

USDA Honey Bee Pests and Diseases Survey Project Plan for 2013

Comprehensive Objective

A national survey of honey bee pests and diseases has been funded annually since 2009 by the USDA Animal Plant Health Inspection Service (APHIS). This survey is being conducted in an attempt to document which bee diseases, parasites, or pests of honey bees are present and/or likely absent in the U.S. Specifically, this survey will attempt to verify the absence of the parasitic mite *Tropilaelaps* and other exotic threats to honey bee populations (e.g., *Apis cerana* and Slow Bee Paralysis Virus). To maximize the information gained from this survey effort, collected samples will be analyzed for other honey bee diseases and parasites known to be present in the U.S. This cross-country survey will be the most comprehensive honey bee pest and health survey to date, and provides essential disease and pest load base line information. This information will help place current and future epidemiological studies in context and thus may indirectly help investigations of emerging conditions such as Colony Collapse Disorder (CCD). Coordination of this survey is in collaboration with USDA Agricultural Research Service (ARS) Bee Research Lab (BRL) and the University of Maryland (UMD).

Primary Objective – Exotics

Tropilaelaps spp., an Asian parasitic mite (several species in the genus *Tropilaelaps* are recognized) of honey bees, feeds on honey bee brood. Their parasitic feeding actions vector viruses, weaken or kill parasitized brood, and can cause infected colonies to abscond which spreads the mites to new areas. *Tropilaelaps* mites can complete their lifecycle in one week, and thus this mite can outcompete the Varroa mite when both mites are present in a hive. Currently, there are no known *Tropilaelaps* mites in the U.S.

The exotic *Apis* species *Apis cerana*, or Asian honey bee, and Slow Bee Paralysis Virus are also NOT known to be in U.S. apiaries; this survey will help confirm their absence. *A. cerana* are now confirmed in northern Australia. It is smaller but very similar in appearance to *Apis mellifera*, is well adapted to warmer climates, builds smaller colonies, and is known to swarm many times during the year. In tropical areas (e.g., Solomon Islands) *A. cerana* has been shown to outcompete *A. mellifera* in nectar and pollen gathering and exhibits a propensity for robbing European honey bee stores. Its honey yield is far less than *Apis mellifera* making it a less valuable bee for commercial honey production.

Secondary Objective – Honey Bee Health Evaluation

A decline in honey bee health has been documented over the past 60 years. The known negative honey bee health challenges are primarily attributable to parasites, diseases, poor nutrition, stress and environmental toxins. There have been no national honey bee health surveys conducted to ascertain the scope of additional unidentified parasites, diseases, and pests that may have a negative impact on honey bee populations in the U.S. The benefit of informing and guiding the direction of honey bee parasite, disease, and pest research and mitigation recommendations to the U.S. apiculture industry would be significant. All of the data collected from the National Survey, is included in the nationwide Bee Informed Partnership (BIP) database. The Bee

Informed Partnership is an extension project currently funded by the USDA National Institute of Food and Agriculture (NIFA). As part of its core mission, the database endeavors to capture honey bee health and management practices from around the country to better inform all beekeepers with the goal of reducing colony losses. The data gathered in these extensive surveys are critical for capturing base line information on the status of honey bee health; this in turn will help place beekeeper disease load data in regional and temporal context.

Over the last 6 years, winter losses have been unsustainably high, averaging just over 30% nationally. This rate of loss threatens the viability of beekeeping operations and – importantly - the production of crops dependent on bees for pollination as well as honey production. Pollination is responsible for over \$15 billion in added crop value, particularly for specialty crops such as nuts, berries, fruits, and vegetables. Of the 2.7 million colonies of bees in the United States, the almond crop in California alone requires approximately 1.3 to 1.6 million colonies, and this need is projected to increase significantly over the next few years. The bee industry is facing difficulty meeting the demand for pollination in almonds because of bee production shortages in California. Consequently, growers depend increasingly on beekeepers from other states to transport honey bee colonies across the country to meet the pollination demand (a phenomenon known as migratory beekeeping).

The USDA has developed a Colony Collapse Disorder (CCD) Action Plan, (http://www.ars.usda.gov/is/br/ccd/ccd_actionplan.pdf). The second goal of this plan is to determine current status of honey bee colony production and health. Objective two of this goal is to develop a long-term annual APHIS survey on the overall health status of U.S. honey bees. This action was designated as a high priority in the CCD Action Plan. Current theories about the cause(s) of CCD and increased colony mortality generally include Varroa mite parasitism; new or emerging diseases, especially mortality by a new *Nosema* species (e.g., *Nosema ceranae*), a newly evolved and more virulent strains of Varroa mite vectored bee viruses; sublethal pesticide exposures (through exposure to pesticides applied for crop pest control or for in-hive insect or mite control); and poor nutrition (due to reduced forage lands caused by increased corn monocropping and changes in agricultural practices). These factors, alone or in combination, are thought to suppress bees' immune systems, making them susceptible to a host of pathogens, which in turn causes increased mortality.

