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INFECTION RATE OF TWO PARASITES BETWEEN BROOD XIII AND BROOD XIX PERIODICAL CICADAS

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ABSTRACT

During the summer of 2024, 74 specimens of Brood XIII cicadas were captured from Illinois and 59 specimens of Brood XIX were captured in Missouri. All specimens were analyzed for infection rate of three parasites, *Wolbachia pipientis, Massospora cicadina*, and *Pyemotes herfsi*. *Wolbachia pipientis* is a rickettsial, alpha proteo-bacterium that infects arthropods and filarial nematodes. *Wolbachia* causes male-killing, parthenogenesis, cytoplasmic incompatibility and feminization. However, it also boosts the immune systems of insects, and can make them resistant to RNA viruses plus insecticides. *Massospora cicadina*, a fungal pathogen, causes infertility, disease transmission and eventual death to the cicadas. No specimens were found to be infected with *Massospora cicadina*. *Pyemotes herfsi* mites are ectoparasites that drain nutrients from the cicadas, and can also transmit diseases. *Pyemotes herfsi* were also analyzed for *Wolbachia* infection. Cicada and mite DNA was extracted through a 24-step process, and ran through an electrophoresis gel to identify *Wolbachia* DNA. *Wolbachia* DNA is identified at 438 bp, and insect plus mite DNA is identified at 709 bp. Cicadas were examined under a dissecting microscope to check for *Massospora cicadina* and *Pyemotes herfsi* mites.

Keywords: Periodical Cicadas, *Wolbachia pipientis, Pyemotes herfsi*

INTRODUCTION

Periodical cicadas were first documented in 1666 (Marlatt,1898). The genus of periodical cicadas, *Magicicada,* is only found in North America (Bryce & Aspinwall, 1975; Dybas & Lloyd, 1974). There are seven *Magiciada* species (Bryce & Aspinwall, 1975). Other periodical cicadas can be found in India and Fiji. The emerge every 13 or 17 years in great numbers and live approximately

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four weeks above ground. The nymphs are subterrain (Ito, et al., 2015). Beings they emerge so seldomly, there is a dearth of research on them. Since the late 1800's, there has been a controversy on the digestive system of these cicadas (Hargitt, 1923; Hickernell, 1923). The system has been described as complete and well organized or degenerate and atrophied. The male and female digestive systems are the same with the exception of the male air chamber. The air chamber is situated in the abdomen and is used to magnify sound (Hargitt, 1923; Hickernell, 1923). The cicadas feed on low nutrient xylem which only contains water and dissolved minerals. There is an enlargement in the alimentary canal that serves as a food reserve allowing the insects to live four weeks without eating (Hargitt, 1923; Hickernell, 1923). The ecological benefits of the emergence is a significant increase of biomass in an area which increases food for many organisms. However, the emergence causes biological harm when the female oviposits which kills tissue and allows pathogens to invade the trees (Ito, et al., 2015; Williams & Simon, 1995).

Pyemotes herfsi, the oak leaf mite, is an ectoparasite that traditionally feed on the oak gall midge larvae, *Polystepha pitulae*. They drop from the trees and attach to animals and humans. The bites on humans cause irritations (Broca, et al., 2006). On insects, the mites suck the hemolymph plus they spread viruses. Cicadas in deciduous forests can become infected with *Pyemotes herfsi.*

