

USDA APHIS Honey Bee Pests and Diseases Survey Project Plan for 2023

Background

An annual national survey of honey bee pests and diseases was initiated by the U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) in 2009. The survey is conducted in collaboration with the University of Maryland (UMD), USDA Agricultural Research Service (ARS), and the Apiary Inspectors of America. The objective of this national survey is to document which bee diseases, parasites, or pests of honey bees are present and/or likely absent in the U.S. Specifically, this survey has verified the absence of the parasitic mite *Tropilaelaps* spp. and other exotic threats to honey bee populations (e.g., *Apis cerana* and *Slow Bee Paralysis virus* (SBPV)). Results of this survey have led to a growing archive of annotated historical honey bee samples and data. These archived samples and data act as a growing and unique resource to perform retrospective studies on the evolution of bee parasites and pathogens and the genetics of honey bees. To date, the NBHDS has published descriptive correlative studies in the past (Traynor et al., 2021, 2016, 2016), identified new viruses (Ray et al., 2020), as well as permitted the identification of new viruses/strains and the timing of their emergence in the US population (Ryabov et al., 2019, Ryabov et al., 2017) as well as distribution of mutations associated with varroa mite resistance to fluvalinate (Millán-Leiva et al., 2021, Hernández-Rodríguez et al., 2022) and amitraz. Collaborators are now exploring the genetic makeup of the studied honey bees (Alburaki et al., 2023), which should allow us to link bee genotype with pathogen prevalence.

The viability of beekeeping operations, honey production, and the production of crops dependent on bees for pollination are at risk from honey bee pests and diseases. Pollination is responsible for over \$15 billion in added crop value, particularly for specialty crops such as nuts, berries, fruits, and vegetables. Of the 2.71 million colonies of bees in the U.S., the almond crop in California alone requires approximately 2.3 million colonies, and this need is projected to continue to increase. Growers depend on beekeepers to transport honey bee colonies across the country, a practice known as migratory beekeeping, to meet the pollination demand.

The Honey Bee Pests and Diseases Survey provides the incidence and distribution of diseases and pest loads in the U.S. through <https://ushoneybeehealthsurvey.info/> where data are accessible to the public in ways that protect the privacy of the individual who participated in our survey. To maximize the information gained from this survey effort, collected samples are also analyzed for other honey bee diseases and parasites present in the U.S. The survey has collected pollen or wax from sampled colonies since 2011 and has documented the pesticide prevalence, diversity, and concentration of those residues found in over 1,000 samples from 2011 and 2017 (Traynor et al., 2021).

A streamlined system for sharing this information as quickly as possible has been developed by sending out individual reports to beekeepers when data is received, and is also presented at the state level (to protect the confidentiality of the beekeepers) with interactive tools at (https://research.beeinformed.org/state_reports/). Past survey results have also been published (Traynor et al., 2016) and annual national reports are published on the APHIS website

<https://www.aphis.usda.gov/aphis/ourfocus/planthealth/plant-pest-and-disease-programs/honey-bees>).

Longitudinal sampling will continue in 2023. Samples taken in the same apiary twice a year will provide information on seasonal changes in honey bee health and will help us determine if we can predict colony health based on earlier inspections (vanEngelsdorp and Meixner, (2010)). In addition, participating beekeepers are encouraged to provide management and mortality data from longitudinally sampled apiaries via an online survey so practices and colony health measures can be linked with operational success (e.g. increased colony survivorship). Further, factors that contribute to the likelihood of disease presence and absence in operations will be identified. This information will help place current and future epidemiological studies in context and thus may indirectly help investigations of emerging conditions.

Primary Objective – Exotics

Tropilaelaps spp., a parasitic mite native to Asia, feeds on honey bee brood. Its parasitic feeding vectors viruses, weakens or kills parasitized brood, and can cause infected colonies to abscond, which spreads the mites to new areas. *Tropilaelaps* can complete its lifecycle in one week, and thus this mite can potentially outcompete *Varroa* when both mites are present in a hive. Currently, there are no known *Tropilaelaps* spp. in the U.S.