Tertiary Objective – Pesticide Analysis within the Hive

Sampling bee bread (bee collected and stored pollen) is included in the 2013 National Honey Bee Survey and will occur concurrently with the pest and disease sampling. Honey bees gather pollen from many floral sources. Some of these sources may be contaminated from direct or indirect pesticide application or from systemic insecticides. Pollen taken back to the hive is stored and used as food for developing brood. Low levels of pesticides in bee bread may have negative effects on bee and colony health. Potential damage is related to the toxicity of individual pesticides, the abundance of different pesticides, the synergism that exists between pesticides and the quantity of pesticides in the pollen. For example, a pesticide sprayed directly on a honey bee may kill her quickly and thus she will not take any of the chemical back to the hive. However, a low level systemic insecticide prevalent in a local pollen source that does not kill immediately will result in that pollen being stored in the hive and used for feeding the brood.

It is unknown at this time whether these consistent, low level pesticides affect brood development, brood mortality, queen reproduction and/or queen mortality. Some recent studies suggest that low level of exposure to some pesticides may make bees more susceptible to disease. Further, combinations of different products, which on their own may not be toxic to bees, may act in synergy creating a toxic brew much more toxic than the products would be on their own.

Scope of work and methodology

The 2013 National Survey has three goals, 1) identify potentially invasive pests such as the exotic mite *Tropilaelaps*, problematic *Apis* species such as *A. cerana* and viruses such as the Slow Bee Paralysis Virus, 2) conduct an epidemiological survey that would meet the goal of developing a long-term overall baseline picture of colony health, and 3) evaluate pollen sampled from the same hives as the pest and disease samples are taken to evaluate pesticide exposure within the hive.

To accomplish these objectives, cooperators will distribute sampling kits, identify stationary and migratory beekeepers who will participate in sampling of their honey bee colonies, collect and preserve samples, quantify parasite loads from bees collected in alcohol and forward live samples for molecular analysis. Additional samples of pollen will be collected from 10 apiaries per participating state to evaluate pesticide occurrence in hives. The results of these analyses will be forwarded to the participating beekeepers and the respective state apiary contacts. Beekeepers participating in this survey should also expect a summary report on the average apiary level Nosema, Varroa loads, presence or absence of *Tropilaelaps* mites, and viral results from the molecular analysis in the sampled apiary within 4 to 6 months of sample collection. All data collected will be maintained at APHIS, ARS and UMD. This data will be entered into the APHIS IPHIS database as well as the BIP database described above.

As part of the survey includes a visual inspection of the hives before sampling, the presence of the following is recorded at the apiaries:

1. American Foul Brood
2. Black Shiny Bees
3. Chalkbrood
4. Deformed Wing Virus
5. European Foul Brood
6. Idiopathic Brood Disease Syndrome (IBDS)
7. Sac Brood
8. Small Hive Beetle Adults
9. Small Hive Beetle Larvae
10. Wax Moth Adults
11. Wax Moth Larvae

The samples taken at the apiary and preserved in alcohol are later inspected using microscopic analysis at UMD for the following:

1. *Nosema* spp. spore count
2. Tropilaelaps Mites
3. Varroa Mite loads
4. *Apis cerana*

The live bees shipped directly from the apiary during collection are sent to USDA-ARS BRL. There, the honey bees are frozen until molecular analyses are conducted. The molecular analyses include the following:

1. Acute Bee Paralysis Virus (ABPV)
2. Chronic Bee Paralysis Virus (CBPV)
3. Deformed Wing Virus (DWW)
4. Kashmir Bee Virus (KBV)
5. Israeli Acute Paralysis Virus (IAPV)
6. Black Queen Cell Virus (BQCV)
7. Slow Paralysis Virus (SPV)

Also, as part of the national survey, training and outreach materials have been developed in the form of videos and written information (http://www.aphis.usda.gov/plant_health/plant_pest_info/honey_bees/survey.shtml). Literature will also be developed for pest identification and finally, information on honey bee health and maintenance will be collected and distributed at the conclusion of the survey.

