# Identification of the Chemical(s) Associated with the CCD and Observed Loss of Bee, Lack of Robbing, and Exclusion of Hive Beetle and Wax Moths

**Project No.:** **07-POLL8-Bromenshenk**

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**Objectives:**

Bee Alert received notice of an award on August 1, 2007. The objective of our project is to continue to work on the identification of the Chemical(s) Associated with the CCD and the Observed Loss of Bee, Lack of Robbing, and Exclusion of Hive Beetle and Wax Moths.

**Interpretive Summary:**

Initial funding for Phase I of the project was received from the National Honey Board. Those funds allowed us to travel to collect initial samples, to develop methods for processing samples, and to begin the process of looking at issues such as the presence of aflatoxins, as well as HMF (hydroxylmethylfurfural), a contaminate of high fructose corn syrups).

A key result of our initial efforts was that as a result of working with the U.S. Army's ECBC laboratory, we now have put in place sample preparation methods necessary for virtual PCR and advanced proteomics work.

Proteomics involves qualitative and quantitative comparisons of expression, functions, and interactions of proteins to further unravel complex biological processes. The separation of protein complexes can be used not only to screen bee samples for a wide array of pathogens and but also for using the expression of proteins as biomarkers of pathology. In essence, we're laying the groundwork for new tools for clinical research regarding bee diseases. And, we've tied these analytical approaches back to screening for viruses, fungi, and bacteria.

In the course of our initial work, we've had opportunities to add some new scientists and laboratories to our investigative team. Dr. Robb Cramer, a fungal specialist, has joined the faculty at Montana State University, our sister campus in Bozeman. Dr. Cramer has launched a research program aimed at better understanding of *Nosema cerana*, a newly discovered fungal pathogen in the United States. *N. cerana* is of particular concern, since many of the bee operations sustaining damage from CCD also showed high levels of infestation by this fungus. In addition, some fungi are known to produce chemical toxins. Therefore, we have partnered with Dr. Cramer, and he will be collaborating with us on our Almond Board project.

In addition, Dr. Rosalind James, the director of the USDA Native Bee Laboratory in Logan, Utah is working with us. She is looking at bacteria in honey combs, and again, she will assist in identifying chemical products that may be produced by bacteria.

Finally, since many of our samples have been stored in freezers since last December-February, we are hesitant to spend much time, effort, or resources on samples that may have degraded with time and storage. Therefore, we have started a new round of sampling to provide fresh samples for our Almond Board project.

This is a critical step, assuming that the chemicals we are looking for are volatile. Volatile chemicals can be rapidly lost from stored samples, even those in freezers. Six months or more is a long time to store samples for these types of analysis. New samples are more likely to contain evidence of the materials that we suspect may be in bee hives and contributing to CCD. Of course, if we do not see any new outbreaks of CCD, we'd have to use stored samples.

In September, we began sampling an organic beekeeping operation that is likely to prove to be partially Africanized. This bee business is location in the arid southwest. These bees are of interest, given claims that Africanized bees are not susceptible to CCD and that CCD may have come into the U.S. from Australia.

In addition, this week, we are currently sampling bee operations in Idaho. In both the Arizona and Idaho cases, the symptoms mirror those of CCD. One change in symptoms is that most of the colonies are completely depopulated.

We've also been talking to beekeepers in Colorado, Washington, and southern

California who are reporting heavy bee losses. In all cases, we will attempt to establish the sampling strategy initially proposed to the Board.

**CCD Overview and Original Proposal:**

We do not know yet what is causing colony failure, but the symptoms are clear – rapid bee disappearance, leaving the queen and young bees behind; few dead bees remaining in the colonies; and ample unused pollen, honey and syrup stores. Also evident and highly unusual, is the absence of any robbing or invasion by typical pests and scavengers after colony failure. This apparent repellant nature of brood nests and food stores is an especially important clue that we at Bee Alert have undertaken to investigate.

***We hypothesize that either a highly toxic or a strongly repellent residual compound is produced during collapse, and then persists for days or weeks after colony failure. Whether this is a chemical produced by a pathogen, a consequence of materials used for mite treatment, a pesticide used on crops, or some form of environmental contaminant is unknown. However, if a chemical exists, we hope to find it.***

Our first objective has been to survey as many affected apiaries as possible to collect and freeze large samples of bees, wax, comb, pollen, and honey for subsequent analysis. Ongoing collapses in California may necessitate additional sampling trips.

Of particular interest is to obtain samples from queen-rearing operations in Louisiana and Alabama, states that to date have not reported CCD. These states also have restrictions on the movement of bees into their beekeeping areas. We'd like to know whether these states truly do not have CCD. If they don't have CCD, then bee colony samples might show some unique difference, providing a valuable clue as to causation.

Because specific and quantitative analyses are time consuming and expensive, we propose **(Phase I)** to first perform a semi-quantitative, generalized chemical survey. Analyzing for presence of broad classes for chemical indications of viruses, bacteria, and fungi, as well as biologically produced toxins, and some of the more common mite management materials can be done at relatively low cost. This will allow us to establish the most likely agent or agents associated with CCD. Then when, and if, a general class of compound common to CCD colonies is determined, we can conduct a more focused **(Phase II)** and quantitative analyses to identify the specific compound and agent responsible for CCD. We propose in **Phase I** to examine bees and wax for the following classes of chemicals:

- Proteins, peptides, and other materials related to pathogens,
- Semi-volatile and volatile organic chemicals (including vapor samples pumped from within hives),
- More persistent pesticides and industrial organics such as PCBs, and

- Select trace elements and heavy metals.

