WOLBACHIA PIPIENTIS AND *VARROA DESTRUCTOR* MITE INFESTATION RATES OF NATIVE VENOMOUS POLLINATOR SPECIES IN THE NORTHERN REGION OF THE CHIHUAHUAN DESERT

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Abstract: A large part of the Northern portion of the Chihuahuan Desert is located in Luna County, New Mexico. Eighty-seven *Xylocopa mican* (Lepeletier, 1841) Mexican Carpenter Bees, eighteen Africanized Honey Bees (AHB) and a small number of wasps, *Pepsis grossa* (Fabricius, 1798), *Pepsis thisbe* (Lucas, 1895), and *Trogus* species, which are all parasitic wasps, were captured during July, 2022 at the base of the Floridian Mountains in the Northern Chihuahuan Desert. These species are native pollinators and tend to be resistant to parasites due to hybrid vigor or heterosis. However, even though large-bodied pollinators have been found to be extremely efficient, they are prone to extinction upon ecological disfunction. The specimens were tested for *Wolbachia pipientis* and *Varroa destructor* mites. *Wolbachia pipientis* is a Rickettsial endosymbiont bacterium that infects arthropods and nematodes. *Wolbachia* 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 population. Identification of *W. pipientis* was based on the 16S gene of rDNA. The gene used for insect DNA identification was the cytochrome C oxidase gene. *Wolbachia* is primarily transferred vertically from mother to offspring, but horizontal transfer does happen especially with parasitic wasps. *Varroa destructor* mites are vectors for at least eighteen viruses linked Colony Collapse Disorder which decimates about 40% of the domesticated bee colonies, *Apis mellifera*, each year. Many beekeepers do not realize the *Apis mellifera* are an invasive species and not native to North America. This study addresses that need for the agricultural community to expand the use to native pollinators and reduce the use of domesticated bees.

Keywords: *Wolbachia pipientis*, Africanized Honey Bees (ABH), *Varroa destructor* mites, native pollinators, heterosis, hybrid vigor, *Xylocopa mican, Trogus* species*, Pepsis thisbe, Pepsis grossa*

INTRODUCTION: Thirty-five percent of the world's crops depend on animal pollinators (Klein, et al., 2007). Traditionally, farmers depend on domesticated honeybees for pollination. Nevertheless, about 40% of domesticated honeybee colonies in the United States are lost each year due to Colony Collapse Disorder (Evison, et al., 2012). Honeybees, especially *Apis mellifera*, are the preferred agricultural pollinators. But the continuing decline of *A. mellifera* highlights the potential risk of sole reliance on one species for pollination (Klein, et al., 2007). Besides, diversity of pollinators has been shown to increase crop yield (Hoehn, Tscharntke, Tylianakis, & Steffan-Dewenter, 2008). Parasites and the overuse of pesticides are responsible for the declining populations of pollinators, both domestic and native (Biesmeijer, et al., 2006). *Varroa destructor* mites, which are ectoparasites, are directly responsible for reduced honey and brood production by *A. mellifera* colonies (Downey & Winston, 2001). Also, they are thought to be major vectors for honeybee viruses (Moore, Wilson & Skinner, 2014). The mite's infestation ranges from all countries with the exception of Australia and have caused a global decline in domesticated bee colonies. Presently, it is not possible to eradicate the mites from infected hives exacerbating the situation (Beverley, 2008). The decline of domesticated pollinators has increased the reliance on diverse communities of wild, native pollinators. Additionally, native pollinators are an important factor in maintaining plant species, but are seldomly relied on for pollination (Klein, et al., 2007). No baseline data exists of native, specie populations (Biesmeijer et al., 2006). Another issue is the transfer of viruses from domesticated bees to wild bees that could potentially be exacerbating the wild bee decline. Pollen from flowers frequented by *Apis mellifera* have been found to harbor viruses from *A. mellifera*, showing how easily viruses can be transferred from domestic bee populations to native pollinators (Dolezal, et al., 2016).

