Honey Bees: (1) Controlling Viruses and Nosema with Essential Oils; (2) Viral Load Relationships to Pests, Parasites, Diseases, and Stress

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Project No.: 10-POLL9-Wick

This project is currently collecting data and processing samples, with most of the work done the last sets of data need to be collected and analyzed. The final data analysis will be delayed by 12 months so that a complete data set may be collected, processed and analyzed.

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Background

➢**INTRODUCTION**

The project is collecting data and processing sample collection and is moving forward. The final data analysis will be delayed by 12 months so that a complete data set may be collected, processed and analyzed.

One of the most significant challenges currently facing the beekeeping industry is to find ways of controlling the viruses that threaten their colonies and livelihoods. A recent and major development in that regard has been the ability to identify and measure viral loads in honey bees, using the Integrated Virus Detection System (IVDS).

However, although even though viruses now detectable and identifiable, what is still needed are reliable treatment methods—and especially comparative analyses of their efficacy.

Using IVDS as a tool, one component of this project is designed to document the use of eight specific essential oils to control viruses, using LeFore commercial essential oil patties. The oils will be tested both individually and in different combinations in patties.

The project's other component, will use IVDS to measure viral loads in bees as part of the USDA/ARS Weslaco Project. IVDS is a means to correlate quantitative and qualitative virus data with measures associated with key factors being tested to improve colony heath and productivity.

The virus nutrition relationship studied in this project is building on previous studies for nutritional quality that has shown to be a beneficial factor in honeybee health and in stimulating population growth, queen quality, and reducing the impact of varroa mites. What has been missing in these studies is the bee virus relationship and factors in bee health. The ability to detect viruses in bees and to observe the change in diversity and titer of each virus over time has been demonstrated by BVS using the Integrated Virus Detection System (IVDS) with the names of several viruses being associated with peak sizes funded by the Almond Board of California. IVDS uses size measurements as identification of individual viruses and has demonstrated multiple virus detections in a single sample as well as the concentration of each virus. Appling this ability to evaluate the nutritional affects on virus diversity and virus concentrations in bee colonies will give us a direct correlation to nutrition on bee viral loads and will help us develop strategies for controlling viral loads in honeybees.

Many of the viruses detected in bees do not show active infection as seen by some symptomatic evidence as noted by the work of L. Bailey but do show up in the IVDS screening and is used as a measurement of how well the bees are fighting off infection. Bee viruses are opportunistic by nature and will increase in the bees as the bee health declines for any of various reasons.

BVS, Inc is working with Bee Alert Technologies, Inc and the related team for a greater understanding of iridescent virus methods for detection and screening.

Results and Discussion:

As a part of this project we addressed some of the pre-collecting and processing of samples to insure data integrity. The quality of samples at various stages of processing, collections, storage and filtration has been a concern in regards to viral intensity and diversity in sample to the degradation of virus integrity in the sample. In cooperation with the University of Montana with BVS, Inc., virus samples were studied for integrity from storage at various temperatures and media. While this is not the focus of this project, sample integrity is important for consistent data comparisons over time. The summary of these projects have shown that there is some titer loss from various filters, the virons are attaching to some of the materials used in the filters or attaching to the debris capture by the filter. We singled out the best filter for our application and have only minimal loss of titer at less than 10% and no diversity loss by using a 20 micron paper filter and syringe holder. The temperature storage and media applications for sample preservation showed no vial titer loss or diversity loss from frozen to fresh samples over time. The largest loss of apparent viron detections as seen on the IVDS detector were from samples stored partially processed in refrigeration but not frozen, the loss was greater than 20% after 72 hrs. but by processing sooner (within 24hrs) there was no apparent loss. The processed and ready for IVDS sample storage time is one week at 35F with no apparent loss. The processed sample ready for IVDS and frozen at -30C does not seem to have any degradation after one year.

The methods used for sample shipping, processing and storage demonstrate integrity to the intact viruses processed by IVDS and increases the project reliability for tracking viral titers and diversity over time using IVDS.

Acknowledgments

BVS, Inc would like to thank the organizations that have brought the IVDS technology to the nations beekeepers.

➢Project Cooperators: Frank A. Eischen, USDA/ARS Weslaco, TX;

➢Michael Stanford, Army Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD;

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- ➢Pat McCubbin,
- ➢Charles Wick,
- ➢Samir Deshpande,
- ➢Rabih Jabbour and
- ➢Michael Stanford.

My thanks to the Almond Board of California for this step forward in technology for honey bees, without your support this project would not have been feasible.

