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Effects of a Neonicotinoid Insecticide on the Growth of Honey Bee Gut Microbes

Macee Mitchell
Eastern Washington University, mmitchell12@eagles.ewu.edu

Daniel Franzese
Eastern Washington University, dfranzese@eagles.ewu.edu

Taylor Morales
Eastern Washington University, taylor@moralesnet.net

Shane Lucht
Eastern Washington University, slucht@eagles.ewu.edu

Jesse Steele
Eastern Washington University, steelejesse34@yahoo.com

See next page for additional authors

Authors

Macee Mitchell, Daniel Franzese, Taylor Morales, Shane Lucht, Jesse Steele, and Jenifer Walke

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EASTERN WASHINGTON UNIVERSITY

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Introduction

- The interactions between microbes and their animal hosts are of fundamental importance for the health of both organisms.[1]
- The gut microbiome in particular plays an essential role in host metabolism, growth and development, immune function, and protection against pathogens.[1]
- The presence and abundance of these microbes may be altered by environmental factors, such as exposure to pesticides.
- The European honey bee, *Apis mellifera*, is an important pollinator for our global food supply and for natural ecosystems. Yet, honey bee colony losses are increasing due to threats such as poor nutrition, diseases, and pesticide exposure.
- Imidacloprid is a neonicotinoid insecticide that has received a lot of attention in regards to pollinators, as it can have detrimental effects on the native and managed bee populations.[2]
- Does this pesticide alter the honey bee gut microbiome, and thus alter host and colony health?

Objectives:

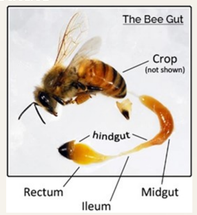
1) **Determine the prevalence of pesticide residues in honey bees in eastern Washington (field study)**
2) **Determine whether the honey bee gut microbiome is impacted by pesticide exposure (field and lab study)**

Methods

Field Study

- Sampled bees from 24 hives among six sites across eastern Washington
  - Pesticide analysis
    - HPLC-MS at WSU-Spokane Core facilities detected the presence of five common agricultural pesticides in the following percentages of bee hives sampled: Imidacloprid (41.7%), Cypermethrin (41.7%), tau-Fluvalinate (12.5%), Chlorpyrifos (4.2%), and Coumaphos (4.2%)
  - Microbiome analysis
    - Multiplexed 16S rRNA gene amplicon sequencing on Illumina MiSeq at Harvard's Dana Farber Cancer Institute

Lab Study



- Sampled bees from two different locations and two time points in eastern Washington
- Sterilely dissected the guts, isolated the gut bacteria, and extracted the bacterial DNA
  - Media: SDA, TSA + 5% sheep blood, MRS, and BHI
- PCR: Amplified and Sequenced 16S rRNA gene
  - Sanger Sequencing using 8F and 1492R primers
  - Analyzed sequences using bioinformatics programs Geneious and BLAST through NCBI
- The growth of the microbe was tested against concentrations of Imidacloprid using 96-well plate bacterial growth assays
  - 2400 µg/liter medium and 6000 µg/liter high
  - SpectraMAX 250 plate reader (OD 600 nm)

