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PREVALENCE OF *WOLBACHIA PIPIENTIS* AND *VARROA DESTRUCTOR* MITES IN AFRICANIZED BEES IN THE DEMING, NEW MEXICO AREA

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Abstract: Africanized Honeybees (AHB) were colonized in New Mexico by 1992. Since then, they have virtually replaced Western honeybees in Luna County, which is adjacent to Mexico's Northern border. AHB are hybrids between *Apis mellifera scutellata*, an African honeybee, and *A. m. ligustica, A.m.iberiensis,* which are both Western honeybees. *Wolbachia pipientis* is a Rickettsial endosymbiont bacterium that infects arthropods and nematodes. *W. pipientis* infestation can manipulate the reproduction of arthropods causing cytoplasmic incompatibility, feminization, parthenogenesis, sterilization, and male killing which decreases the number of progeny and skews the male/ female ratios in arthropod populations. *Varroa destructor* mites also impact honeybee populations by spreading viral infections such as the deformed wing virus (DWV). Honeybees, Africanized or not, are the main pollinators of many crops grown in Southern New Mexico and are suppliers of honey, royal jelly, wax, and bee venom. A decline in honey bee populations impacts the entire agricultural industry of New Mexico. In this study 88 AHB were tested for *W. pipientis* and 92 AHB were inspected for *V. destructor* mites. Twenty-six percent of the bees were infected with *W. pipientis*, which is 50% less than the national Western honeybee infection rate. Plus, none of the AHB were infected with *V. destructor* mites compared with an estimated 80% of Western honeybees infected in the U.S. This research also proposes that hybrid vigor or heterosis may be a factor in the difference between the infection rates.

Keywords: *Wolbachia pipientis,* Africanized Honey Bees (AHB), *Varroa destructor,* heterosis.

INTRODUCTION

The Western honeybee industry was an integral part of Southwest New Mexico. Honeybees have a significant influence on the honey, wax, royal jelly, and bee venom industries (Dukku, 2016). The honey industry alone is worth over 150 million dollars annually. The top 20 honey producing countries in 2011 combined produced approximately 1.26 million metric tons of honey estimated at 3.16 billion dollars (Dukku, 2016). Also, honeybees pollinate approximately 80% of angiosperms (Calderone, 2012). In 2009, the U.S. alone benefitted from 11.68 billion dollars' worth of crops pollinated by the honeybee subspecies (Calderone, 2012; Dukku, 2016). For example almonds, a popular crop in America, depend solely on

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honeybees for pollination (American Bee Keeping Federation, 2018) which led to $5,468,040,000 production in the almond industry (United States Department of Agriculture, 2018). However, problems have plagued the honeybee industry, which solely depends on Western honeybees. From October 1, 2018 to April 1, 2019 it is estimated that 37.7% of Western honeybee colonies in the United States were lost due to Colony Collapse Disorder (CCD). This is an increase of 7% compared to the previous year's losses (Bruckner, et al., 2019). CCD and its effects on the Western honeybee population began to be identified in 2006 along with some of the hypothesized causes (Environmental Protection Agency, 2018). The main trait of CCD is rapid loss of adult worker bees. Despite research, a single variable has not been identified as the cause, but it has been determined that collapsed colonies have been exposed to a greater number of pathogens and insecticides than non-collapsed colonies (Vanengelsdorp, et al., 2009). *Varroa destructor* mites are thought to be a major vector of viruses associated with CCD (Moore, Wilson & Skinner, 2014). Western honeybees can be plagued by a host of viral, bacterial, fungal and insect pathogens. In 2018, 56.4 % of operations with 5 or more Western colonies were infected with *V. destructor* mites (United States Department of Agriculture, 2019).

