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UNDERSTANDING THE FACTORS CONTRIBUTING TO THE ABSENCE OF VARROA DESTRUCTOR MITES IN AFRICANIZED HONEYBEES IN DEMING, NEW MEXICO

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Abstract

The honeybee industry is an essential component of agriculture, providing pollination services to many crops. However, the Western honeybee population has been continuously declining due to Colony Collapse Disorder and Varroa destructor mites, which have become major threats to honeybee colonies. Africanized honeybees (AHB) were introduced to the United States in 1990 and have since been spreading throughout the southern states. They are a hybrid of the African honeybee and two Western honeybees and have replaced Western honeybees in Luna County, New Mexico. This study aimed to investigate the prevalence of Wolbachia pipientis and Varroa destructor mites in AHB in the Deming, New Mexico area. The study found that AHB had a significantly lower infection rate of both W. pipientis and V. destructor mites compared to Western honeybees. None of the AHB were infected with V. destructor mites, which is in contrast to an estimated 80% of Western honeybees infected in the United States. The low infection rates of AHB may be attributed to hybrid vigor or heterosis, which increases disease resistance and decreases the expression of undesirable traits. The study sheds light on the resilience of AHB to the environmental and parasitic stresses currently affecting Western honeybees.

Introduction

Honeybees play a crucial role in pollination and agriculture, making them an essential component of the world's food production systems. However, over the past few decades, there has been a steady decline in the population of Western honeybees due to various environmental and parasitic factors. Varroa destructor mites, which serve as a major vector of viruses associated with Colony Collapse Disorder, have been a significant threat to the Western honeybee populations. In addition, the Africanized Honeybee (AHB), a hybrid of the African and two Western honeybees, has increasingly replaced Western honeybees in the southern United States. Little is known about

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AHB populations, which could be helpful in understanding the current honeybee decline. This study aims to compare infection rates of AHB and Western honeybees in the Deming, New Mexico area. We hypothesize that AHB may be less infected with W. pipientis and V. destructor mites than Western honeybees due to the effect of heterosis on AHB's resistance against environmental and parasitic stresses.

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%

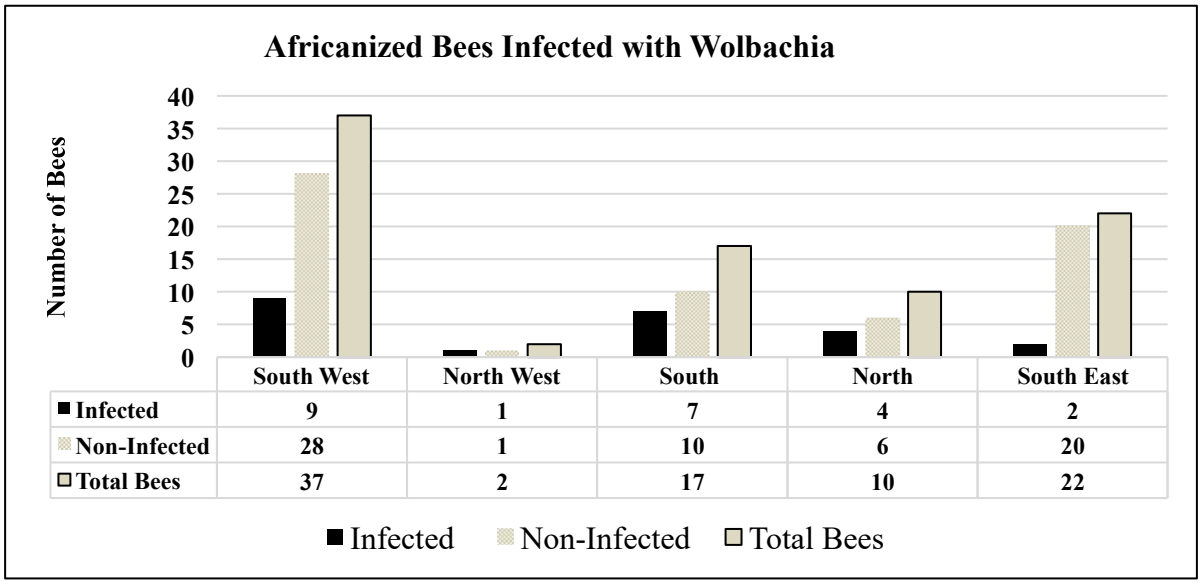

Figure 1. Bar graph of *Wolbachia* infection rate of AHB in five areas of Deming, New Mexico

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). Ninetytwo 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).

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