Wolbachia pipientis is a wide-spread endosymbiont that tends to be parasitic in insects and mutualistic in filarial nematodes (Slatko, Luck, Dobson, & Foster, 2014). In 2015, it was estimated that 52% of arthropods and 47% of nematodes were infected with *Wolbachia* (Weinert, Araujo-Jnr, Ahmed, & Welch, 2015). One hundred percent of domesticated honeybees in Germany tested positive for *Wolbachia*, and 75% of domesticated honeybees in South Africa have tested positive showing the extent of the infestation (Pattabhiramaian, Brueckner, Witzel, & Reddy, 2011). No papers testing *A. mellifera* in North America were found. *Wolbachia* is known for manipulating the reproduction of hosts to cause cytoplasmic incompatibility, parthenogenesis, and feminization (Zhou, Rousset, & O'Neill, 1998). It is known specifically to cause parthenogenesis in parasitic wasps (Werren, 1997). However, in the last several years, *Wolbachia* has been viewed as a mutualistic endosymbiont in insects. *Wolbachia* has been shown to facilitate resistance to RNA viruses in its host (Pimentel, Cesar, Marcos, & Cogni, 2021). In some cases, the bacterium has increased resistance to insecticides. This fluctuates with the insect species, the insecticide, the population density and the strain of *Wolbachia* (Liu & Guo, 2018). *Wolbachia* has been associated with resistance to organophophate pesticide in *Culex pipiens* (Linnaeus) mosquitoes. Buprofezin, commonly used in China on rice paddies, prevents the formation of chitin needed for insect exoskeletons. Leaf hoppers, *Laodelphax striatellus* (Fallen) infected with high densities of *Wolbachia*, have developed resistance to the pesticide. Also, *L. striatellus*, treated with anti-biotics and cleared of *Wolbachia,* were not resistance to buprofezin (Li, Liu, & Guo, 2018). The success of *Wolbachia* infestation is attributed to efficient maternal transmission and manipulations of the host reproductive system to favor females.

Wolbachia is usually transmitted vertically from mother to offspring through the female germline, but it can also be transmitted horizontally from prey to predator (Werren, 1997).

Until 2006, Mexican Carpenter Bees, *Xylocopa mican* (Lepeletier, 1841) were only found year-round in the Eastern and Southeastern sections of the United States than specimens were collected from Arkansas (Warriner, 2010). *Xylocopa mican* has also been found in the lower Rio Grande Valley since 1981 during the warmer months (Porter, 1981). One scenario for the migration was purposed by Tripodi and Szalanski (2011). Beings *X. mican* build their nests in wood, they proposed lumber transported across the country facilitated the migration inadvertently via the interstate highway system. An example to support this theory, *Xylocopa* species from Hawaii arrived in San Francisco via balsa wood (Tripodi & Szalanski, 2011). *Xylocopa mican* are polylectic and efficient pollinators of several crops including tomatoes, eggplants and peppers. Peppers are a major crop in Luna County, New Mexico (United States Department of Agriculture, 2021). The habitats of *Xylocopa mican* may be changing due to climate change and/or anthropogenic behavior (Tripodi & Szalanski, 2011).

Africanized Honey Bees (AHB) entered North American in 1990, and have since colonized throughout the southern United States, replacing *Apis mellifera*. AHB are a cross between an African honeybee and two subspecies of domesticated Western honeybees (Winston, 1992). They were created in 1956 by two geneticists in Brazil. The objective was to create a gentle bee that produced more honey. However, 26 queen bees that were produced were more aggressive than the African bee and they did not produce more honey than the Western bees. They were accidently released into the wild (Winston, 1992). AHB moved into Luna County in 1992, and systematically replaced all of the Western bees. Domestic beekeeping rapidly declined in Luna County (Blandford, 2019; Sutherland, 2019). AHB are slightly smaller, produce more offspring, and have a shorter life span than *Apis mellifera* resulting in rapid population growth (Tribe & Fletcher, 1977). The African bee traits are dominant over the Western bee traits. These traits include swarming excessively, absconding the hive quickly, replacing other bee colonies, and excessively defending their hives (Eimanifar, Brooks, Bustamante, & Ellis, 2018).

