The Longitudinal Effects of Supplemental Forage on Honey Bee Colony Growth, Immunity, and Pathogen Resistance

Project No.: **17-POLL14-McFrederick/Anderson**

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Objectives:

1. Determine if supplemental forage affects honey bee immunity by measuring pathogen load.
2. Determine if the availability of supplemental forage affects honeybee immunity by measuring production of immune compounds.
3. Determine if rapini cover crops and native plant cover crops provide the same quality of forage in regard to honey bee immunity.

Interpretive Summary:

Results in 2015-2016 showed large effects on colony survival, where colonies that were given supplemental forage before the almond bloom had greater survival later in the year. To test whether supplemental forage can help bees fight of disease, we are measuring the disease load and immune system function of honey bees exposed to two types of supplemental forage or no supplemental forage. We currently have the immune function data collection finished and are close to finishing the pathogen screens and quantification. These results will be crucial for our ability to identify benefits of supplemental forage.

Materials and Methods:

As weak colonies can obtain Black Queen Cell virus, Lake Sinai virus, and Sacbrood virus during the almond pollination (Cavigli et al. 2015), we will screen for these viral pathogens. Deformed Wing Virus (DWV) is also prevalent worldwide (Wilfert et al. 2016), so we will additionally screen for this virus. We will use the RNA portion of the TRIzol extraction to make complimentary DNA using random hexameric primers. We will then use specific primers to determine which samples contain viruses (de Miranda et al. 2013). To quantify viral load in samples positive for virus, we will conduct quantitative PCR using standard protocols (de Miranda et al. 2013). We will run both positive and negative controls for all reactions and run each reaction in triplicate.

We will follow a similar protocol for eukaryotic pathogens, but instead of using the RNA portion of the TRIzol extractions we will use the DNA portion of these extractions. We will screen for the common honey bee pathogens *Nosema ceranae*, *Nosema apis*, *Crithidia mellificae*, and *Apicystis bombi.* We will first test for pathogen presence using PCR, followed by quantitative PCR to determine absolute abundance in the positive samples (Meeus et al. 2010, Fries et al. 2013). We will again follow standard protocols including all appropriate controls and triplicate qPCR reactions. McFrederick will conduct nucleic acid extractions as well as virus and eukaryotic pathogen screens in his lab at UC Riverside.

To determine if *Varroa* load differs between treatments, we will measure mite levels at each sampling point. We will follow the COLOSS BeeBook standard methods for measuring mite infestation rate (Dietemann et al. 2013). We will collect 300 bees per colony into a jar with 2 mm hardware mesh screen on the lid, add one tablespoon of icing sugar, roll the bees and let them stand for 1 minute, shake the jar upside down onto a white cloth for one minute, then count the number of mites that have been dislodged.

To quantify bee immune response to the different cover crop treatments, we will use quantitative PCR to measure expression of two immune genes that have been shown to be upregulated in bees fed honey compared to those fed sucrose: the gene that encodes the antimicrobial peptide abaecin (Mao et al. 2015) and pale (Wheeler and Robinson 2014). We will additionally measure expression of genes that are known to be involved in bee immunity such as defensin, apidaecin, and hymenoptaecin, and the immune signaling gene Toll (Evans 2006, Evans et al. 2006, Chaimanee et al. 2012). We will run each PCR in triplicate, along with the honey bee housekeeping gene RPS5 as a baseline against which to measure relative expression (Evans 2006). Anderson will conduct immune gene expression analyses in his aboratory at the Carl Hayden Bee Research Center.

We will analyze longitudinal data using repeated measures ANOVA in the program R. We will check the residuals for homoscedasticity with normal quantile plots.

Results and Discussion:

The field work and colony health measurements for this project were conducted by the Niño lab and these components are now complete (see Niño project report).

We have completed DNA/RNA extraction, eukaryotic pathogen screening, and host immune gene expression of 30 bees per colony for each timepoint (32 colonies, 6 timepoints). We have also completed cDNA construction and will finish RNA virus screening this summer/fall. We will then analyze all data and present our final results at the Almond Conference.