Slow Bee Paralysis virus (SBPV) is present throughout Europe, however of low prevalence. Honey bees that are infected with SBPV exhibit paralysis in their front two pairs of legs and ultimately succumb to the virus. When SBPV is present within a colony that also has high *Varroa* loads, it can result in increased colony mortality (Carreck et al., 2010). The APHIS Honey Bee Pests and Diseases survey validates the absence of SBPV within the U.S.

This survey also confirms the absence of the exotic *Apis* species *Apis cerana*, or Asian honey bee from U.S. apiaries. *A. cerana* is smaller but very similar in appearance to *Apis mellifera*, is well adapted to tropical 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. Due to smaller colony size and lower honey production, *A. cerana* is not well suited to migratory beekeeping for pollination compared to *A. mellifera*.

Secondary Objective – Honey Bee Health Evaluation

A decline in managed honey bee populations has been documented over the past 60 years (Traynor, et al., 2021). Honey bee health is at risk from factors such as parasites, diseases, poor nutrition, stress, and environmental toxins. We have conducted the National Honey Bee Pests and Diseases Survey since 2009 to ascertain the scope of parasites, diseases, and pests that may have a negative impact on honey bee populations in the U.S. This information also informs and guides the direction of honey bee parasite, disease, and pest research. Additionally, it informs recommendations to the U.S. apiculture industry. All of the data collected from this survey are included in the nationwide database (programmatic details here: <https://beeinformed.org/aphis/>, diagnostic data provided here: https://research.beeinformed.org/state_reports/ and viral data

provided here: https://research.beeinformed.org/state_reports/viruses/). The data gathered in these extensive surveys are critical for capturing baseline information on the status of honey bee health; this in turn will help place beekeeper disease load data in regional and temporal context.

Tertiary Objective – Longitudinal Pest and Disease Monitoring

Summarized data from multiple years of the National Honey Bee Pests and Diseases Survey has demonstrated seasonal variation in honey bee health. *Varroa* populations consistently increase in the fall and *Nosema* spore loads are higher during the spring months. Similarly, many of the honey bee viruses tested for in the survey also display seasonal variations that are present across survey years. This baseline information is valuable in itself, but its impact would be even greater if variation in seasonal disease levels could be linked to colony losses. Longitudinal monitoring will serve to bridge the gap between the seasonal honey bee health measures and annual colony mortality.

Longitudinal monitoring samples, a subset of beekeepers (n=5), are collected twice– in the spring before or at the start of the honey flow, and in the fall after honey flow. The longitudinal monitoring will include a full survey assessment for exotics, pests and disease, viruses, and in-hive pesticides. Additionally, the beekeepers who manage these apiaries will provide management information, such as feeding and mite treatment practices, and annual colony mortality rates by committing to taking the Colony Loss and Management Survey conducted annually in April by BIP. This information will be used to identify how beekeeping events (e.g. migratory pollination, package production, honey flow), can affect seasonal honey bee health and colony mortality.

Scope of Work and Methodology

The 2023 National Honey Bee Pests and Diseases Survey has three goals: 1) early detection of potentially invasive pests such as the exotic mite, *Tropilaelaps*, *Slow Bee Paralysis virus*, and problematic *Apis* spp. such as *A. cerana*; 2) continue to build the honey bee health surveillance dataset which provides critical long-term historical perspective of colony health; and 3) identify risk and protective factors that predict colony health and operational success by connecting honey bee health measures over time and annual colony losses.

The results of analyses will be forwarded to the participating beekeepers and the respective state apiary contacts as well as the State Plant Regulatory Officials (SPRO), and APHIS State Plant Health Directors (SPHD). Beekeepers participating in this survey should expect a summary report on the average apiary level of *Nosema* spore loads, *Varroa* loads, presence or absence of *Tropilaelaps*, *Slow Bee Paralysis virus*, and *A. cerana*, and viral results from the molecular analysis in the sampled apiary and pesticide residue detections, where applicable, within six months of sample collection and/or receipt of complete samples for diagnostics. Although report turnaround time is not designed to provide real-time actionable results for beekeepers, processing and reporting for *Varroa* and *Nosema* are usually sent within one month of receipt. However, viral diagnostics and pesticide analyses are often backlogged due to the batch nature of sample analysis protocol, occasional technical issues, and large volumes of samples. After all sample analysis, SPHDs, SPROs, and state apiary specialists will receive a summary report for their

state. A report with the national-level results will be published on the APHIS honey bee website. All data collected are handled by UMD and then stored and maintained in the BIP database which adheres to strict security protocols. Additional information regarding protocols, reports, data collection, blogs and extension materials can be found at the new National Honey Bee Pests and Diseases Survey website: <https://ushoneybeehealthsurvey.info/>The samples taken at the apiary and preserved in alcohol will be inspected using visual and microscopic analysis at UMD for the following:

1. *Tropilaelaps* presence or absence
2. *A. cerana* presence or absence
3. *Varroa* loads
4. *Nosema* spp. spore count

Live bees taken from each apiary should be immediately mailed to the UMD Honey Bee Lab. There, the honey bees will be frozen at -80°C and molecular analyses are conducted. These analyses include the following:

1. Lake Sinai virus-2 (LSV-2)
2. Acute bee paralysis virus (ABPV)
3. Chronic bee paralysis virus (CBPV)
4. Deformed wing virus-A (DWV-A)
5. Deformed wing virus-B (DWV-B; formerly known as Varroa destructor virus)
6. Kashmir bee virus (KBV)
7. Israeli acute paralysis virus (IAPV)
8. Slow bee paralysis virus (SBPV)
9. Moku Virus (MKV)
10. *Nosema ceranae*

Additionally, ~3 grams of beeswax collected from brood frames in the 5 apiaries undergoing the longitudinal survey sampling will be tested for 199 known pesticides (full list in Appendix) by the USDA Agricultural Marketing Service (AMS) in Gastonia, NC. Longitudinal beeswax samples will be collected in the spring and in the fall when other longitudinal samples are collected. Inspectors will collect a total of 10 beeswax samples per state.

The survey includes a visual inspection of the hives before sampling. The presence of the following is recorded at the apiaries and entered into the BIP database, but not included in analysis. Since visual identification of these diseases and pests are dependent on the training and experience of the sampling personnel, they are not used to confirm the presence or absence of disease:

1. American Foul Brood
2. Black Shiny Bees
3. Chalkbrood

4. Deformed Wing Virus
5. European Foul Brood
6. Parasitic Mite Syndrome (PMS)
7. Sac Brood
8. Small Hive Beetle adults
9. Small Hive Beetle larvae
10. Wax Moth adults
11. Wax Moth larvae

Training and outreach materials for the National Honey Bee Pests and Diseases Survey are available at: <http://www.aphis.usda.gov/plant-health/honey-bees-survey>

Project Management, Cooperators and Other Participating Institutions

Sampling is conducted under cooperative agreements between USDA APHIS and states. Samples are collected by state apiary specialists and university scientists. Some beekeepers may also participate in conducting the survey. BIP will assist with sample collection in Colorado, Louisiana, North Dakota, and a portion of California. The 42 states and territories that will be sampled in the 2023 National Honey Bee Pests and Diseases Survey are:

Alabama	Kentucky	Ohio
Alaska	Louisiana	Oklahoma
Arkansas	Maine	Pennsylvania
California	Maryland	South Carolina
Colorado	Massachusetts	South Dakota
Commonwealth of the Northern Marianas Islands (CNMI)	Michigan	Tennessee
Delaware	Minnesota	Texas
District of Columbia	Montana	Utah
Florida	Nebraska	Vermont
Georgia	Nevada	Virginia
Guam	New Jersey	Washington
Hawaii	New Mexico	West Virginia
Idaho	New York	Wisconsin
Indiana	North Carolina	
	North Dakota	

UMD personnel are responsible for the sample kit fabrication and distribution. USPS mailing labels for returning samples are included with the kits. States/territories are responsible for purchasing postage. States/territories should scan the sample datasheets and email them to nhbs@umd.edu. All live bee samples, alcohol bee samples, *Tropilaelaps* samples and apiary data sheets should be sent to UMD. These items should be addressed to:

Rachel Fahey

University of Maryland
 4291 Fieldhouse Drive
 Plant Sciences Bldg. Rm. 4112
 College Park, MD 20742

All live bees are immediately frozen at UMD and are screened for a suite of honey bee viruses and *Nosema ceranae* by quantitative PCR (polymerase chain reaction). Pesticides samples are prepared and sent to USDA AMS for processing. All other samples including alcohol samples are processed at UMD. UMD is responsible for all pest, diseases (including viruses) and exotic species and subspecies. UMD will report summary results to the beekeeper, the apiary contacts for the selected states, the SPRO, and the SPHD at the appropriate level of detail for each recipient. UMD is responsible for entering and maintaining the data in the BIP database and providing an annual national-level report to USDA APHIS.