Components and Results

Objectives:

Document if use of specific essential oils reduce viral loads in honey bees as measured by the Integrated Virus Detection System (IVDS). Also assess if essential oils provide Nosema control. Essential oils will be administered to bees via LeFore essential oil patties.

As a separate project component, measure viral loads using IVDS in the existing USDA/ARS Weslaco Project bee colonies. This project is assessing an integrated approach to controlling hive pests and improving honey bee health. IVDS will be a tool to determine if test practices reduce viral loads.

- Determine the viral diversity change over time from adding nutrition to the bee diet.
- Determine the viral titer change over time from adding nutrition to the bee diet.
- Evaluate the nutrition levels needed to affect these changes.
- Determine which viruses are most affected by nutritional changes.
- Determine the timing intervals for adding nutrition that will have the highest impact on viral loads.

Sample Collection Continuity – Time of Day Test

A time of day sample collection for variables in viral load changes was conducted with statistical analysis by Brian Steele, Department of Mathematical Sciences, the University of Montana, Missoula MT, 59812, USA; Time of Day Sampling Design was by Bee Alert Technologies, Inc., Missoula, Montana; the Sampling Coordination was done by Dr. V Sivaram, the University of Montana.

The data originated from 9 hives.
Collected at three times of day (morning, afternoon , and evening).
Total of 27 samples on numbers of particles belonging to a particular size class.
To determine if there are patterns in the counts associated with time of day.

Summary:

There is *convincing* evidence of the presence of the sacbrood virus in every hive.

There is *unconvincing* evidence of differences in number of virus particles among different times of day.

Participation with the study for the interaction of nutritional stress, varroa parasitism, nosematosis and their consequences for viral, bacterial and fungal infections with the USDA Weslaco Lab.

The current project to investigate the Affects of Nutrition on Virus Diversity and Titers, our data set and sampling have increased beyond the original estimations, but will yield a better statistical analysis for this project that will not be available until next year. The initial data is consistent with previous work and is showing titer and diversity changes over time. This has not been correlated with the nutritional data nor with the other components of this project such as varroa mite levels and Nosema relationships, this would be premature.

This project is in conjunction with the USDA Weslaco laboratory where we have processed over 800 samples to date and are nearly at the half way point of this portion of the study.

We have preliminary data that shows the diversity frequency and comparative titer data on over 800 samples processed.

Time of Day Test

Counts for each hive plotted against size, and for each time of day.
The peak associated with the sacbrood virus is apparent in each panel.
Clear differences among time of day with respect to counts are not visible.
Morning counts are not greater than the afternoon and evening counts.

There is a fairly consistent difference in the particle counts over the course of a day;
- Morning counts are less than afternoon and evening counts.
- Differences between afternoon and evening counts are not consistent.

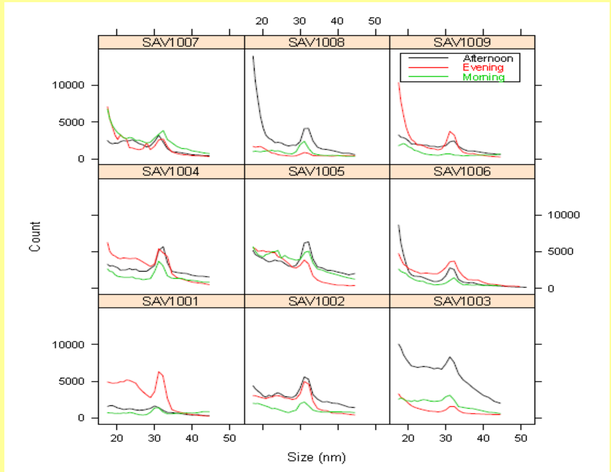
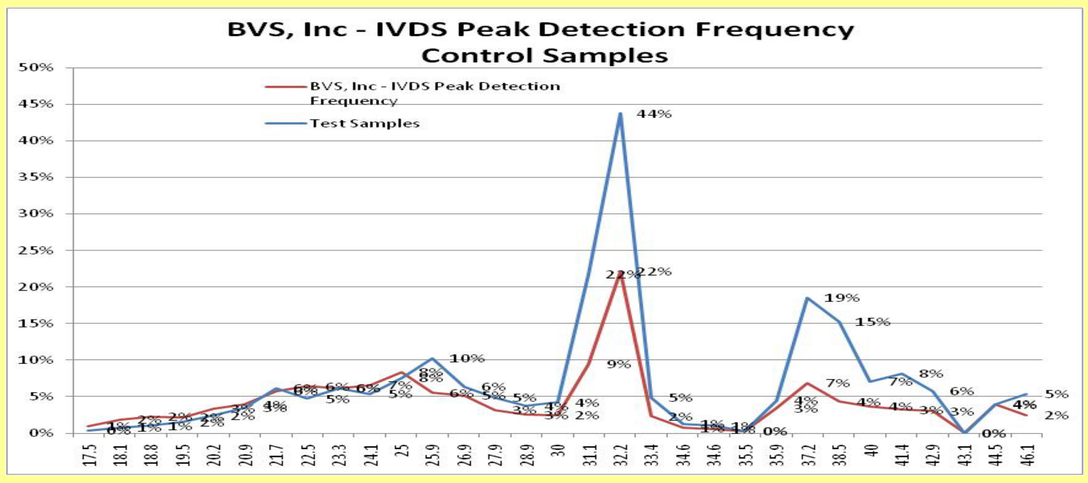