Research Effort Recent Publications:

We published two papers from previous ABC support in 2018:

Rothman, J. A., Carroll, M. J., Meikle, W. G., **Anderson, K. E.**, & **McFrederick, Q. S.** (2018). Longitudinal effects of supplemental forage on the honey bee (*Apis mellifera*) microbiota and inter- and intra-colony variability. *Microbial Ecology*, *274*(80), 1–11. http://doi.org/10.1007/s00248-018-1151-y

Meikle, W. G., Holst, N., Colin, T., Weiss, M., Carroll, M. J., **McFrederick, Q. S.**, & Barron, A. B. (2018). Using within-day hive weight changes to measure environmental effects on honey bee colonies. *PLoS ONE*, *13*(5), e0197589. http://doi.org/10.1371/journal.pone.0197589

References Cited:

Cavigli, I., K. F. Daughenbaugh, M. Martin, M. Lerch, K. Banner, E. Garcia, L. M. Brutscher, and M. L. Flenniken. 2015. Pathogen prevalence and abundance in honey bee colonies involved in almond pollination. Apidologie.

Chaimanee, V., P. Chantawannakul, Y. Chen, J. D. Evans, and J. S. Pettis. 2012. Differential expression of immune genes of adult honey bee (Apis mellifera) after inoculated by Nosema ceranae. Journal of Insect Physiology 58:1090–1095.

de Miranda, J. R., L. Bailey, B. V. Ball, P. Blanchard, G. E. Budge, N. Chejanovsky, Y. P. Chen, L. Gauthier, E. Genersch, D. C. De Graaf, M. Ribière, E. Ryabov, L. De Smet, and J. J. M. van der Steen. 2013. Standard methods for virus research in Apis mellifera. Journal of Apicultural Research 52:1–56.

Dietemann, V., F. Nazzi, S. J. Martin, S. J. Anderson, B. Locke, K. Delaplane, Q. Wauquiez, C. Tannahill, E. Frey, B. Ziegelmann, P. Rosenkranz, and J. D. Ellis. 2013. Standard methods for varroa research. Journal of Apicultural … 52:1–54.

Evans, J. 2006. Beepath: an ordered quantitative-PCR array for exploring honey bee immunity and disease. Journal of Invertebrate Pathology 93:135–139.

Evans, J. D., K. Aronstein, Y. P. Chen, C. Hetru, J. L. Imler, H. Jiang, M. Kanost, G. J. Thompson, Z. Zou, and D. Hultmark. 2006. Immune pathways and defence mechanisms in honey bees *Apis mellifera*. Insect Molecular Biology 15:645–656.

Fries, I., M.-P. Chauzat, Y. P. Chen, V. Doublet, E. Genersch, S. Gisder, M. Higes, D. P. McMahon, R. Martin-Hernandez, M. Natsopoulou, R. J. Paxton, G. Tanner, T. C. Webster, and G. R. Williams. 2013. Standard methods for *Nosema* research. Journal of Apicultural Research 52:1–29.

Mao, W., M. A. Schuler, and M. R. Berenbaum. 2015. A dietary phytochemical alters caste-associated gene expression in honey bees. Science Advances 1:e1500795–e1500795.

Meeus, I., D. C. De Graaf, K. Jans, and G. Smagghe. 2010. Multiplex PCR detection of slowly evolving trypanosomatids and neogregarines in bumblebees using broad-range primers. Journal of Applied Microbiology 109:107–115.

Wheeler, M. M., and G. E. Robinson. 2014. Diet-dependent gene expression in honey bees: honey vs. sucrose or high fructose corn syrup. Scientific Reports 4.

Wilfert, L., G. Long, H. C. Leggett, and P. Schmid-Hempel. 2016. Deformed wing virus is a recent global epidemic in honeybees driven by Varroa mites. Science 31:594–597.