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

The 2023 National Honey Bee Pests and Diseases Survey sampling in each participating state will be divided into two sections, 1) longitudinal sampling of five beekeepers, and 2) 14 general survey surveillance samples split into three or more sampling trips throughout the year. Because the longitudinal sampling will be conducted twice for each of the five beekeepers, each state should have a total of 24 samples at the end of sampling season.

Longitudinal Sampling	General Sampling
<ul style="list-style-type: none"> -Select five (preferably at least two commercial migratory) beekeepers and their respective apiaries to sample. The colonies selected should be easy to locate on the next sampling event. <u>First samples (May or June*)</u> -Conduct regular sampling and collect pesticide sample -Mark hives with APHIS survey stickers (provided) -Have beekeepers fill out pre-sampling survey <u>Second sampling (September or October*)</u> -Locate previously marked colonies (if a dead out occurred, complete sample size) -Conduct regular sampling -Have beekeepers fill out new pre-sampling survey -Remind beekeeper that they must take the BIP Loss and Management survey April of next year 	<ul style="list-style-type: none"> -Select 14 beekeepers and their respective apiaries to sample -Preferentially select beekeepers who have large operations, are queen or package producers -Plan three (for northern states) or four (for southern states) sampling periods: <ol style="list-style-type: none"> 1) pre-honey flow (May or Jun.) 2) mid-season (Jul. or Aug.) 3) fall (Sep. or Oct.) 4) For southern states only: winter (Dec.-Feb.) -Randomly assign beekeepers (a mix of different types including migratory, queen producers, and stationary) to one of these sampling groups so that you are approximately sampling the same number of beekeepers per period (~four to five beekeepers per period in northern states and ~three to four beekeepers per period in southern states)

	-Have beekeepers fill out pre-sampling survey at time of sampling -Encourage them to take the BIP Loss and Management survey April of next year
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*For states and territories where colonies are active year-round, these months may be adjusted.

General Requirements for National Honey Bee Pests and Diseases Survey Sampling

- Apiaries should have at least ten colonies. Eight colonies will be sampled. The remaining two colonies will be sampled if the inspector encounters a dead out or queenless colonies during inspection. Dead outs and queen-less colonies should not be included in the survey sampling.
- Prioritize queen producers, package/nuc producers, honey producers, and apiaries used for crop pollination.
- Prioritize apiaries in areas at high risk for invasion of exotic pests and diseases (near deep water shipping ports, international airports, high traffic areas for migratory beekeeping).
- 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 four quadrants with apiaries randomly chosen from each quadrant.
- When sampling an apiary, it is critical to select colonies at random rather than haphazardly or regularly spaced. When sampling an apiary, it is critical to select colonies at random, which is different than haphazardly or regularly spaced. Colonies should **not** be preferentially selected because they seem “healthy” or “sickly”. To help select colonies as random, we will provide sheets of randomly generated numbers. Instructions on the use of this will included in the sampling kit.

Suggested National Honey Bee Survey Sampling Calendar:**

May	June	July	Aug.	Sep.	Oct.	Total # Samples
1 st longitudinal sampling trip (n=5)				2 nd longitudinal sampling trip (n=5)		10
1 st general sampling trip (n=5)		2 nd general sampling trip (n=4)		3 rd general sampling trip (n=5)		14

**This schedule is just a suggestion and not a strict sampling plan. Please adjust your schedule to best accommodate when honey bees are active in your region. If you would like assistance in creating your state’s personalized sampling plan please reach out to Project Manager Rachel Fahey by email: faheybrl@umd.edu.

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