IMPROVING HONEY BEE HEALTH BY USING A SYSTEMS BIOLOGY APPROACH

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Abstract

Honey bee (*Apismellifera*) populations around the world are being reduced by several factors recently. Many previous studies have focused on investigating a single or two factors at a time in well controlled laboratory studies. However, the stressors faced by bees are environmental in nature. Therefore, we conducted a study to determine how these environmental factors (disease conditions, pesticides) affect bee health, as many technologies have been developed to identify the relation between those factors and how they induce synergistic decline in bee health. In this project, we work on characterizing the exposome of the honey bee and search for associations between the exposome and *Nosema spp*, Foulbrood, and viral diseases and how this varies with pesticide exposure. We identified 31 compounds that could serve as biomarkers for monitoring bee health, several of which are found in highly conserved fatty acid metabolic pathways, suggesting that these may be used as robust biomarkers of bee health.

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1. Introduction

The western honey bee is one of the most important animal pollinators in the agricultural and food industry.[1] Although they are managed intensely, their population size is decreasing because of multiple stressors such as bacterial, parasitic or viral infections, habitat loss, nutrition deficiency, and pesticide exposure.[2,3] All of these factors affect the bee health at the individual and colonial level. In addition, these factors may interact with one another and this interaction may result in synergistic or antagonistic effects on the hive health.[4]Although many of these factors have been well studied, most have been in isolation or just a combination of two factors in well-controlled laboratory experiments. Many correlations have been identified between pesticide exposure and acquiring honey bee diseases.[5] One of those diseases is the microsporidian gut pathogen *Nosema cerenae*. Bees that are exposed to fungicides consistently are more susceptible to suffer from *Nosema sp* disease.[6] Other examples of stressors include the relation between Foulbrood disease that is a fatal bacterial disease that affects the brood of the honey bee and is caused by the spore forming bacterium *Paenibacillus larvae*. Because there is no cure available for this disease yet, the best solution to contain the infection is to destroy the whole bee hive before it spreads to others.[7] Moreover, Siviter and colleagues (2018) demonstrated that there is an inverse proportion between pesticide exposure and learning and memory of honey bees.[8] Because all of these stressors are environmental in

nature, a new approach is needed to study the bee hive in the field from a holisitic perspective so that all environmental factors can be measured and considered.

Therefore, a systems biology approach is needed to address the issue of honey bee population decline.Traditional big-data analyzing methods (genomics, transcriptomics, proteomics etc) can complement a new field; exposomics.[9]The exposome is firstly defined as "how environmental exposures affect the genetic factors under disease condition". Basically, the exposome is all of the chemicals that an individual encounters in its lifetime. The exposome can be expanded into 3 sub-categories[10] in honey bee context such as; general external (climate),specific external (pollution, nutrition, communication signals between the hive members etc) and internal exposome (metabolism, activity of microbiome and oxidative stress).

In our project, our aim is to characterize the exposome of honey bee and identify significant associations with disease infections and pesticide exposure to establish reliable and robust biomarkers that will accurately predict a decline in bee health. We examined exposomic data generated from 30 different bee hives sampled from the Philadelphia area in the Unites States. We integrated this exposomic data with health data (disease and pesticide status) to identify biomarkers for specific diagnoses of diseases like *Nosema spp* and Foulbrood. Ultimately, with established volatile biomarker profiles, we can use our findings to monitor hive health, using an absorbent silicone wrist band to sample hive air.

2. Materials and Methods

2.1. Normalizing the Disease and Pesticide Data

For analysis, we used MassProfiler Professional software (Agilent Technologies) and we conducted an initial analysis, a statistical analysis and then afold-change analysis. Then we enetered all significant compounds into the online KEGG Pathway Database to understand the possible mechanistic effects on bee metabolic pathways and bee health.Each disease (viral, bacterial, and fungal pathogen) was normalized such that it forms an index that are comparable to one another. Each disease could then be added to a normalized pesticide exposure index to yield an overall health index.

2.2.Initial Analysis

We created new experiment on Mass Profiler Professional software by Agilent Technologies. We set analysis type as "Mass Profile Professional" and experiment type as "Identified + Non-identified". The workflow was set to "Analysis: Significance Testing and Fold Change". We selected data source as MassHunterQual/ Profinder because .CEF files were obtained from previous part of the project from MassHunter associated with GC Q-TOF analyses. For organism selection for the integrated KEGG pathway analyses, we chose *Apis mellifera* through a Custom choice. We then imported .CEF files generated from our colleagues. For filtering minimum absolute abundance was set to 5000 counts, minimum retention time was set to 15 minutes, maximum retention time was set to 37 minutes, and minimum number of ions was set to 2. No normalization criteria were selected as this was previously conducted

using MassHunter. For baselining options, we selected “Baseline to the median of all samples”.