Results

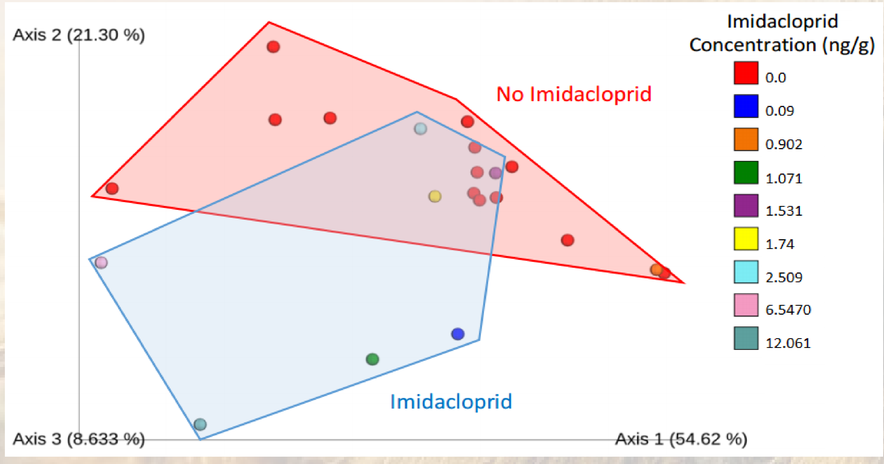
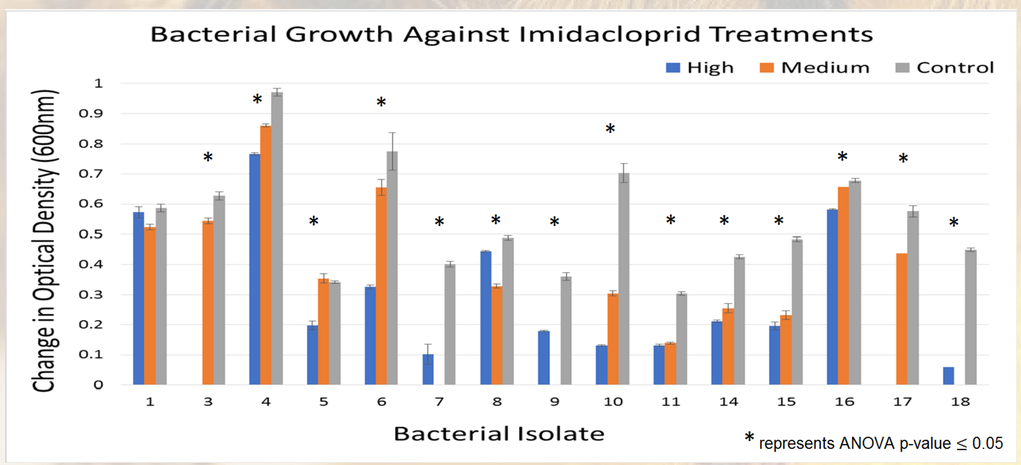


Figure 1: Non-Metric Multidimensional Scaling (NMDS) ordination of weighted UniFrac distance matrix of microbiome data. PERMANOVA: p = 0.04, indicating a significant effect of Imidacloprid concentration on microbiome structure.



Figures 2-3: Bacterial growth against Imidacloprid treatments in 96-well plate assays.

- Imidacloprid reduced the growth of all isolates tested except 1, 23, and 40, which were not affected by imidacloprid.

Figure 4: *Bacillus spp*. grown on MRS and blood agar.

- We isolated 56 morphologically-distinct isolates, 40 of which we sequenced and treated against Imidacloprid, excluding the isolates that were visibly fungi.

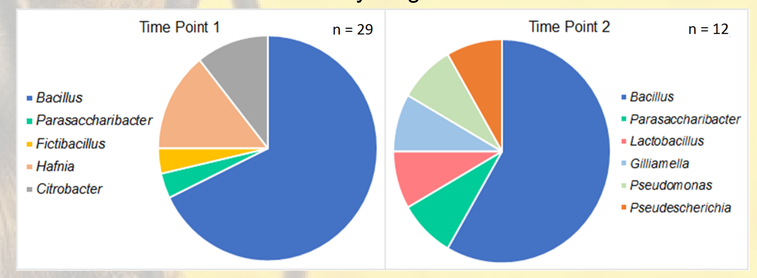


Figure 5: Cultured bacterial diversity among two hives at two time points.

Discussion

Field study: Bees with different levels of Imidacloprid had significantly different gut microbiomes (Figure 1). Future work will determine how these shifts in the gut microbiome affect host health.

Lab study: The majority of isolates tested exhibited reduced growth in the presence of even relatively low concentration of the pesticide. However, some isolates were robust to the pesticide (Figs. 2 and 3).

- These shifts in growth can lead to a dysbiosis within honey bee guts due to the suppression of beneficial symbionts, or the enhancement of these symbionts, leading to beneficial bacteria becoming pathogenic.

Identification: *Bacillus spp.* appeared to be the dominant culturable bacteria in the hives used for this study (Figure 5).

- *Bacillus spp.* are typically found in low abundance; however, they are very resistant and can survive storage in the freezer. This does not reflect actual relative abundance in honey bee guts, which are dominated by *Lactobacillus spp*.

- There is potential for implementing regulations on the application timing and use of Imidacloprid to protect pollinators.
- Our combined field and laboratory results about the host-microbe-pesticide interactions can have important implications for bee, and thus ecosystem, health.

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