Wobachia pipientis is a Rickettsial, alpha proteo-bacterial endosymbiont which can be parasitic or mutualistic. *Wolbachia* tends to be parasitic in insects. *Wolbachia* can cause cytoplasmic incompatibility, feminization, parthenogenesis, sterility, male killing and shorter life spans in arthropods (Knight, 2001; Werren, 1997). *Wolbachia* modifies host reproduction to enhance its own spread (Southhamer, Breeuwer, & Hurst, 1999). Sixty-seven percent of all insect species are infected with *Wolbachia.* There are two types of cytoplasmic incompatibility, unidirectional and bidirectional. In unidirectional, if the male is infected and the female is not, there will be no offspring. All other scenarios will produce offspring. In bidirectional, if the male and female are infected with different strains of *Wolbachia,* there will be no offspring (Dobson, Fox, & Jiggins, 2002). In feminization, *Wolbachia* manipulates the DNA of male arthropod and nematodes to produce ovaries and estrogen. *Wolbachia* can completely feminize male arthropods and nematodes into fully functioning females (Asgharian, Chang, Mazzoglio, & Negri, 2014).The manipulation of the organisms' reproductive system is skewing the male/female sex ratio of the populations and decreasing their gene pool (Jiggins, Hurst, & Majerus, 2000). *Wolbachia* can be transferred vertically or horizontally. Horizontal transmission occurs between organisms of the same or different species due to cannibalism or food supply (Ahmed, Breinholt, & Kawahara, 2016). Vertical transmission occurs between mother and offspring. *Wolbachia* can also boost an insects' immune system and make it resistant to RNA viruses and insecticides (Brennan, Keddie, Braig, & Harris, 2008; Eleftherianos, Atri, Accetta, & Castill, 2013). *Wolbachia* can prevent the transmission of diseases such as Zika virus, dengue virus, malaria, yellow fever, and more (Cook

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& McGraw, 2010; Frentiu, Robinson, Young, McGraw, & O'Neill, 2010; Iturbe-Onmaetxe, Walker, & O'Neill. 2011). Some countries such as Australia, Saudi Arabia, Columbia and Vietnam are releasing infected males to combat disease.

Capture Sites

Brood XIX Periodical cicadas were captured in two separate parks in Missouri. The first park, Greenfelder Park, is located on the eastern border. The forest is comprised of deciduous trees, mostly Oaks and Cotton Woods, with some pine trees interspersed. All of the cicadas from both locations were captured on deciduous trees, none were observed on pine trees. The second park, Meramec Park, is located 80. 47 kilometers to the north of Greenfelder Park. Brood XIII were captured in Jubilee State Park in Illinois, which is 9.65 kilometers west of Peoria. As with Brood XIX, Brood XIII specimens were captured on Oak and Cotton Wood trees.

METHODS

DNA extraction and PCR protocols for the Cicadas: Two millimeters (mm) were removed from the posterior of 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 95°C water bath for 5 minutes. After heating, the tube was opened briefly to release pressure then centrifuged for 5 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 same speed and time as previously stated. Another clean microfuge tube was obtained and 300µL of supernatant was transferred. Four-hundred µL of isopropanol were added and then centrifuged for 5 minutes at 10,000 rpm 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 5 minutes. Two-hundred µL of TE/RNase was added. The pellet was disturbed by pipetting then tube was centrifuged at 10,000 rpm for 1 minute. The DNA was frozen until PCR amplification. PCR amplification was done with a Biorad thermocycler t100. 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. Two percent agarose electrophoresis gels were run at 150 V for 30 minutes. Five µL of SYBR safe green was added to each gel. Lithium bromide buffer was used. An Edvotek TruBlu 2 Transilluminator was used to view the DNA.

DNA Extraction and PCR Protocols for *Pyemotes herfsi:*

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The same DNA extraction procedure was used for both the cicadas and the mites, however, for the mites we decreased the compounds used by 75%. Beings the mites are eukaryotes and have mitochondria, the same Cytochrome C oxidase gene was targeted for mite DNA, and obviously the 16S rDNA gene was targeted for the *Wolbachia* DNA.

Species Identification of the Broods:

The broods are identified by the area of emergence, the intervals of emergence, the venation of the wings, and the orange marking patterns of the ventral side of the abdomen.

Brood XIII and XIX Species:

Figure 1. (L to R) *Magicicada cassini*, Magicicada *septendecula*, and *Magicicada septendecim*

Magicicada cassini has no orange marking on its abdomen, *Magicicada septendecula* has small orange markings on its abdomen separated by a black area, and *Magicicada septendecim* has orange stripes on its abdomen with no black separation, *Magicicada cassini* tends to be smaller than the other two species. Brood XIII emerges every 17 years. All of the species inhabit hard wood, deciduous trees. Brood XIII emerges in Illinois.