2.3.Statistical Analysis

For each parameter,representing each index,3 groups were made as high, medium, and low. In addition, due to the low prevenlence of the disease we divided the Foulbrood index data just into two groupings: infected and non-infected.All of the entities we obtained from the initial analysis were included. For indices with 3-groupings, we conducted a One-Way ANOVA with unequal variance, for indices with 2-groupings, we conductedan Unpaired t-Test. After conducting the ANOVA and Unpaired t-Test, we applied Tukey post hoc test tocomparethe three groupings across all entities in the exposome. Multiple Testing Correction was accounted for aswe applied a Benjamin Hochberg(FDR) correction method.

2.4.Fold-Change Analysis

After statistical analyses, a fold-change analysis was used to generate figures and a correlational analysis across the high, medium, and low groupings for each index (Figure 1-2-3-4-5)**.**

2.5.Pathway Identification

After the results are obtained from several analyses, each significant entity was searched on the KEGG (Kyoto Encyclopedia of Genes and Genomes) database for *Apis mellifera* to see if the compound has a role in honey bee metabolic pathways or not. In addition, we searched every compound in the literature to identify their possible impact on honey bee health decline.

3. Results

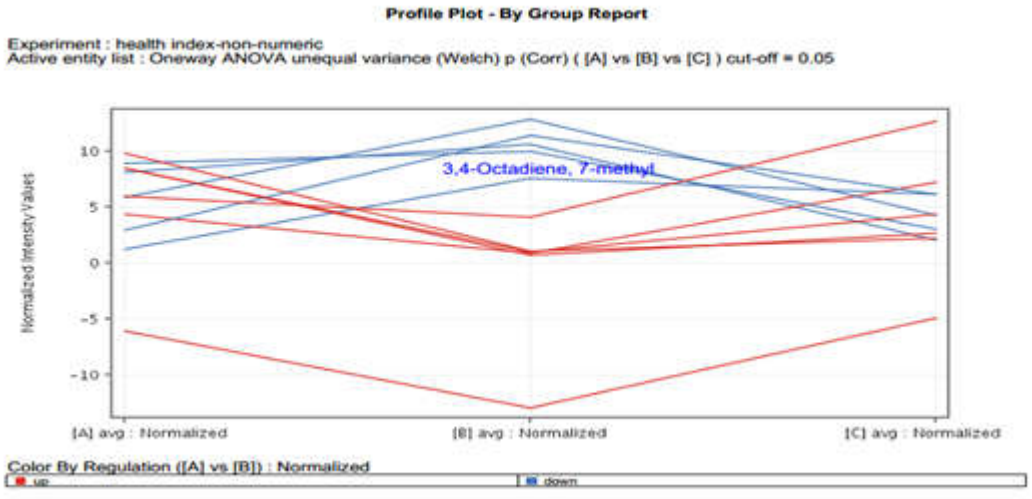

Figure 1: Health Index Fold Change Analysis; A,B and C indicates high, medium and low infection,respectively

In the above health index results (Figure 1), 11 compounds were identified. Only 1compound (3,4 Octadiene,7 methyl-) had a consistent down regulation from highly infected samples to lowly infected ones.

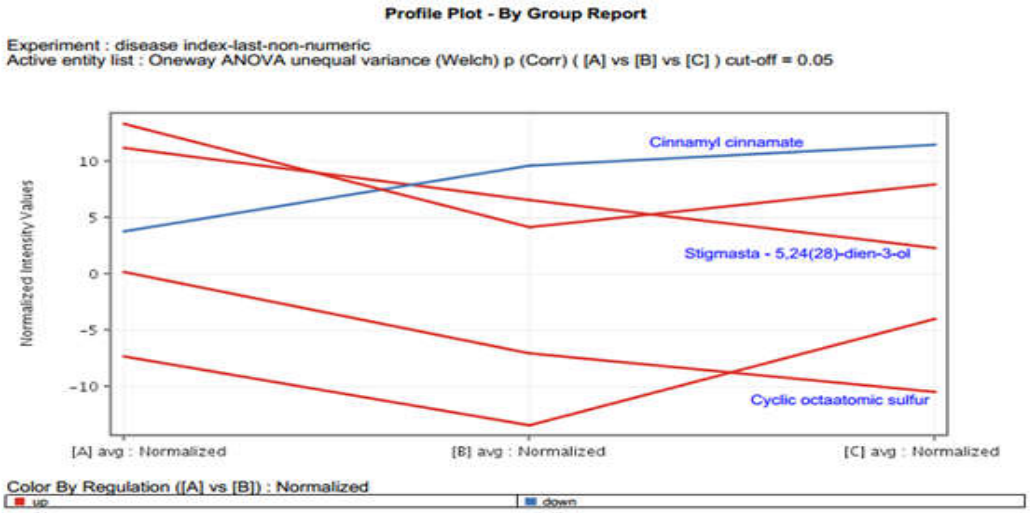

Figure 2: Fold change analysis of Pesticide Index; A,B and C indicates high, medium and low infection,respectively

In disease index fold change analysis(Figure 2),5 significant compounds were found. Two of them (Stigmasta-5,24(28)-dien-3-ol, and (3.beta.,24Z)- and Cyclic octaatomic sulfur) were upregulated and one of them (Cinnamyl cinnamate) was down-regulated from when going from high infection to low infection across the pesticide index.