This two-stage approach allows us to efficiently narrow our search for the cause of CCD. By deferring expensive quantitative analyses until after we have narrowed the list of possible causes, we can broaden initial sampling. This improves our ability to generalize about the causative agent. Sample collection is relatively inexpensive and sufficient sample quantities can be collected and stored in a freezer to provide for both the general survey and later specific analyses.

**Preliminary Results:**

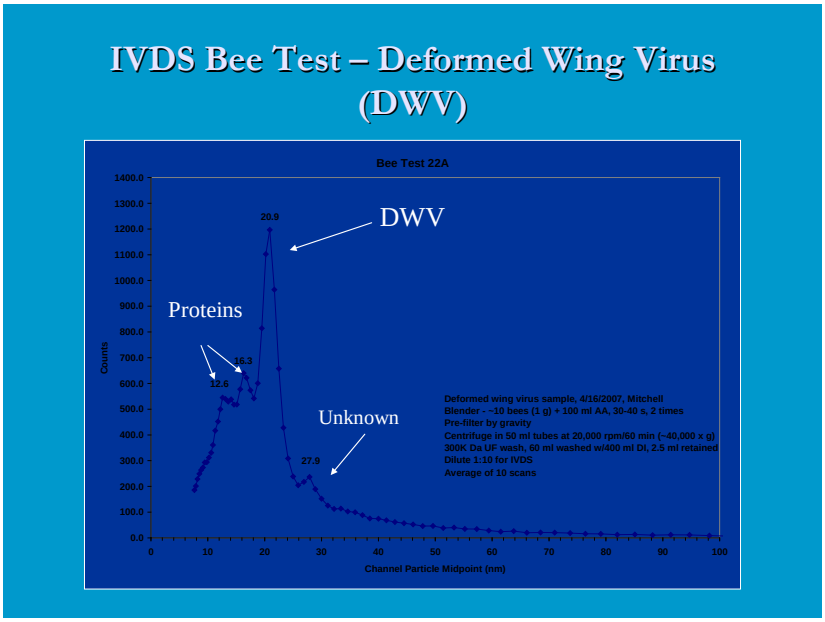
In Phase I, we found that some beekeeping operations did have a problem with excessively high levels of HMF in corn syrup. That certainly contributed to their losses, although HMF was not a factor in many other CCD operations, especially since over half the affected bee companies did not feed their bees.

We also noticed elevated levels of dichlorobenzene, notably in some east-coast based operations. Beekeepers use Para-dichlorobenzene to control wax moth. Our results indicate that over-dosing may be leading to wax contamination.

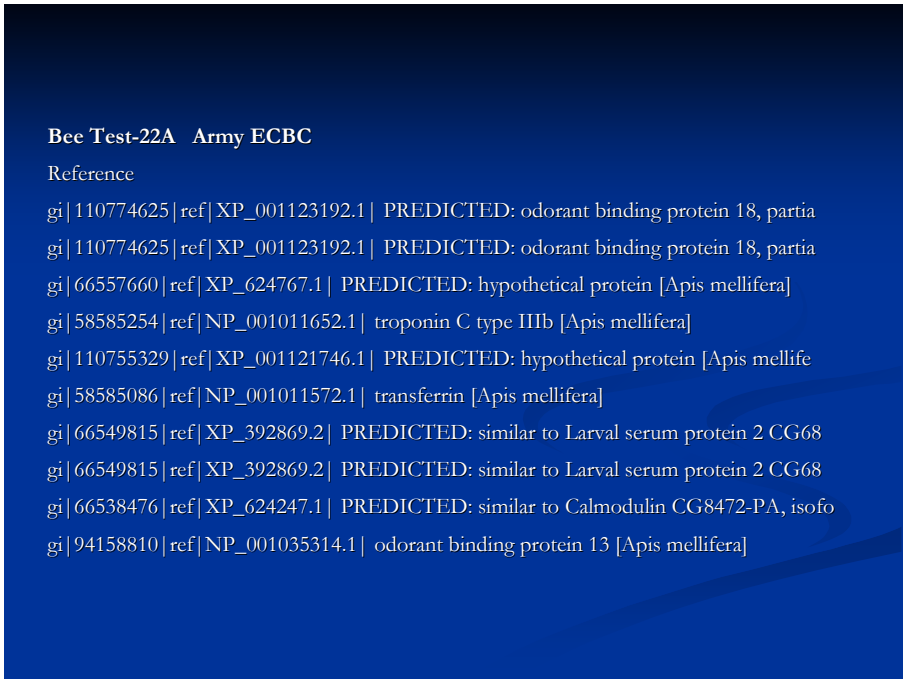
Initial screening by the U.S. Army has also documented between 200 and 300 proteins and other chemicals per sample. These results have been matched against virus screening results for all samples. At this point in time, we're looking at approximately 10,000 chemical identifications. The outcome of this work should be ready for publication by December, and we hope to present a summary at the December meeting of the Almond Board in California.

A sample of the analysis output from just one bee samples appears below. The Army ECBC laboratory has provided both virus detection and proteomics results for bees from 30 colonies covering a variety of CCD scenarios.

The first chart shows the output of an IVDS (Integrated Virus Detection System) scan for of a bee sample. In this system, viruses appear as peaks of specific nanometer size. The height of each peak reflects the titer or concentration of the virus. The second chart shows the first ten of approximately 300 chemical/proteomics identifications for this same sample




Deformed Wing and unknown bee virus detection by IVDS.




GC/MS Proteomics results for Deformed Wing Virus bee sample.

**Summary and Conclusions:**

Bee Alert is currently obtaining new samples from recently reported cases in the western states. Some samples are being processed at The University of Montana, others have been sent to external laboratories. Collection of samples of at least ten colonies from at least ten different bee operations is ongoing. Preliminary results should be available and will be presented at the Almond Board Meeting in December, 2007.