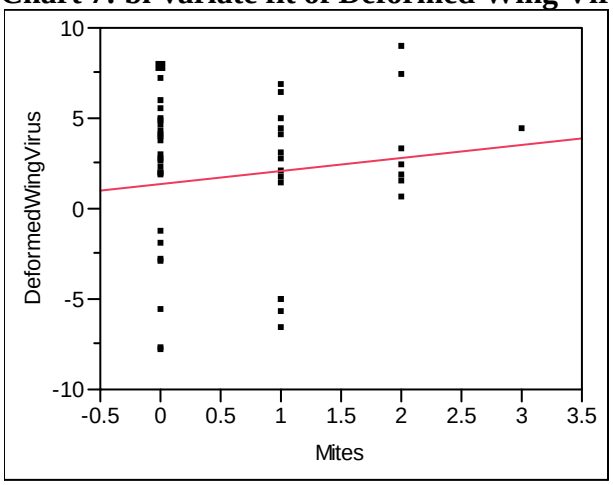
Bees from different source colonies did not differ in DWV levels. As demonstrated by this one-way Analysis of DWV by source, the level of DWV from source colony 308 did not differ significantly from those of 316.

Chart 6: one-way analysis of Deformed Wing Virus by Age

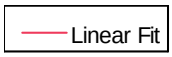


Bees that were younger when placed into the cups had significantly higher levels of DWV at the end of the study. Since they also had higher levels of Varroa mites, one can conclude that the Varroa mites served as vectors for DWV which is why the emerging bees tested for higher levels of the virus.

Chart 7: bi-variate fit of Deformed Wing Virus by mites



There was a trend toward more DWV in bees with more mites on their bodies, suggesting transfer of DWV by mites on adult bees. According to this Bivariate fit, levels of DWV are statistically correlated to levels of mites, demonstrated by the linear fit in the graph above. These results confirm the major hypothesis that feeding by Varroa mites on adult honeybees leads to higher virus exposure.



Linear Fit

Deformed Wing Virus = 1.355773 + 0.7151184*Mites

DISCUSSION

Bee health is impacted by a wide variety of negative factors ranging from environment, nutrition, parasites and viruses. This research focused on the negative impact of *Varroa* mites on honeybees and the correlation between mite levels and subsequent levels of Deformed Wing Virus. This research provides insight into which types of bees if any are more attractive to mites and whether this higher level of attraction means a higher susceptibility to DWV. Since it is recognized that *Varroa* mites are effective vectors of viruses (Shen et al. 2005), especially DWV, this research confirmed prior research by finding that bees with increased numbers of mites had higher levels of DWV than bees with fewer mites, confirming the suggested link between mites and viruses. However, this research also looked into the *Varroa* mite as a vector by studying whether or not source colony and bee age are correlated with attractiveness to mites. While results showed no significant correlation between colony source and mite levels, this research shows that emerging honeybees were more attractive to mites than bees already working in the hive, and the younger bees also had higher levels of DWV. This result indicates that younger bees are at a higher risk for viral exposure than are older bees.

The fact that emerging bees are more attractive to mites than older bees poses a great risk for colony health because this means that there is a high likelihood that bees will be exposed to viruses right from their emergence, weakening the health of new worker honeybees. During the summer, bees typically live for 30 days, the last half of which is spent flying to collect food and defend the nest. More critically, bees produced toward the end of the fall must survive over an entire winter in temperate areas (5 months in Maryland), before emerging to forage in the spring and provisioning a new cohort of spring bees. When mite levels are high in the fall, these bees are likely exposed to mites both within their development cells (honeycomb) and as adults. This

experiment clearly suggests that adult exposure to mites can increase the risk of viral infection, a result that has been shown previously for younger bees (in the pupal stage, e.g., Chen et al., 2006).

In our first experiment, the bees derived from one colony showed higher mite levels than did bees from a second colony during these cup trials. This does not reflect their initial mite loads, since bees entering the study were mite-free, but does suggest that mites find particular bees more attractive than others. Alternatively, particular bees might be better able to clean their bodies of mites (hygienic behavior, Evans and Spivak, 2009). For honeybees, hygienic behavior consists of the response by adult honeybees to the presence of virus, disease, and parasites in brood within the hive, and especially a defense mechanism against Varroa mites (Peng et al., 1979b) which could offer an explanation as to why one colony may have been more vulnerable to Varroa mite infestation than another.