The parasitic wasps captured are divided into two groups: spider wasps (*Pepsis grossa* and *Pepsis thisbe*) and caterpillar wasps (*Trogus* species). *Pepsis grossa* and *P. thisbe* lay their eggs on tarantulas, while *Trogus* species lays its eggs on swallowtail butterfly larvae (Sime, 2005). Both behaviors facilitate horizontal transfer of *Wolbachia*. Having less setae than bees, they are less efficient pollinators but still are pollen vectors and are essential to the native pollinating population (Hooks & Espindola, 2023).

Parasitoid wasps largely depend on chemical cues released from both their herbaceous hosts and the damaged plants (Tumlinson, Turlings, & Lewis, 1992). Parasitic wasps are also native pollinators and a common inhabitant of deserts in the southwestern United States.

MATERIALS AND METHODS:

Capturing specimens: Proper safety measures were observed during specimen collection. AHB, especially, are known to be very aggressive if their hives are approached. The specimens 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 specimens were refrigerated before handling. The centrifuge tubes were labeled with the location and date. The specimens were frozen until screening for *V. destructor* mites and *W. pipientis.*

Identification of specimens: Hurd's revision of the Nearctic species of the pompilid genus *Pepsis* was used to identify the wasps in the *Pepsis* genus, *Pepsis grossa* and *Pepsis thisbe* (1952). *Trogus* species was identified using Wahl and Sime revision of the genus *Trogus* (2006**).** However, the specimens were damaged during tissue extraction. We were unable to determine whether the species were *T. pennator* or *T. flavipennis*. *Xylocopa micans* was identified using the Hurd and Moure key for large carpenter bees (1963). Africanized Honey Bees were identified by Carol Sutherland, Entomologists for New Mexico State University and Jack Blandford, Director of Agriculture at New Mexico State University.

Wolbachia and Insect DNA extraction and PCR protocols: Two millimeters (mm) were removed from the specimen's posterior 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 10,000 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 rpm's 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 10,000 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 pellet was air dried for 10 minutes. Two-hundred µL of TE/RNase was added. The pellet was disturbed by pipetting and then tube was centrifuged at 10,000 rpm for 1 minute. The DNA was frozen until PCR amplification. PCR amplification was done with a Bio-Rad thermocycler t100; PuReTaq™ Ready-To-Go™ PCR beads were used. The DNA was thawed. Twenty microliters of primer was added to the PCR bead along with 5 μL of extracted DNA. Primer for 16S rDNA was used to identify *W. pipientis*, and primer for the Cytochrome C oxidase gene was used to identify insect 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 150V for 30 minutes. SYBR safe green loading dye was used with lithium bromide buffer. An EDVOTEK TruBlu2 DNA illuminator was used to view the DNA. *Wolbachia pipientis* DNA is identified at 438 kilo-basepairs (kbp) and insect DNA is identified at 708 kbp.

Varroa destructor Mite Screening

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

RESULTS

Between 2:00 pm and 3:00 pm during July of 2022, collection sweeps were made at random sites in a 5-hectare area of the Northern Chihuahua Desert. Venomous pollinators were collected mostly from purple sage (*Salvia carduacea*), desert willow trees (*Chilopsis linearis*), creosote bushes (*Larrea tridentata*), honey mesquite trees (*Prosopis glandulosa).*

Table 1. Results of Daily Sweeps during July of 2022

Insect Species	Numbered Captured	% of Insects Captured	Number Infected with *W. pipientis*	Number Infected with *V. destructor*
Xylocopa micans	69	70%	10	0
AHB	21	21%	0	0
Pepsis grossa	2	2%	0	0
Pepsis thisbe	6	6%	0	0
Trogus spp.	1	1%	0	0
Total	99	100%	10%	0