Africanized honey bees (AHB) entered the United States in 1990 and have been spreading through the southern states ever since. They were created in 1956 by two geneticists in Brazil. The objective was to produce a gentle, highly-productive honeybee. The geneticists crossed an aggressive African honey bee, *Apis mellifera scutellata,* known for being highly-productive with two gentle Western honey bees, *A. m. ligustica*, and *A.m.iberiensis*. However, the cross did not produce the desired effects (Winston, 1992). The 26 queen bees that were produced were more aggressive then the African bee, but they did produce more honey than the Western bees. Unfortunately, they were accidently released into the environment and began mating with Western drones (Winston, 1992). AHB moved into Luna County in 1992, killed, mated with or replaced the European bees and took over the hive areas. As a result, all the honey bees in Luna County are AHB (Blandford, 2019; Sutherland, 2019). The introduction of AHB into Luna County has caused a steep decline in beekeeping. Beekeepers often found AHB too difficult to handle (Blandford, 2019; Sutherland, 2019). The quick takeover of potential hive areas by AHB is due to several characteristics. AHB are slightly smaller, produce more progeny, mature quicker and have a shorter life span then the Western bees resulting in rapid population growth (Tribe & Fletcher, 1977). During mating rituals, there are simply more AHB drones to mate with the queens. DNA studies have

shown that the undesirable African traits tend to be dominant over the more desirable Western traits (Eimanifar, Brooks, Bustamante & Ellis, 2018). The undesirable traits include swarming excessively, absconding the hive quickly, usurping other bee colonies, and excessively defending their hives (Eimanifar, Brooks, Bustamante, & Ellis, 2018). AHB's reputations as high honey producing bees comes from their ability to produce more honey when growing conditions are poor (Winston, 1992), which may be an advantage in a time of climate change.

W. pipientis is known to be a master manipulator of insect reproductive systems in order to enhance its own spread and survival (Weeks & Breeuwer, 2001). Cytosine methylation is one type of epigenetic reversible DNA modification attributed to *W. pipientis* infection. A study in 2013 found ~1000 *Aedes aegypti* genes of infected specimens were methylated and exhibited reduced transcription. These genes are highly conserved and are present in honeybees along with aphids (Ye, et al., 2013). Methylation is one method that *W. pipientis* is thought to use when modifying reproduction in insects (Saridaki, et al., 2011). Most female producing parthenogenesis host species are characterized by haplo-diploid sex determination. *W. pipientis* is the most frequent parthenogenesis inducing endosymbiont to date (Ma & Schwander, 2017). It is also capable of providing some protection to the host against some other pathogens (Newton, et al., 2016). Additionally, *W. pipientis* infection inhibits mosquitoes from transmitting certain viruses such as Dengue, Chikungunya, Yellow Fever, West Nile plus it inhibits *Plasmodium* in filarial nematodes. Interestingly, most filarial nematodes have a mutualistic symbiosis with *W. pipientis*. They require *W. pipientis* within their tissues for fertility, development and survival (Slatko, Luck, Dobson, & Foster, 2014). *W. pipientis* can be transmitted both vertically, from mother to offspring, and horizontally, through feed or cannibalism (Ruiz-Guzman, Ramos-Castaneda, & Hernandez-Quintero, 2016). Since 1997, the *W. pipientis* infestation rate in insects has gone from 17% (Werren, Winsor, & Guo, 1995) to 67% **(**Hilgenbocker, Hammerstein, Schlattmann, Telschow, & Werren, 2008**).** *W. pipientis* uses different reproductive manipulation on select species. The object of reproductive manipulation is to ensure the optimal spread of the endosymbiont. *W. pipientis* is spread through the maternal line, therefore, reproductive manipulation that decreases or eliminates the male progeny are to its advantage. There are two sub-groups of *W. pipientis*, type A and B, which diverged 58-67 million years ago (O'Neill, Gordiano, Colbert, Karr, & Robertson, 1992). Feminization is one reproductive manipulation strategy of *W. pipientis.* Two examples of feminization are documented in a few species of isopods

and butterflies. *W. pipientis* manipulates the reproductive chromosomes in males to become full-functioning females (Juchault, Rigaud & Mocquard, 1992; Kageyama, et al., 2017). Beings *W. pipientis* manipulates the reproductive strategies differently depending on the arthropod species, more research is needed to exactly pinpoint which species undergo feminization.