Brood XIX Species:

Figure 2. (L to R) *Magicicada tredecassini*, *Magicicada tredecula*, *Magicicada tredecim*, and *Magicicada neotredecim*

Magicicada tredecassini has no orange markings on the abdomen, *Magicicada tredecula* has minor orange stripes, *Magicicada tredecim's* ventral abdomen is completely orange, and *Magicicada neotredecim* has broad orange stripes on the abdomen. Brood XIX emerges every 13 years

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Controversy About the Species:

The most common definition of a species in Biology is a group of organisms that share genetic heritage, interbreed, and produce fertile offspring. Entomologists differ on whether there are three or six species. *Magicicada neotredecim* is an exception because it was just discovered this year. But for the other species, they can interbreed and produce fertile offspring. The only problem was they usually emerge at different times and different places. That did not happen this year. But for the other species, they can interbreed and produce fertile offspring. For example, *M. cassini* and *M. tredcassini* are identical with the exception of being in different broods. Their markings, and wing venation are the same, and they can interbreed to produce fertile offspring.

RESULTS

Results of Specimens Captured from Brood XIII and XIX

Table 1: Infection Rate from Brood XIII

Species	Male	Female	*Pyemotes herfsi* Infection Rate & Percentage	*Wolbachia pipientis* Infection Rate & Percentage
M. cassini	20	22	2 5%	24 57%
M. septendecula	5	17	3 14%	9 41%
M. septendecim	1	8	1 11%	5 56%

Table 2: Infection Rate from Brood XIX

Species	Male	Female	*Pyemotes herfsi* Infection Rate & Percentage	*Wolbachia pipientis* Infection Rate & Percentage
M. tredecassini	9	35	9 20%	11 25%
M. tredecula	8	4	1 8%	1 8%
M. tredecim	0	0	0	0
M. neotredecim	2	1	1 33%	2 67%

Results of *Pyemotes herfsi* infected with *Wolbachia pipientis*

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Table 3: *Pyemotes herfsi* Mites Infected with *Wolbachia pipientis* in Brood XIII

Species	Number of Mites	Number of Mites Infectesd with *Wolbachia*	Prey Cicadas Infected with *Wolbachia*	Percentage of Mites Infected with *Wolbachia*
M. cassini	2	1	2	50%
M. septendecula	3	1	2	33%
M. septendecim	1	1	1	100%

Table 4: *Pyemotes herfsi* Mites Infected with *Wolbachia pipientis* in Brood XIX

Species	Number of Mites	Number of Mites Infected with *Wolbachia*	Prey Cicadas Infected with *Wolbachia*	Percentage of Mites Infected with *Wolbachia*
M. tredecassini	9	6	1	67%
M. tredecula	1	1	0	100%
M. tredecim	0	0	0	0
M. neotredecim	1	1	0	100%

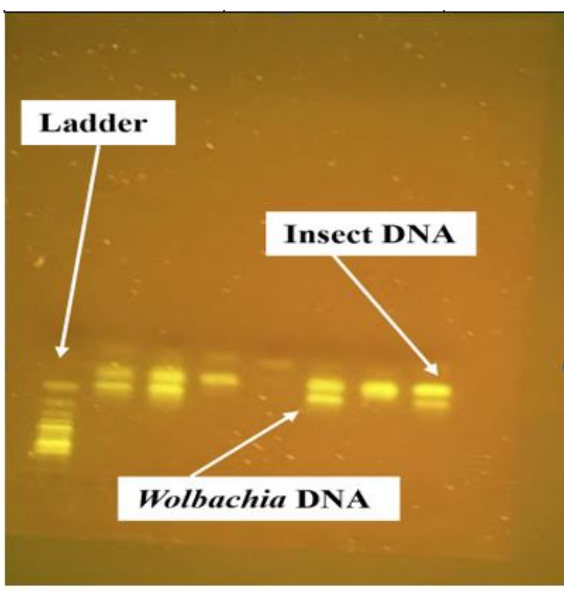

Figure 3. Electrophoresis gel of Brood XIII cicadas. (L to R) The ladder, and specimens 37 to 43. Specimens 37, 38, 41, and 43 are infected with *Wolbachia.*

