Effects of Nutrition on Virus Diversity and Titers

Project No.: **09-POLL9-Wick**

Project Leader: David Wick
BVS, Inc.
5501 HWY 93 N., Suite 6
Florence, MT 59833
(406) 369-4214
E-mail: mrwick@bvs-inc.us

Project Cooperators and Personnel:
Frank A Eischen
Research Entomologist
2413 E. Highway 83 #213
Weslaco, TX 78596
(956) 969-5007
E-mail: frank.eischen@ars.usda.gov

Dr. Michael Stanford
Deputy Team Leader
Point Detection Team
ECBC
AMSRD-ECBC-RT-DD BLDG E3160
5183 Blackhawk Road
APG, MD 21010-5424
(410) 436-6887
E-mail: mfstanfo@apgea.army.mil

Objectives:

- 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.

Interpretive Summary:

The initial project was to use the existing bee colonies at the Carl Hayden Bee Research Center for both the controls and the experiments. A nutrition schedule was planned to be implemented for nutritional levels and timing. The colonies were then to be sampled and sent to BVS for IVDS screening to determine the viral loads. This project was in cooperation with Dr. Gloria DeGrandi-Hoffman, Research Leader at the Carl Hayden Bee Research Center.

The project location and cooperators were changed to the USDA – ARS laboratory at Weslaco, Texas with Dr. Frank Eischen and his team. This was in cooperation and notification to the Almond Board of California and Dr. Gloria DeGrandi-Hofman at the Carl Hayden Bee Research Center, USDA – ARS labroratory, Tucson, AZ. The reasoning for this change was to increase the scope of this investigation by combining the viral diversity and titer changes over time to an existing project being conducted at the Weslaco lab that included nutritional aspects that are compilmentry to this project. The advantages are an increased number of samples run and a greater number of interrelationships to bee health and productivity, we felt that this would have a greater impact for application of research to the commercial beekeeper. We have processed over 800 samples at this date whereas the project proposal only called for a total of 500 samples. The scope of this project has grown to include more samples, address more variables, and has increased the time frame of the project from 10 months to 22 months.

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.

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 honey bee 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 honey bees.

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.

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.

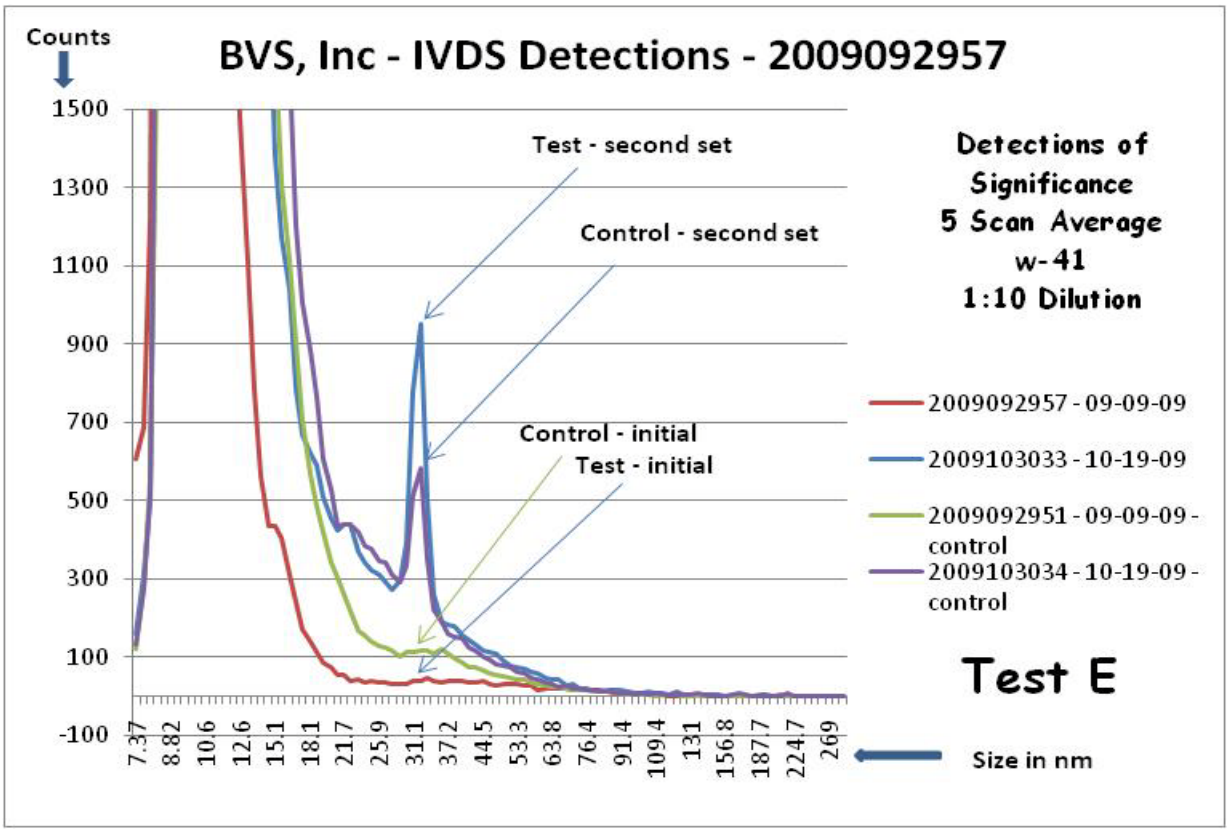

Figure 1

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.