W. pipientis is transmitted through the cytoplasm. Cytoplasmic incompatibility is thought to be a post-fertilization modification of the paternal genome that renders embryos inviable or unable to complete diploid development between crosses of infected males and uninfected females or infected females and males of a different strain (Bordenstein & Werren, 2007). There are two types of cytoplasmic incompatibility (CI), bidirectional and unidirectional. In unidirectional CI, if the male is infected and the female is not, CI occurs and there are no progeny. In bidirectional CI, if the male and female are infected with two different strains of *W. pipientis*, there are no progeny. In diploid organisms, CI leads the embryonic mortality. In haplodiploid organisms, CI usually results in haploid progeny (Bordenstein & Werren, 2007). Sperm are modified in the testes by *W. pipientis* which results in abnormal processing after fertilization if the appropriate *Wolbachia* strain is not present to rescue the sperm (Werren, 1997). In *Nasonia vitripennis,* a parasitoid wasp, CI is caused by the delayed breakdown of the nuclear envelope in the male pro-nucleus resulting in asynchrony between the male and female pro-nuclei (Tram & Sullivan, 2002). The culmination of these reproductive manipulations results in skewed male/ female ratios of arthropod populations.

V. destructor, an ectoparasite infecting honeybee colonies, serves as a vector for many viruses. The exact mechanism for the transfer of viruses is not known, however, the mites are suspected of host immunosuppression and viral amplification (Yang & Cox-Foster, 2005). It is estimated that 80% of the Western bee hives are infected with *V. destructor* (Moore, Wilson, & Skinner, 2014). The mites reproduce on the honeybee brood cells, feed on the hemolymph of the pupa, and spend most of the life in the colony (Moore, Wilson, & Skinner, 2014). Africanized bees show tolerance to *V. destructor* for several reasons. In Western honeybees, *V. destructor* reproduces in both worker and drone brood cells causing an increasing mite population that ultimately kills the colony. Whereas in AHB, *V. destructor* only reproduces in the worker brood cells thus limiting population growth. Therefore the population of mites never reaches a threshold that would kill the colony (Martin & Medina, 2004). Other factors contributing to AHB tolerance and/or resistance to *V. destructor* are their smaller brood cells, the absconding rate of AHB colonies, plus the hygienic and

grooming behavior of the bees. The duration of the location of an AHB colony is much shorter than that of a Western honeybee colony thus not giving the mites time to overpopulate in a colony. Then, AHB clean both their hives and their bodies by removing mites and dead or virus infected bees more often than Western honeybees (Gebremedhn, Amssalu, De Smet, & de Graaf, 2019).

Heterosis or hybrid vigor occurs when two genetically distinct organisms produce offspring that are superior in disease resistance, biomass, stature and fertility. Inbreeding or crossing two organisms of similar or like genetic make-up increases the probability of homozygosis. This, in turn, leads to the expression of recessive traits. Many undesirable traits are recessive. Outbreeding leads to heterozygosis which decreases the probability of undesirable traits being expressed (Bircher, Yao, & Chudalayandi, 2006).

MATERIALS AND METHODS

Capturing AHB

Proper safety measures were observed during specimen collection. AHB are known to be very aggressive if their hives are approached. The bees were captured away from hives limiting the threat of swarm attacks. Proper attire was worn. Fifty mL centrifuge tubes and insect nets were used to capture the bees. The tubes were labeled with the location and date. The specimens were frozen until screening for *V. destructor* mites and *W. pipientis*.