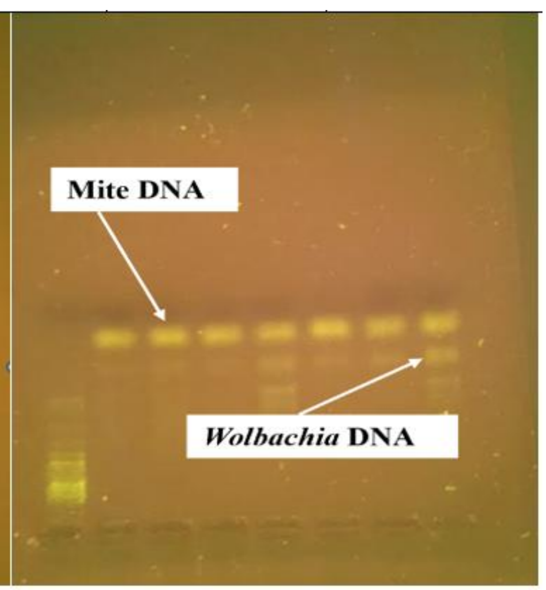

Figure 4. Electrophoresis gel of *P. herfsi* mites. (L to R) The ladder, and specimens 39a, 4a, 11a, 61a, 3a, 71a, and 3b. Mites 61a, 3a, 71a, and 3b are infected with *Wolbachia.*

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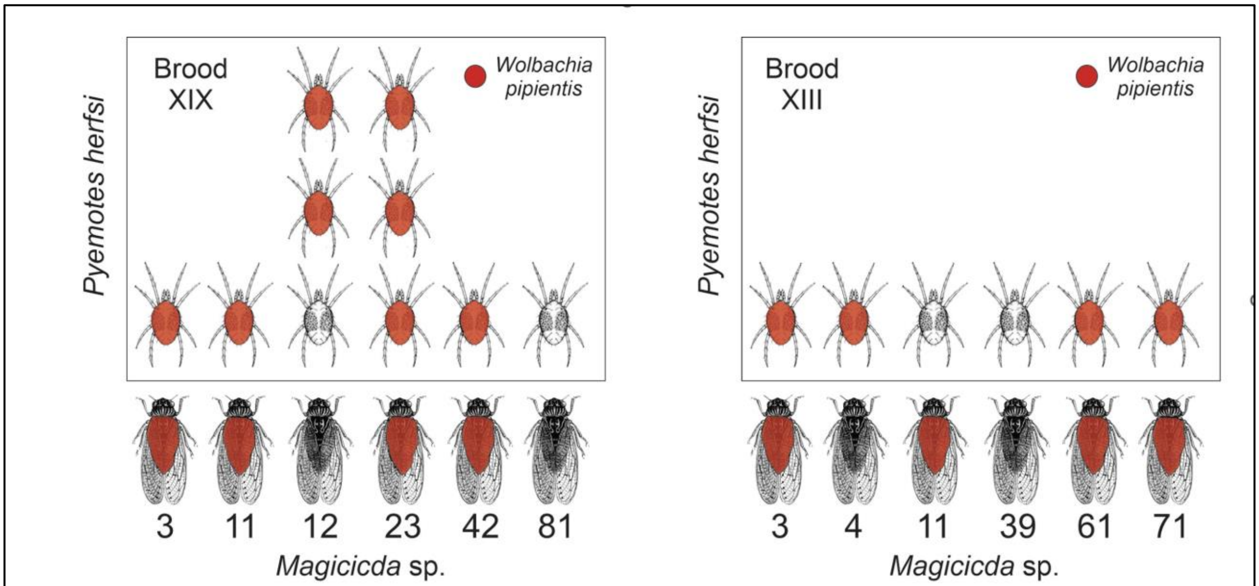

Figure 5: Pictograph of Cicadas and Mites infected with *Wolbachia pipientis*.