Project Management, Cooperators and Other Participating Institutions

This National Survey is funded and coordinated by USDA APHIS and ARS and the University of Maryland. A Steering committee comprised of personnel from APHIS, ARS and UMD determined the sampling protocol, and determined the optimal distribution of sample analysis. Sampling is conducted under cooperative agreements with states. Samples are collected by state apiary specialists and university scientists who identify beekeepers whose colonies will be used for sampling. Some of these beekeepers may also participate in conducting the survey. The states being sampled in the 2013 National Survey are:

Alabama	Minnesota	Oregon
Arkansas	Mississippi	Pennsylvania
California	Montana	South Carolina
Florida	North Carolina	South Dakota
Georgia	North Dakota	Tennessee
Hawaii	New Jersey	Texas
Iowa	New Mexico	Utah
Illinois	New York	Virginia

Indiana
Maryland
Michigan

Nevada
Ohio

Wisconsin
West Virginia

Puerto Rico, Guam and Grenada will also be sampled in order to provide baseline data of pest and disease loads. These islands are considered sentinel bee communities. We anticipate that new invasive diseases and pests may appear first in these locations on account of lax bee import laws and or high trade and traffic with other areas that have known bee diseases not thought to be in the continental USA. University of Maryland personnel, in close collaboration with USDA ARS personnel are responsible for the sample kit fabrication and distribution. They are also the contact for receiving all alcohol samples and apiary data information forms from the field. The alcohol samples containing any dislodged mites from the hive frames are forwarded to USDA ARS Bee Research Laboratory (BRL) where scientists microscopically analyze the sample for the presence of *Tropilaelaps* mites.

USDA-ARS BRL scientists are the contact for receiving all live bee samples. The bees are immediately frozen and held until molecular analysis is conducted. Pollen samples will be mailed to the University of Maryland who will forward the samples for pesticide analysis to USDA Agricultural Marketing Service (AMS). UMD is responsible for all pest, diseases and exotic species and subspecies, as well as pesticide reporting to the beekeeper and the apiary contact for the selected states.

Guidance for Choosing Apiaries and Hives to Sample for the USDA Honey Bee Pests Survey

- 24 apiaries should be sampled in all states except California where 48 apiaries should be sampled.
- 8 hives should be sampled in each apiary.
- Apiaries sampled should have at least 10 colonies.
- If you are sampling an apiary with a large number (hundreds or thousands of hives), please try to provide a composite sample from all locations within the apiary. This may require that you divide the apiary into quadrants and then sample a few hives in each quadrant. It is critical that hives that appear 'sick' are sampled along with hives that appear 'healthy'.
- 10 queen producers should be sampled unless there are fewer than 10 willing queen producers in your state.
- Of the remaining apiaries sampled, when possible 1/2 of the apiaries sampled should be from migratory operations (move out of state and return prior to sampling) and 1/2 should be from stationary operations (Do not move out of the state but move within the state).
- Apiaries should be chosen in order to give as close to an equal representation of the entire state as possible. Ideally, a state will be sectioned into 4 quadrants with apiaries randomly chosen within a quadrant.
- If there are a limited number of beekeepers within a state, it is allowable to sample up to 50% of the same beekeepers as in previous years.
- Additional apiaries that may occur near ports or other areas that could be considered high risk should also be considered for sampling.

Milestones and Project Timeline

A pilot survey program funded from the APHIS Farm Bill funding was initiated in 2009 and samples were collected from three states to test the inspection and collection process, assess the infrastructures related to shipping, storing and analyzing the specimens, and to gather baseline data for a survey of honey bee pests and pathogens. Hawaii, Florida and California were part of this pilot program. These are high-risk areas that have key ports, long growing seasons and diverse agricultural crops. A summary report of the 2009 pilot survey is available on the APHIS website

at http://www.aphis.usda.gov/plant_health/plant_pest_info/honey_bees/downloads/twg_report_july_2010.pdf.

The 2010 limited national survey, focusing on 13 states, was performed to expand and augment the baseline pest and pathogen data collected from the pilot study conducted in 2009. A summary report of the 2010 Survey is available on the APHIS website

at www.aphis.usda.gov/plant_health/plant_pest_info/honey_bees/downloads/2009_Pilot_Summary_Report.pdf.

In 2011 and 2012, full-scale national surveys were funded for 34 and 32 states respectively, and these represent the most comprehensive U.S. honey bee pest and disease surveys to date. A summary report of the 2011 Survey is available on the APHIS website at http://www.aphis.usda.gov/plant_health/plant_pest_info/honey_bees/downloads/2011_National_Survey_Report.pdf. The primary focus of the survey was to verify the absence of the parasitic mite *Tropilaelaps* and other exotic threats to the U.S. bee population (e.g., *Apis cerana*).

Establishing the absence of threats to honey bee populations not thought to be present in the U.S. was the primary objective of this effort; however, to capitalize on the information gathered from this survey, samples were analyzed for other honey bee diseases and parasites known to be present in the U.S.

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