Figure 2: Distribution of counts over size class, by time of day and hive.

Viral Interaction Study

Figure 4 compares the frequency of detections from the total database set with the test sample set. The test sample set is showing the same trends as we have in the overall data set with the frequency increases in the test set shown. There is a noted increase in the frequency trend of Israeli Acute Paralysis Virus in the test set vs. the database set. With the additional data from nutritional factors we should be able to correlate a relationship between viral and nutrition levels.



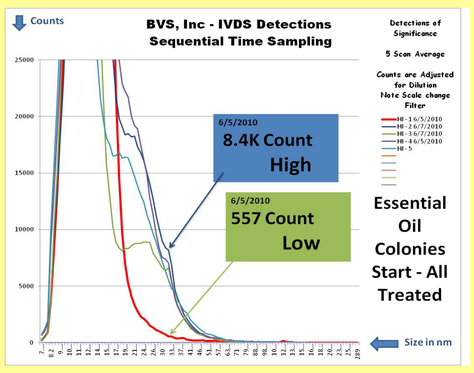
Number of samples containing these viruses	29	94	85	181	88		545	48		420	118
Virus Name	Satellite Virus	Deformed Wing Virus	Kashmir Bee Virus	Isralli Accute Paralysis Virus	Acute Bee Paralysis Virus	NO ID	Sacbrood Virus	Black Queen Cell Virus	NO ID	Chronic Paralysis Virus	Not identified

Figure 5 is a correlation table with the number of detections for the named viruses in the database. This is a product of a project to name the peaks funded by the Almond Board of California.

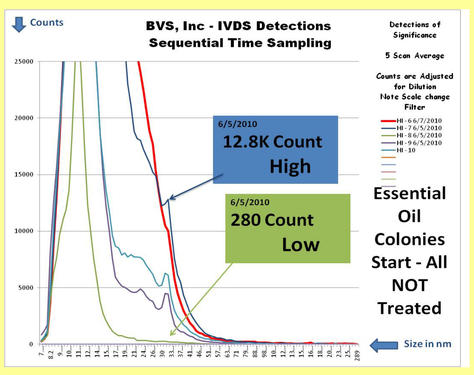
Materials and Methods:

Bees were collected and delivered to BVS for processing.
Initial processing for BVS processing was done at Weslaco that provided a 50ml solution of 100 bees ground and mixed with 50ml of deionized water.
Samples were then frozen and shipped to BVS for processing.
At BVS each sample is filtered though standard cheesecloth to remove non soluable bee parts.
30ml of the sample is centrifuged for 60 minutes at 20,000 X g.
The supernatant is recovered and Ultrafilterd through a 500,000 Dalton hollow fiber filtration system and a 250ml RO wash which is then reduced to ~ 2ml. This produces a concentration of the viues in the sample.
The solution is prepped for IVDS by a 1:10 dilution using with Ammonium Acetate (AA) as the salt for controlled conductivity in IVDS.
Each sample is filtered through a w-41 20um paper or a .45um PTFE filter, for removal of fatty and pollen residues that tend to float in the solution after centrifucation.
IVDS uses a 5 scan average and is then saved in the IVDS database.
Charts and tables are created from the exported data from IVDS.