V. destructor Screening

V. destructor screening was performed under a dissecting microscope by carefully examining the areas between the sclerites where the mites normally reside. The AHB were examined for mites before being screened for *W. pipientis.*

DNA extraction and PCR protocols

Two millimeters (mm) were removed from the specimen's abdomen. The abdominal segment was then placed in a 1.5 milliliters (mL) microfuge tube with 200 microliters (µL) of lysis buffer. The abdominal segment was macerated for 1 minute. Eight-hundred µL of lysis buffer was added to the microfuge tube then vortexed. The tube was placed in a 99°C water bath for 5 minutes. After heating, the tube was opened briefly to release pressure then centrifuged for 8 minutes at 8900 rpm. Another microfuge tube was obtained and 400 µL of the supernatant and put into the new tube. Forty µL of 5.0 M NaCl was added and placed on ice for 5 minutes. Tubes were placed in the centrifuge at the same voltage and time as previously stated. Another clean microfuge tube was obtained and 300µL of supernatant was transferred. Four-hundred microliters of isopropanol was added and then centrifuged at 8900

rpm for 8 minutes. The supernatant was carefully poured out and the mouth of tube was tapped lightly to remove most of the liquid. The tube was centrifuged for 1 minute and the rest of the liquid was pipetted out. The pellet was air dried for 10 minutes. Two-hundred of TE/RNase was added. The pellet was disturbed by pipetting then tube was centrifuged at 8900 rpm for 1 minute. The DNA was frozen until PCR amplification.

PCR amplification was done with a Biorad thermocycler t100. PuReTaq™ Ready-To-Go™ PCR beads were used. The DNA was thawed. Twenty microliters of *wsp* primer was added to the PCR bead along with 5 µL of extracted DNA. PCR cycles included 95 degrees for 2 minutes, 30 cycles of: 94 degrees for 30 seconds, 55 degrees for 45 seconds, 72 degrees for 1 minute, then 72 degrees for 10 minutes, and finally left at 4 degrees for the rest of the allotted time.

One point two percent agarose electrophoresis gels were run at 100 V for 30 minutes. SYBR safe green loading was used with lithium bromide buffer. A pearl biotech DNA illuminator was used to view the DNA.

RESULTS AND DISCUSSION

The present study was conducted to ascertain the differences in infection rates of *W. pipientis* and *V. destructor* in Africanized honeybees (AHB), which are hybrids, as opposed to Western honeybees, which tend to be purebreds, due to the problems the United States is having with Colony Collapse Disorder (CCD). The national infection rates of *W. pipientis* in insects and *V. destructor* in Western honeybees are 67% (Hilgenbloeker et al., 2008) and 80% respectively (Moore, Wilson, & Skinner, 2014). This study revealed that the infection rates of AHB in the Deming, New Mexico area are 26% and 0% respectively.

Table 1. Comparison of Infection Rates between AHB and Western Honeybees

Infectious Agent	Percentage of Africanized Honeybees Infected in Deming	Percentage of Western Honeybees Infected in the United States
W. pipientis	26%	67%
V. destructor	0%	80%

Table 2. Percentage of AHB infected with *Wolbachia* in the Deming, NM Area

Total Number of AHB Captured In Each Area		Number of AHB Infected	Number of AHB not Infected	Percentage of AHB Infected
Southwest	37	9	28	24%
Northwest	2	1	1	50%
South	17	7	10	41%
North	10	4	6	40%
Southeast	22	2	20	9%
Total	88	23	65	26%