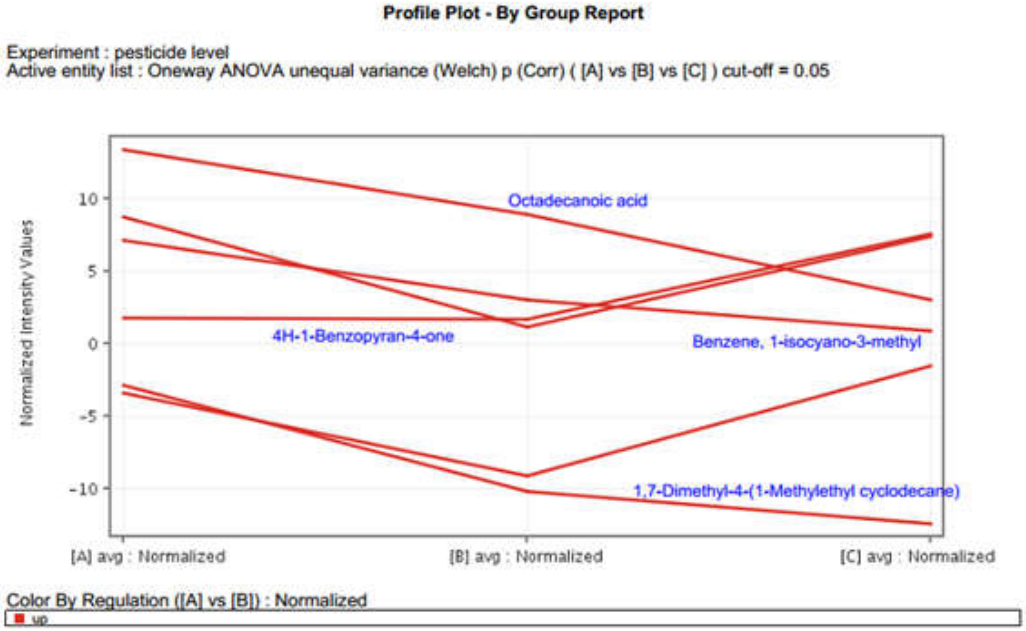

Figure 3:Fold Change Analysis of Pesticide Index; A,B and C indicates high, medium and low infection,respectively

In the pesticide index data (Figure 3) analysis, 6 significant entity associations were identified. Because 4 of the entities: (1,7-Dimethyl-4-(1-methylethyl) cyclodecane; Octadecanoic acid;4H-1-Benzopyran-4-one, 2,3-dihydro-5,7-dihydroxy-6,8-dimethyl-2-phenyl-, (S)- and Benzene, 1-isocyano-3-methyl-) show consistency from high infection to low infection and could be potential biomarkers, these entities are highlighted. All of the entities are down-regulated from high infected samples to low infected samples.

