

Survivorship of Apis mellifera in Response to Bacterial Challenge McKenzie Gray, Jonah Heller, Elizabeth Young, Justin Butera, Dr. Hartmut Doebel, Dr. Ioannis Eleftherianos Department of Biological Sciences, The George Washington University

Introduction: European honey bees (*Apis mellifera*) serve an important role in ecosystems as a pollinator. In the last 10 years *Apis mellifera* populations have been suffering from a condition known as Colony Collapse Disorder (CCD), a condition when honeybee colonies seemingly spontaneously abandon their hives. There are many hypotheses as to the stressors that lead to CCD including a parasite, *Varroa destructor*, and pesticides. An investigation into honey bees' innate immune response and functional assays could provide a better understanding of the contributing factors to CCD. For this study we chose to analyze the immune response induced by *E.coli*, a bacterium that is nonpathogenic to honey bees.

Aims

1. Develop a method to keep bees alive separate from the hive for extended time periods (24 hrs.+) to achieve Aim 2.

2. Develop methods to establish an infection model for a

nonpathogenic bacteria to serve as a baseline for further analysis.

Materials and Methods

AIM 1: Adult worker bees between 0 to 7 days old were obtained from a single hive and injected as described below. Two bees were kept in each box and fed water and honey ad libitum via feeders. Boxes were kept in an incubator at 35° C. Humidity was controlled in the incubator, and sponges soaked with water humidified the boxes, as well.





AIM 2: Bees were inoculated with 5μ L of ampicillin resistant *E. coli* solution (~100 bacterial cells) using an insulin needle inserted underneath the 4th tergum. Control bees were injected with 5 μ L of PBS. We then recorded how many bees were alive at certain time points after injection.

AIM 2: A second group of bees were injected as described above, washed in ethanol, and homogenized using a mortar and pestle at specific time points. This product was washed and centrifuged with PBS and spread on ampicillin bacterial plates. Bacterial colony counts of these plates will indicate the number of ampicillin-resistant bacteria that were injected previously that remain alive in the bees at each time point.





Figure 1. Survival curves for bees injected with PBS and *E.coli* and kept for a maximum of 48 hrs. post injection.



Figure 2. Survival curves for bees injected with PBS and *E. coli* and kept for a maximum of 96 hrs. post injection.



Figure 3. Bacterial plates for *E. coli* injected bees homogenized at A. 0 hr., B. 1 hr., C. 2 hr., and D. 24 hr. post injection.

Discussion

In the 48 hr. group, there is no statistically significant difference between the average number of hours survived for bees injected with PBS (p<0.05, $\mu=39.8$ hrs) and bees injected with E. coli (µ=41.3 hrs). In the 96 hr. group, there is no statistically significant difference between the average number of hours survived by bees injected with PBS $(p<0.05, \mu=86.5)$ and bees injected with E. *coli* (μ =77.9). This indicates that there is no effect of the needle injection injury on survivorship and supports that E. coli is nonpathogenic to honeybees. Also, the homogenization method could be affective in analyzing the ability of bees' immune systems to clear a bacterial challenge, but more consistent results are necessary.

Future Direction

- Continue modifying the homogenization and plating method to obtain bacterial colony counts at specific time points.
- Repeat survival trials using a pathogenic
- bacteria Pseudomonas aeruginosa.
- Repeat both methods using bees treated with sub-lethal doses of pesticides.
- Use molecular methods to analyze the immune response to these bacteria at the molecular level.

Acknowledgements : We would like to thank the Harlan Summer Research Program for funding and training. Also we thank Dr. Hartmut Doebel and Dr. Ioannis Eleftherianos for guidance.