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 degragation 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 consistant 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 apperent 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 apperent loss. The processed and ready for IVDS sample storage time is one week at 35F with no apperent loss. The processed sample ready for IVDS and frozen at -30C does not seem to have any degregation after one year.

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

We are currently investigating the time of day sample collection for variabity in viral load changes.

As for the current project to investigate the Effects 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 consistant with previous work and is showing titer and diversity changes over time. This has not been correlated with the nutritional data or with the other components of this project such as varroa mite levels and Nosema relationships, this would be premature.

The following in **Figure 2** is an example data chart for the work done thus far, this chart is showing the difference in titer for a single marked virus and **Figure 3** is showing the diversity and changes over time.

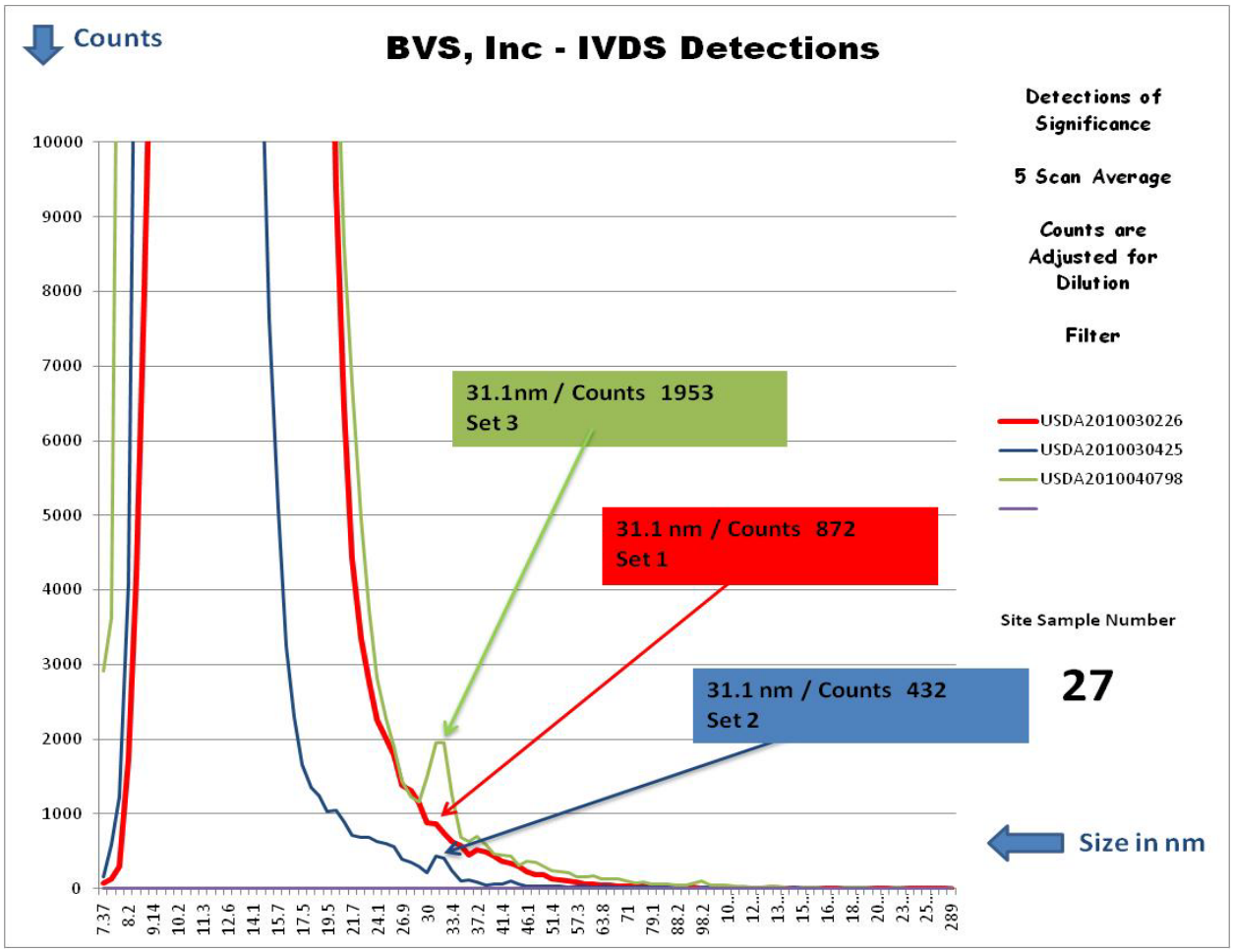

Figure 2

BVS, Inc
5501 Hwy 93 N, Suite 6
Florence, MT 59833
406-369-4214

Virus Detection Report