Research Limitations and Future Research

This study suggests a clear link between the presence of mites in colonies and susceptibility to viruses and it indicates an effect of Varroa mites on adult bees that might relate to the roles of these mites in Colony Collapse Disorder and bee declines generally. Limitations to this research included time constraints and sample pool. Future date could include samples of honey bees from more than two different hives, with more bees and mites in each bee cup cage for a larger sampling. Ongoing experiments will aim at the transfer of other viruses, such as Kashmir Bee virus or Israeli Acute Paralysis virus, by mites to adult bees, and the effects of these mites and their viruses on bee survival.

Conclusion

This study demonstrates possible implications of virus transmission within honeybee hives and its relationship to Colony Collapse Disorder suggesting a correlation between honey bee age, vulnerability to mites, and subsequent susceptibility to viruses. Finding whether or not certain honey bees are more attractive to mites than other honeybees may be the first step towards determining how to protect these bees from these health-threatening challenges which is important today because Colony Collapse Disorder threatens the survival of the international Agriculture system and the economy.

Acknowledgments

I would like to thank my mentor, Jay Evans and the US Department of Agriculture Bee Research lab for guiding me in my research and providing me the opportunity to conduct my research. I would also like to thank Mr. Inglis and Ms. Dyer for their guidance and support for the past three years. I would especially like to thank my parents for their support of my scientific endeavors.

Bibliography

Chen, Y.P., Pettis, J.S., Collins, A., Feldlaufer, M.F.(2006). Prevalence and Transmission of Honeybee Viruses. Applied and Environmental Microbiology 606-611

Chen, Y.P, Evans, J., Feldlaufer, M. (2006). Horizontal and Vertical Transmission of Viruses in the Honeybee, Apis mellifera. Journal of Invertebrate Pathology 92, 152-159

Chen, Y.P. et al. (2004). Transmission of Kashmir bee virus by the ectoparasitic mite *Varroa destructo*.Apidologie 35. 441-448

Chen, Y.P., et al. (2004). Multiple virus infections in the honeybee and genome divergence of honeybee viruses. Journal of Invertebrate Pathology. 84-93

Chen, Y.P., et al. (2005) Detection of multiple viruses in queens of the honeybee Apis mellifera L. Journal of Invertebrate Pathology. 90, 118-121

Chen, Y.P., et al. (2009) Effects of Host Conditions on Susceptibility to Virus infection in Honey Bees, Apis mellifera. Submitted to Insect Molecular Biology

Chantawannakul, P., et al. (2006) A Scientific note on the detection of honeybee viruses using real time PCR (Taqman) in Varroa mites collected from a Thai honeybee apiary. Journal of Invertebrate Pathology. 91, g9-73

Cox-Foster, et al. (2007). A Metagenomic Survey of Microbes in Honey Bee Colony Collapse Disorder.

Evans, J. (2006). Beepath: An ordered quantitative-PCR array for exploring honey bee immunity and disease. Journal of invertebrate Pathology. 135-139

Evans, J. and Spivak, M. (2009): Socialized Medicine: Individual and Communal Disease Barriers in Honey bees. Journal of Invertebrate Pathology.

Evans, J. (2009). Unpublished Project summary 2009-2013

Fievet, J., et al. (2006). Localization of deformed wing virus infection in queen and drone Apis mellifera. Virol J. 3-16

Fujiyuki, T., et al. (2006). Prevalence and Phylogeny of Kakugo Virus, a Novel Insect Picorna-Like Virus that Infects the Honeybee, under Various Colony Conditions. Journal of Virology. 11528-11538

Kukielka, D, et al. (2008) A sensitive one step real time RT-PCR method for detection of deformed wing virus and black queen cell virus in honeybee Apis mellifera. Journal of Virological Methods 147, 275-281

Lanzi, G., et al. (2006). Molecular and Biological Characterization of Deformed Wing Virus of Honeybees. Journal of Virology. 4998-5009

Shen, M., et al. (2005). Intricate transmission routes and interactions between Picorna-like viruses with the honeybee host and the parasitic Varroa mite. Journal of General Virology, 86, 2281-2289

Shimanuki, H., and Knox, D. Diagnosis of Honey Bee Diseases. Agriculture Handbook Number 690