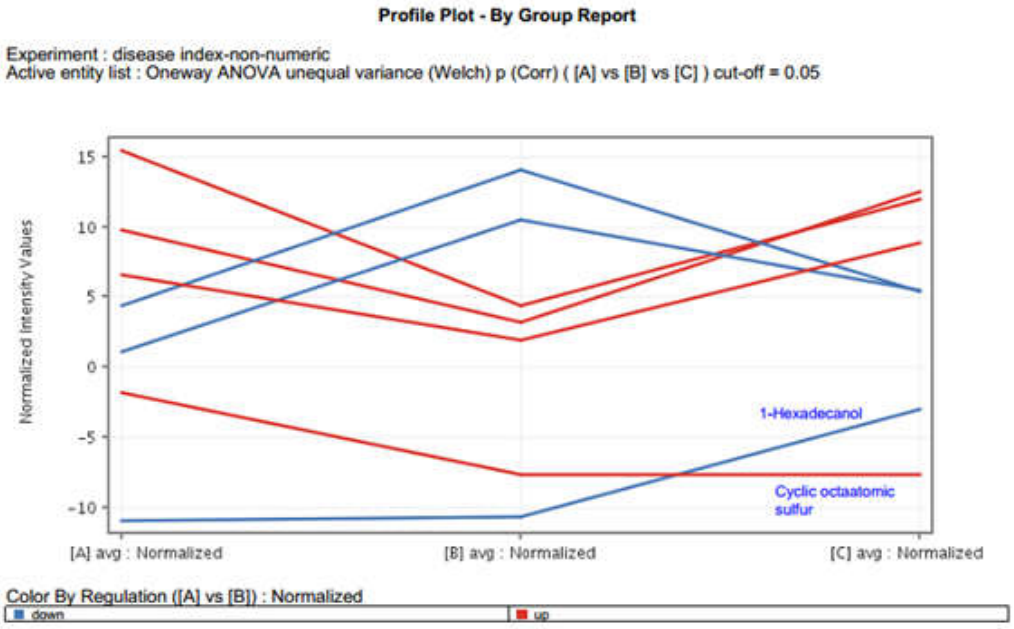

Figure 4: Viral Index Fold Change Analysis; A,B and C indicates high, medium and low infection,respectively

In Figure 4, viral index data is analyzed and 7 possible biomarkers were identified. One compound, 1-Hexadecanol, is down-regulated and one compound, cyclic octaatomic sulfur, is down regulated from high infection to low infection.

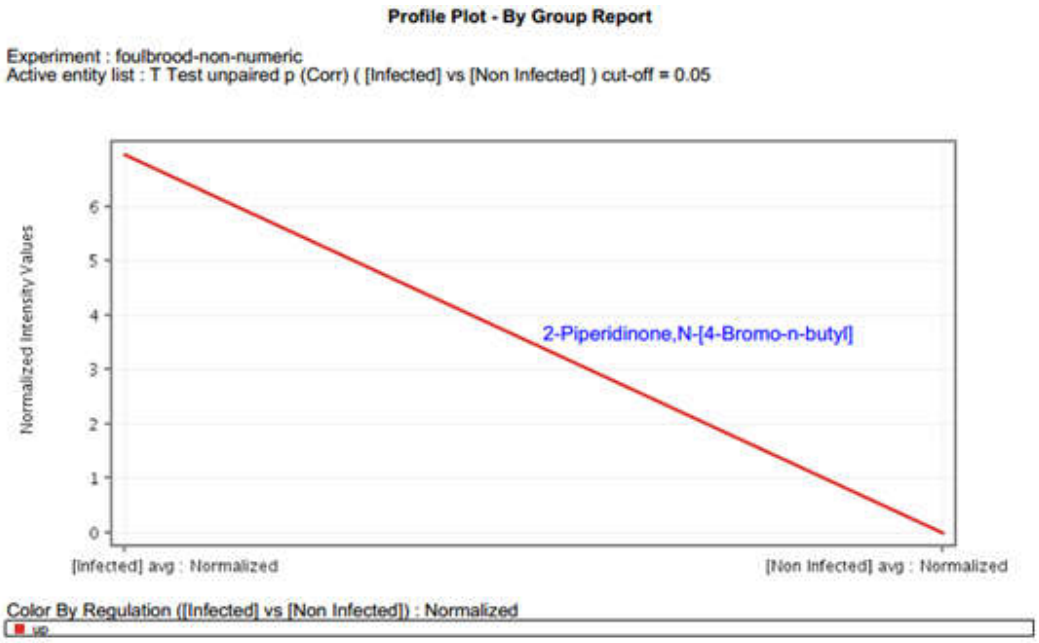

Figure 5: Foulbrood Index Fold Change Analysis

Lastly in Figure 5, when the Foulbrood index is analyzed there is only one up-regulated compound (2-Piperidinone,N-[4-bromo-n-butyl]) identified.

4. Conclusion and Future Work

After several statistical and fold-change analyses we identified biomarkers that might be placed in several different metabolic pathways and determined if they could have potential synergistic effects on the decline in bee health by interacting with one another.

Some potential biomarkers play a role in metabolic pathways such as phototransduction, unsaturated fat biosynthesis, and fatty acid degradation of *Apis mellifera* metabolism. For example, octadecanoic acid is found in phototransduction pathways of honey bee (*Apismellifera*) and1-Hexadecanol is also found in the fatty acid degredation pathway of *Apis mellifera*.

For future work, there is an ongoing study to develop a better understanding of the impact the up-regulation and down-regulation of these metabolites on bee health. In addition, there is a goal to develop a new technology that will capture these recently-identified biomarkers to monitor bee health on a regular basis. All of these biomarker identifications will help researchers build a biomarker library that can increase the accuracy and precision of bee health diagnoses by correlating exposome data and health data. These assays can contribute to improving bee health by using absorbent silicone wrist bands to sample hive air to be analyzed in the lab for volatile chemical biomarkers that are linked to a decline in bee health.

5. References

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