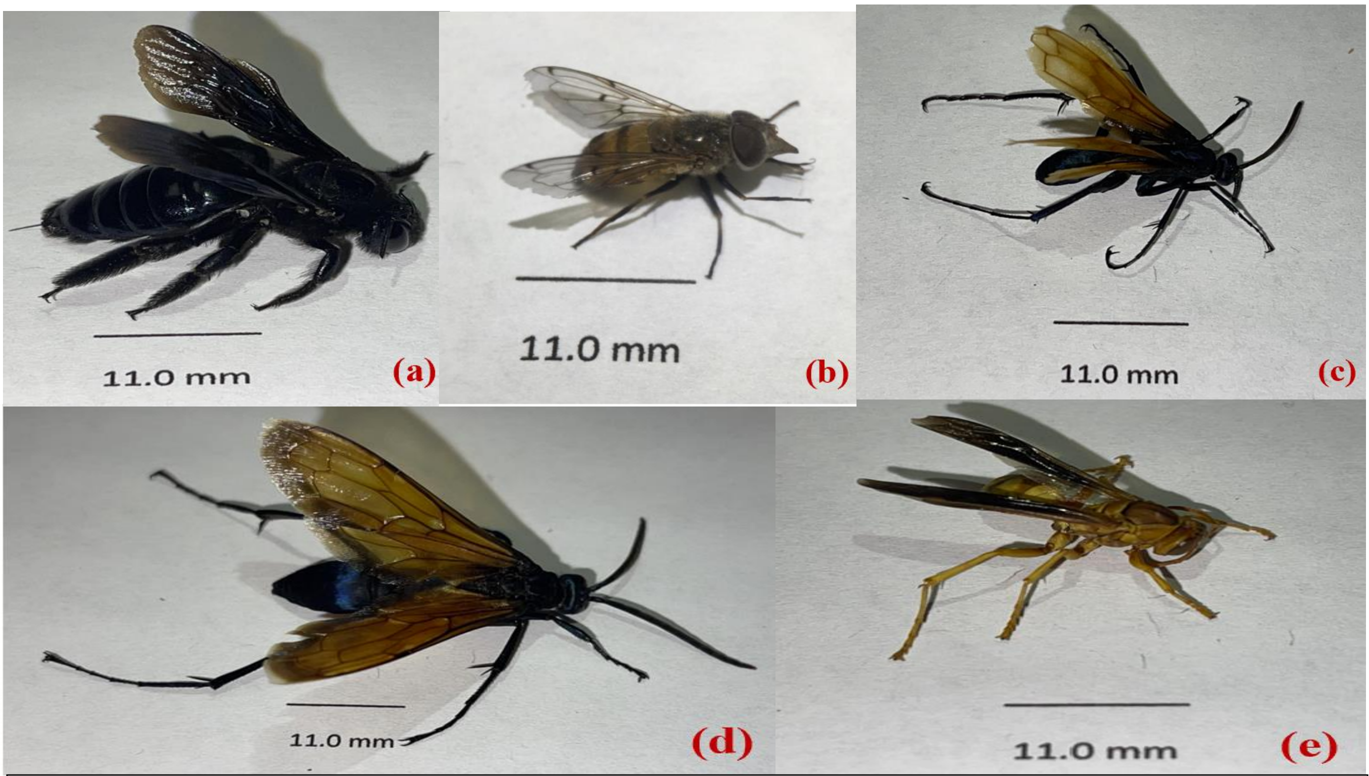

Figure 1. **(a)** *Xylocopa micans*, **(b)** Africanized Honey Bees, **(c)** *Pepsis thisbe*, **(d)** *Pepsis grossa,*

(e) *Trogus spp.* Photographs by Jethro Cline LeBaron

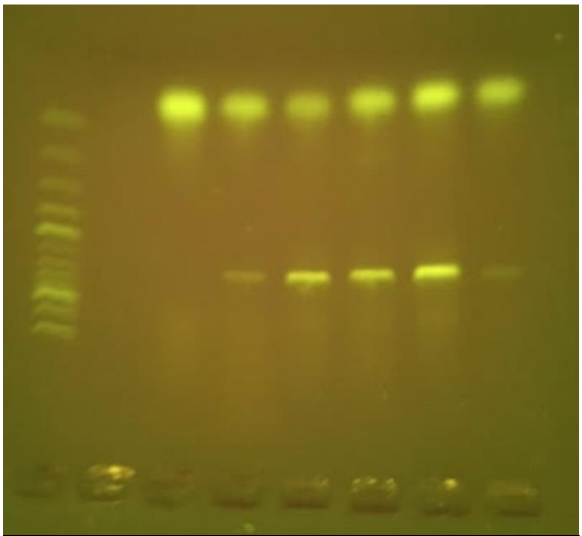

Figure 2. Electrophoresis gel showing *W. pipientis* infection in specimens 2 through 6.