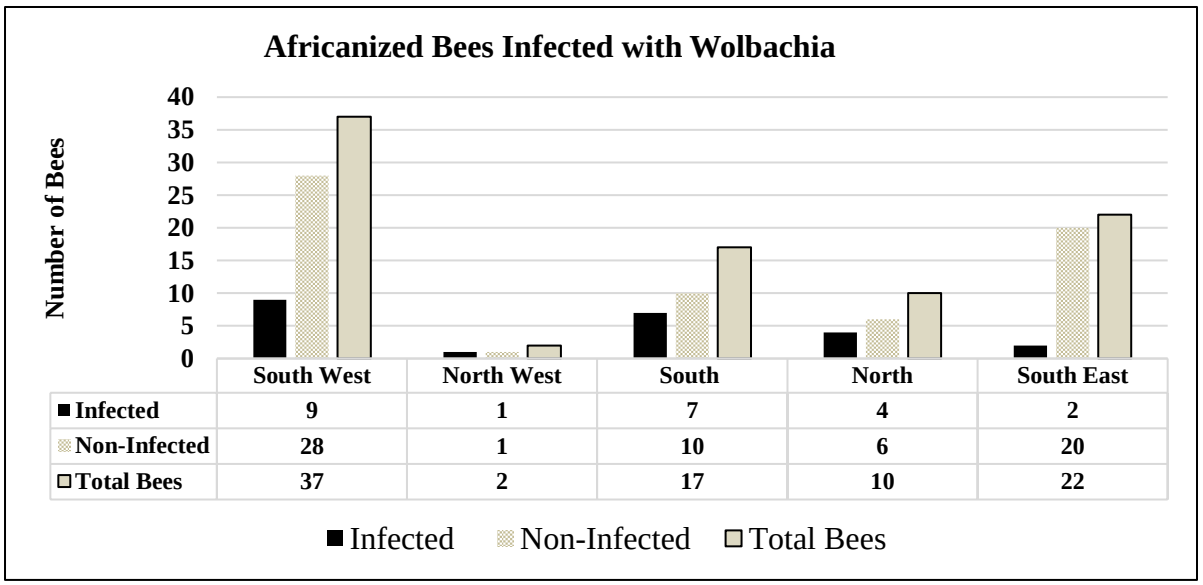

Figure 1. Bar graph of *Wolbachia* infection rate of AHB in five areas of Deming, New Mexico

The Deming area was divided into five areas depicting the highest to lowest concentration of angiosperms. Logically, more bees were captured in these areas with the higher density of flowering plants. Luna County is located in the Chihuahuan Desert therefore the surrounding area of the City of Deming have a lower concentration of vegetation. Domestic gardens and field crops are abundant in the city. Our results show a significant difference between the infection rates of Western honeybees and AHB. Comparing the infestation rates of *V. destructor* mites between the two populations (table 1) none of the AHB captured were infected with mites as compared with the literature published values of 80% for Western honeybees. In reference to this, the AHB hives were not examined. The bees are too aggressive. However, if there is a mite infestation in the hive, normally some adult bees will

be infected and no evidence of this was found. Some reasons for the discrepancies between the two mite infestation rates could possibly be (1) AHB have smaller brood cells allowing for less room for mite occupation, (2) AHB abscond their hive faster than Western bees giving mites less time to propagate and develop a population that would lead to Colony Collapse Disorder, and (3) most importantly AHB are hybrids which gives them the benefit of hybrid vigor or heterosis. Hybrids have a higher probability of being heterozygous for many traits. Homozygosis leads to the expression of recessive traits which can tend to be undesirable. Due to this, hybrids tend to be resistant to diseases, pathogens, and infections of parasitic organisms. Another factor influencing the lack of *V. destructor* mites in the Deming area is the environment. *V. destructor* mites prefer a humid, less elevated environment. Deming, being in the Chihuahuan desert, is quite arid and has an elevation of 1321 meters.

Eighty-eight AHB were tested for *W. pipientis* and only 23 were infected (table 1). Ninety-two AHB were inspected for *V. destructor* mites and none of them were infected. *Wolbachia* is known to be a reproductive manipulator of insects causing the increase of female offspring which amplifies the spread of *Wolbachia*. Honeybee hives are predominantly female with a ratio of 1 male to 100 females therefore the danger of *Wolbachia* in honeybees is not the reproductive manipulation but through the spread of *Wolbachia* via cannibalism and parasitic transfer. A correlation has been made between increased infection rates of parasites and Colony Collapse Disorder. Presently, CCD is an ongoing problem in the United States. No documentation has been found of CCD in AHB, however, AHB have been treated as pests. This topic definitely deserves more research. In light of climate change, the resilience AHB seem to have may be an advantage.