Unusual Results in Brood XIX

Figure 6. Examples of abdomens portions missing in Brood XIX. (L to R) Specimen 73, *M. tredecula*; Specimen 44, *M. tredecula*; Specimen 43. *M. tredecassini*.

Eleven percent of *M. tredecassini* and 62% of *M. tredecula* were missing portions of their abdomens. This was not the case with Brood XIII. One specimen was missing its entire abdomen and was alive. This was possibly a *Massospora cicdina* infection, but it could not be verified because the abdomen had dropped off

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DISCUSSION

This was an interesting and arduous study. Due to the infrequency of periodical cicada emergence, there are not many published research papers on them. The number of each species captured varied greatly. *Magicicada cassini* and *Magicicada tredecassini* were the most abundant. Depending on which entomologist you consult, they are either the same or different species. They can mate and produce fertile offspring. They just happen to emerge in different places and usually at different times. The number of specimens captured in the other species were very small. Identifications were made mainly using the orange pattern of the ventral side of the abdomen (Figure 1, Figure 2). Statistically, our results in those species are not relevant due to the low numbers. The *Wolbachia* infection rate of *Magicicada cassini* aligns with other global studies of insect infection rates, however, *Magicicada tredecassini* does not (Table 1, Table 2). *Magicicada cassini's Wolbachia* infection rate was 57%. Global studies usually find population infection rates between 50% and 70% (Rech, et al., 2020; Rech, et al., 2023). *Magicicada tredecassini's* infection rate was 25%, well below the global average. Gel electrophoresis (Figure 3) shows examples of cicada specimen's infected with *Wolbachia.* Periodical cicadas only feed on xylem fluids which contain inorganic ions and water, so if they are infected with *Wolbachia* it must have been vertical transmission.

Mites are different. They feed on the hemolymph, the body fluids of insects. They are hematophagous ectoparasites and *Wolbachia* could have been transferred through the hemolymph. Studies (Rasgon, Gamstone, & Ren, 2006) have shown that *Wolbachia,* though usually found in the gametes, can be found in the hemolymph. Six mites were found on cicadas in Brood XII, and 11 were found in Brood XIX (Table 3, Table 4). Again, relevancy can be questioned due to the low numbers, but the results are an indicator. We found two incidences where the cicada was not infected but, the mites on the cicadas were infected with *Wolbachia* (Figure 5). This could have been either vertical or horizontal transfer because mites do change hosts. *Wolbachia* in mites was identified by electrophoresis gel (Figure 4) by targeting the C oxidase gene in the mitochondria.

The unusual results in Brood XIX were puzzling. Eleven percent of *Magicica tredecassini* and 62% of *Magicicada tredecula* were missing parts of their abdomen (Figure 6). We have no explanation for this. None of Brood XIII specimens had parts of their abdomens missing. All of the specimens were captured alive. Specimen 43 (Figure 6) had its entire abdomen missing. A possible explanation for this could be *Massospora cicadina*, the fungus that infects periodical cicadas. It does cause the insect's abdomen to fall off. However, with the abdomen gone, it was impossible to make an accurate assumption.

CONCLUSION

The *Wolbachia* infection rate for Brood XIII was 52%, and aligned with global studies. However, Brood XIX did not. The *Wolbachia* infection rate was 24%, well below the global average. The majority of specimens captured were species *Magicicada cassini* of Brood XIII and *Magicicada tredecassin*i of Brood XIX. There were only a small number of the other species captured. *Pyemotes herfsi* infection rate for Brood XIX was 73%, and the mite infection rate for Brood XIII was 50%. We could find no research papers on *Pyemotes herfsi* relating to *Wolbachia* infections, so we could not make any comparisons. We also could not find any research papers relating to the missing abdomens parts in Brood XIX. Much more research is needed to done on both the periodical cicadas and the *Pyemotes herfsi* mites.

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