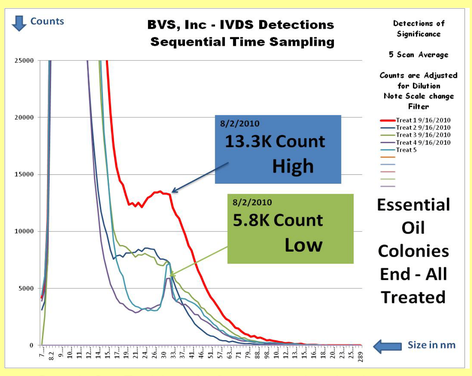
Essential Oil Study



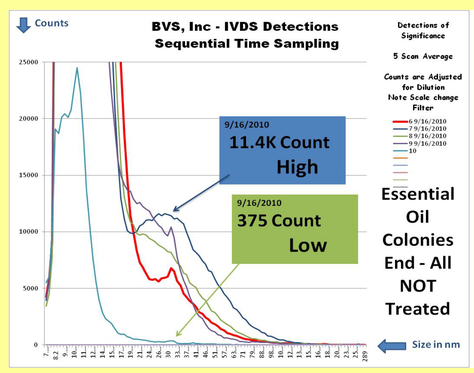
Graph 1 – Start levels for treated colonies.



Graph 2 – Start levels for non-treated colonies.



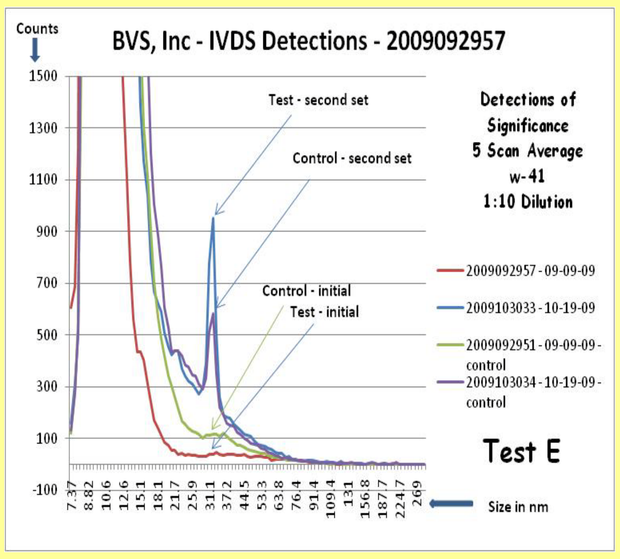
Graph 3 – End levels for treated colonies.



Graph 4 – End levels for non-treated colonies.

Sequential Sampling Test

The following chart (figure 1) represents some of the initial test samples compared to the control sample with only the initial and second data sets represented and only from one apiary. The correlations to other variable factors will we added into the analysis of the entire study, but this demonstrates the viability of the Intergrated Virus Detection System (IVDS) to be able to track changes in viral diversity and intensity.



Time of Day Test

Counts are greater in the afternoon compared to the morning.

If viruses *besides sacbrood are absent from the hives, then the* pattern of greater counts in the afternoon compared to morning may be attributable to particles besides the sacbrood virus.

In conclusion, there is no clear evidence of differences in numbers of sacbrood viral particles by time of day.

The sample taking location for this study was from the front of the hive, collecting foragers.

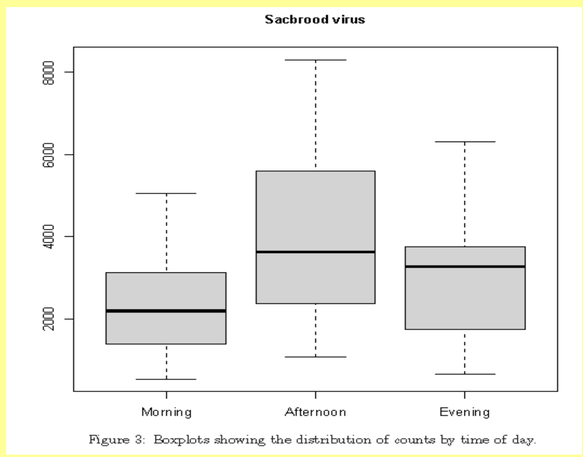


Figure 3: Boxplots showing the distribution of counts by time of day.

The samples were taken from existing colonies for this project which brought existing viral loads and nosema levels into the project baseline.

It is important to note that Essential Oils work as a preventative measure on viral loads and nosema levels. Not as a cure.

Essential Oils works over time and this is the first year on these colonies.

No significant viral load differences between these two groups of colonies were observed.

No changes were observed for nosema levels from the treated and non-treated colonies from start to end.

This project is continuing through next year, with these and with new colonies for both control and experimental groups.