Beekeepers in the southern regions of the U.S. are coming to the realization that they must adapt to the reality that the presence of AHB is not going to change. Suggested management techniques for AHB include (1) isolating the hives so they are no threat to livestock or the public, (2) the hives should be kept 200 to 300 m from roads, agricultural fields and dwelling often behind fences or vegetation, (3) hives should be well separated, (4) beekeepers should wear protective clothing and use ample smoke to calm the bees, and (5) hives should be worked quickly and infrequently (Winston, 1992).

References

[1] American Bee Keeping Federation. (2018). *Honey Bees are Pollinators*. Retrieved August 2019, from American Bee Keeping Federation: abfnet.org/page/Pollinator/ Facts

[2] Birchler, J., Yao, H., & Chudalayandi, S. (2006). Unraveling the genetic basis of hybrid vigor. *Proceedings of the National Academy of Sciences, 103*(35), 12957-12958.

[3] Blandford, J. (2019, October 11). County Program Director of Agriculture New Mexico State University. (N. Rech, Interviewer)

[4] Bordenstein, S., & Werren, J. (2007). Bidirectional incompatibility among divergent *Wolbachia* and incompatibility level differences among closely related *Wolbachia* in *Nasonia*. *Heredity, 99*, 278-287.

[5] Bruckner, S., Steinhauer, N., Aurell, D., Caron, D., Ellis, J., Fauvel, A., et al. (2019). Honey bee colony losses 2018-2019 preliminary results. *Bee Informed Partnership*, 1-6.

[6] Calderone, N. (2012). Insect pollinated crops, insect pollinators and US agriculture: trend analysis of aggregate data for the period 1992-2009. *PloS One*, 1-27.

[7] Dukku, U. (2016). Evaluation of morphometric characters of honeybee (*Apis mellifera* L.) population in the Lake Chad Basin in Central Africa. *Advances in Entomology*, 75-89.

[8] Eimanifar, A., Brooks, S., Bustamante, T., & Ellis, J. (2018). Population genomics and morphometric assignment of western honey bees (*Apis mellifera* L.) in the Republic of South Africa. *BMC Genomics*, 1-26.

[9] Environmental Protection Agency. (2018, January 19). *Pollinator Protection*. Retrieved March 12, 2020, from Colony Collapse Disorder: epa.gov/pollinator-protection/colony-collapse-disorder

[10] Gebremedhn, H., Amssalu, B., De Smet, L., & de Graaf, D. (2019). Factors restraining the population growth of *Varroa destructor* in Ethiopian honey bees (*Apis mellifera simensis*). *PloS One, 14*(9), 1-19.

[11] Grau, T., Brandt, A., DeLeon, S., Meixner, M., Strauss, J., Joop, G., et al. (2017). A comparison of *Wolbachia* infection frequencies in *Varroa* with prevalence of deformed wing virus. *Journal of Insect Science, 17*(3), 1-23.

[12] Hilgenboeker, K., Hammerstein, P., Schlattmann, P., Telschow, A., & Werren, J. (2008). How many species are infected with *Wolbachia* - a statistical analysis of current data. *FEMS Microbiology Letters, 281*(2), 215-220.

[13] Juchault, P., Rigaud, T., & Mocquard, J. (1992). Evolution of sex-determining mechanisms in wild population of *Armadillidium vulgate* Latr. (Crustacca, Isopoda): competition between two feminizing parasitic factors. *Heredity, 69*, 382-390.

[14] Kageyama, D., Ohno, M., Sasaki, T., Yoshido, A., Konagaya, T., Jouraku, A., et al. (2017). Feminizing *Wolbachia* endosymbiont disrupts maternal sex chromosome inheritance in a butterfly species. *European Society for Evolutionary Biology*, 232-243.

[15] Ma, W., & Schwander, T. (2017). Patterns and mechanisms in instances of endosymiont-induced parthenogenesis. *Journal of Evolutionary Biology, 30*, 868-888.