High levels	1
Medium levels	2
Low Levels	0

Reference Number		Virus diversity per sample	Satellite Virus				Deformed Wing Virus			Kashmir Bee Virus		Israili Accute Paralysis Virus			Acute Bee Paralysis Virus			Sacbrood Virus			Black Queen Cell Virus			Chronic Paralysis Virus												
							DWV			KBV		IAPV			ABPV			SBV			BQCV			CPV												
			Virus size in nm																																	
			17.5	18.1	18.8	19.5	20.2	20.9	21.7	22.5	23.3	24.1	25	25.9	26.9	27.9	28.9	30	31.1	32.2	33.4	34.6	34.6	35.5	35.9	37.2	38.5	40	41.4	42.9	43.1	44.5	46.1
USDA2010030226	27	0												2													0							
USDA2010030425	27	2					2							0						0															
USDA2010040798	27	0																		2						2									

Figure 3

What we do have for this project is the diversity frequency and comparative titer data on over 800 samples processed.

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 Israli 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.

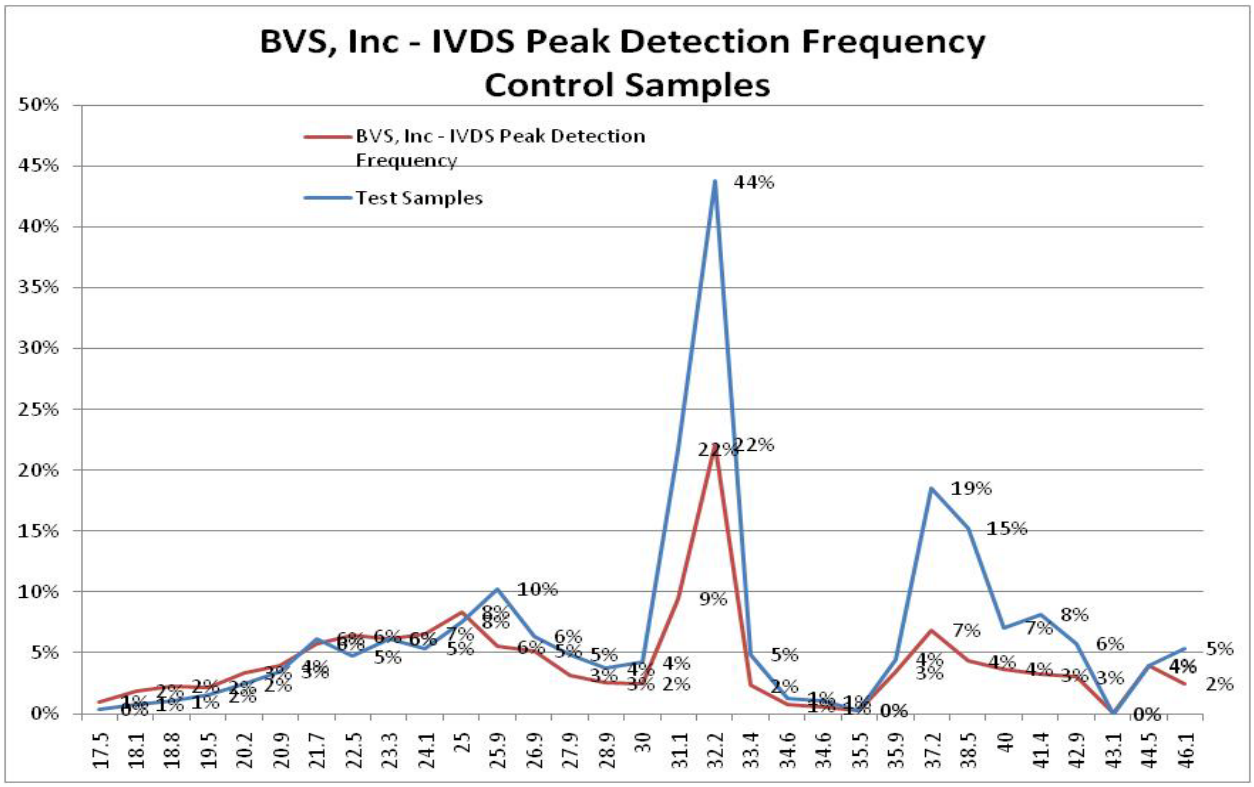

Figure 4

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.

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	Israli Accute Paralysis Virus	Acute Bee Paralysis Virus	NO ID	Sacbrood Virus	Black Queen Cell Virus	NO ID	Chronic Paralysis Virus	Not identified

Figure 5

Research Effort Recent Publications:

No current publications.

References Cited:

none