The electrophoresis gel to the left shows speciemens 1through 6 of *Xylocopa micans*. The 100 basepair (bp) ladder is visible on the left. Specimens 2 through 4 show high-density infections *Wolbachia pipientis.* Specimens 2 and 6 show a low-density infection. None of the specimens of any specie tested positive for *V. destructor* mites. *W. pipientis* is identificable at 438 bp and insect DNA is identificable at 708 bp. *Xylocopa micans* was the only specie that tested positive for *Wolbachia.*

DISCUSSION

Native pollinators are extremely important to the ecological stability of habitats. Relinance of the agricultural community on a single species, *Apis mellifera*, may prove to be in error especially due to the decline of domesticated colonies because of CCD. Research shows that diversity of pollinators improves indiginous plant and crop production. The northern portion of the Chihuahuan Desert relies heavily on native pollinators for the successful replenisment of plant species. The health and density of these species are essential to the environmental sustainability of the desert. The purpose of this study was to evaluate the *V. destructor* mite and *W. pipientis* infection rates in the populations, however, it also revealed the population density of some species. Of course seasonal variations happen in insect populations, but both *Xylocopa micans* and Africanized Honey Bees were the most populous venomous pollinating species during our time frame. *Xylocopa micans* is an efficient pollinator of peppers, which are a major economic crop in this area.

Most of the specimens were caught on purple sage bushes (*Salvia carduace*), desert willow trees (*Chilopsis linearis*), creosote bushes (*Larrea tridentata*), and honey mesquite trees (*Prosopis glandulosa*). *Xylocopa micans* made up seventy percent of the specimens caught. *Xylocopa micans* are solitary bees that boor holes in the stalks of the *Agave parri* to make nests. *Agave parri* are common in this area. They were not expected to harbor *Varroa destructor* mites due to their solitary existance. Africanized honey, which are social, made up the second largest percentage, 21%. Even though AHB tend to be more resistant to parasites than *Apis mellifera*, we thought that some of the AHB might harbor *V. destructor* mites. The mites tend to locate themselves between the sclerites of the insects. The wasps, a minor percentage of the specimens, were not expected to be hosts. *Varroa destructor* mites were not found on any of the species.

As for *Wolbachia pipientis*, we had projected that a percentage of all of the species would be infected. Globably, *Wolbachia* is a ubiquitous endosymbiont. The carpenter bees, *X. micans*, had a 10% infection rate, much lower than other studies researched. It was speculated that *Wolbachia* infection in the bees would have been by vertical transfer. Beings the wasps lay their eggs on other species, horizontal transfer of *Wolbachia* was conceiable. However, none of the AHB or wasp specimens were infected. Possible reasons for this are the low specimens numbers obtained, or the resistance to parasites due to hybird vigor or heterosis. *Wolbachia* has been known to improve the immune systems of some insects, and even make some species resistant to insecticides. But as it decreases the male population in a species, it decreases the gene pool and thus decreases the variation in a population. Variation in a population allows a species to adapt to changing environments, as we are experiencing today.

The identification of the *Trogus* species was problematic. Very little research has been done on the genus. We did obtain an revision of the taxonomic key for the genus. During the extraction of tissue for *Wolbachia* testing, the specimens were slightly mutalated and the exact identification was impossible. We believe the *Trogus* specimens are either *T. pennator* or *T.flavipennis.*

More research is needed both in the area of parasite infection rates of native pollinators, and the density of the pollinating species in the Northern Chihuahuan Desert.

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