[16] Martin, S., & Medina, L. (2004). Africanized honeybees have unique tolerance to *Varroa* mites. *Trends in Parasitology, 20*(3), 112-114.

[17] Moore, P., Wilson, M., & Skinner, J. (2014). Honey bee viruses, the deadly *Varroa* mite associates. *Bee Health*, 1-19.

[18] Newton, I., Clark, M., Kent, B., Bordenstein, S., Qu, J., Richards, S., et al. (2016). Comparative genomics of two closely related *Wolbachia* with different reproductive effects on hosts. *Genome Biological Evolution*, 1526-1542.

[19] O'Neil, S., Gordiano, R., Colbert, A., Karr, A., & Robertson, H. (1992). 16S rRNA phylogenetic analysis of the bacterials endosymbionts associated with cytoplasmic incompatiibility in insects. *Proceeding of the National Academy of Sciences, 89*, 2699-2702.

[20] Ruiz-Guzman, G., Ramos-Castaneda, J., & Hernandez-Quintero, A. (2016). Costs and benefits of vertical and horizontal transmission of Dengue virus. *Journal of Experimental Biology*(219), 3665-3669.

[21] Saridaki, A., Sapountzis, P., Harris, H., Batista, P., Biliske, J., Pavlikaki, H., et al. (2011). *Wolbachia* prophage DNA adenine methyltransferase genes in different *Drosophila Wolbachia* associations. *PLoS One, 6*(5), 1-18.

[22] Slatko, B., Luck, A., Dobson, S., & Foster, J. (2014). *Wolbachia* endosymbionts and human disease control. *Molecular & Biochemical Parasitology, 195*(2), 88-95.

[23] Sutherland, C. (2019, November 7). Entomologist, New Mexico State University. (N. Rech, Interviewer)

[24] Tram, U., & Sullivan, W. (2002). Role of delayed nuclear envelope breakdown and mitosis in *Wolbachia*-induced cytoplasmic incompatibility. *Science, 296*(5570), 1124-1126.

[25] Tribe, G., & Fletcher, D. (1977). Rate of development of the workers of *Apis mellifera adansonii* L. In African bees: Their taxonomy, Biology and Economic Use . *Pretoria: Apimondia*, 115-119.

[26] United States Department of Agriculture. (2019, August). *National Agricultural Statistics Service*. Retrieved August 2019, from United States Department of Agriculture: nass.usda.gov/Surveys/Guide_to_NASS_Surveys/B_and_Honey/#colony_loss

[27] Vanengelsdorp, D., Evans, J., Saegerman, C. C., Haubruge, E., Nguyen, B., Frazier, M., et al. (2009). Colony collapse disorder: a descriptive study. *PloS One*, 1-2.

[28] Weeks, A., & Breeuwer, J. (2001). *Wolbachia*-induced parthenogenesis in a genus of phytophagous mites. *The Royal Society*, *268*(1482), 2245-2251.

[29] Werren, J. (1997). Biology of *Wolbachia*. *Annual Review of Entomology*, *42*, 587-609.

[30] Werren, J., Windsor, D., & Guo, L. (1995). Distribution of *Wolbachia* among neotropical arthropods. *Proceedings of the Royal Society*, *262*(1364), 197-204.

[31] Winston, M. (1992). The biology and management of Africanized honey bees. *Annual Review of Entomology*, *37*, 395-406.

[32]Yang, X., & Cox-Foster, D. (2005). Impact of an ectoparasite on the immunity and pathology of an invertebrate: evidence for host immunosuppression and viral amplification. *Proceedings of the National Academy of Science*, *102*(21), 7470-7475.

[33] Ye, Y., Woolfit, M., Huttley, G., Rances, E., Caragata, E., Popovici, J. O., et al. (2013). Infection with a virulent strain of *Wolbachia* disrupts genome wide-pattern of cytosine methylation in mosquito *Aedes aegypti*. *PloS One*, *8*(6).