USDA APHIS National Honey Bee Pests and Diseases Survey Project Plan for 2024

Background

Since 2009, the U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) has funded an annual national survey of honey bee pests and diseases. The survey is conducted in collaboration with the University of Maryland (UMD), USDA Agricultural Research Service (ARS), state apiary specialists, and university scientists 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., Slow Bee Paralysis Virus (SBPV), *Apis cerana*, and other exotic threats to honey bee populations.

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 2.7 million colonies of bees in the U.S., the almond crop in California alone requires most of those colonies for pollination. Growers depend increasingly on beekeepers from other states to transport honey bee colonies across the country, a practice known as migratory beekeeping, to meet the pollination demand.

Since its inception, the National Honey Bee Pests and Diseases Survey has been the most comprehensive honey bee pest and health survey in the U.S. The survey provides the incidence and distribution of known diseases and parasite loads. Archived samples from the survey were used to identify the presence and distribution of Deformed Wing Virus B (DWV-B), also known as Varroa Destructor Virus 1 (VDV), as well as *Apis mellifera* Solinvivirus-1 (AmSV-1).[1,2] Furthermore, archived samples have helped identify mutations associated with mite resistance to fluvalinate and Amitraz.[3,4] 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.[5]

The information and results collected during the survey are shared in individual reports to beekeepers as quickly as possible.

Longitudinal sampling will be required in 2024. 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. In addition, participating beekeepers will provide management and mortality data from longitudinally sampled apiaries 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. *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.

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*. It 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[3]. 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. 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. Additionally, it informs recommendations to the U.S. apiculture industry. Refer to the APHIS Survey of Honey Bee Pests and Diseases webpage for more programmatic details.

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, 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.

For longitudinal monitoring samples, a subset of beekeepers (n=5), are collected twice – once in the spring before or at the start of the honey flow, and once in the fall after honey flow. This sampling schedule is adjustable for states and/or territories in tropical regions outside of the temperate United States. 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 90 days prior to sampling by taking an online survey all participants are asked to complete. 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 National Honey Bee Pests and Diseases Survey has three goals: 1) early detection of potentially invasive pests such as the exotic mite, *Tropilaelaps*, 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 with 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* mites and *A. cerana*, viral results from the molecular analysis, 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* mites and *Nosema* are usually sent within one month of receipt. 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. All data collected will be handled by UMD and then stored and maintained in the APHIS database which adheres to strict security protocols. Additional information regarding protocols, reports, data collection, blogs and extension materials can be found at the National Honey Bee Survey and the APHIS Survey of Honey Bee Pests and Diseases webpages.

The samples preserved in alcohol will be inspected using visual and microscopic analysis at UMD for the following:

1. *Tropilaelaps* spp. presence or absence
2. *A. cerana* presence or absence
3. *Varroa* spp. 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 until molecular and visual analyses are conducted. The molecular analyses will include the following:

1. Acute bee paralysis virus (ABPV)
2. Chronic bee paralysis virus (CBPV)
3. Deformed wing virus-A (DWV-A)
4. Deformed wing virus-B (DWV-B; formerly known as Varroa destructor virus)
5. Kashmir bee virus (KBV)
6. Lake Sinai virus-2 (LSV-2)

7. *Apis mellifera* Solinvivirus-1 (AmSV-1)
8. *Nosema ceranae*
9. Israelí acute paralysis virus (IAPV)
10. Slow bee paralysis virus (SBPV)

Additionally, ~3 grams of wax collected from brood frames in the five apiaries undergoing the longitudinal survey sampling will be tested for pesticides by the USDA Agricultural Marketing Service (AMS) in Gastonia, NC. Longitudinal wax samples will be collected in the spring and in the fall when other longitudinal samples are collected. Inspectors will collect a total of 10 wax 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 database, but not included in analysis. Visual identification of these diseases and pests is dependent on the training and experience of the sampling personnel:

1. American Foul Brood
2. European Foul Brood
3. Sac Brood
4. Chalkbrood
5. Parasitic Mite Syndrome (PMS)/Snotty brood
6. Deformed wings
7. Black shiny bees
8. Small Hive Beetle adults or larvae
9. Wax Moth adults or larvae
10. Queen cells
11. Drone laying queen
12. Queen right (queen or eggs are viewed)
13. Queenless (no eggs or queen viewed)

Training and outreach materials for the National Honey Bee Pests and Diseases Survey are available at the National Honey Bee Survey and the APHIS Survey of Honey Bee Pests and Diseases webpages.

Project Management, Cooperators and Other Participating Institutions

Sampling is conducted under cooperative agreements. Samples are collected by state apiary specialists and university scientists. Some beekeepers may also participate in conducting the survey.

UMD personnel are responsible for the sample kit fabrication and distribution. U.S. Postal Service mailing labels for returning samples are included with the kits. States/territories are responsible for purchasing postage. All live bee samples, alcohol bee samples, *Tropilaelaps* spp. samples and apiary data sheets should be sent to UMD. Refer to the Sampling Protocol and the U.S. Postal Service Mailable Live Animals webpage more information on mailing live bees. These items should be addressed to:

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

UMD sends pesticides samples to USDA AMS National Science Labs for processing. All other samples (alcohol preserved samples and live bee samples) are processed at UMD. UMD is responsible for quantifying all pests, diseases (including viruses), and exotic species and subspecies. UMD will report summary results to the beekeeper, the apiary contact 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 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 Pests and Disease Survey

The 2024 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 the sampling season.

Longitudinal Sampling*

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.

First samples (May or June)

- Conduct regular sampling and collect pesticide sample
- Mark hives with APHIS survey stickers (provided)
- Have beekeepers fill out pre-sampling survey

Second sampling (September or October)

- Locate previously marked colonies (if a dead out occurred, complete sample size)
- Conduct regular sampling
- Have beekeepers fill out new pre-sampling survey

General Sampling

Select 14 beekeepers and their respective apiaries to sample. Preferentially select beekeepers who have large operations and are queen or package producers. Plan three (for northern states) or four (for southern states) sampling periods:

* For states and territories where colonies are active year-round, these months may be adjusted.

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 the pre-sampling survey at the time of sampling.

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 queen-less 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. Colonies should **not** be preferentially selected because they seem "healthy" or "sickly". Use a random number generator app on your phone to select each colony you will sample.

Suggested National Honey Bee Survey Sampling Calendar**

May & June	July & August	September & October	Total # Samples
1st longitudinal sampling trip (n=5)		2nd longitudinal sampling trip (n=5)	10
1st general sampling trip (n=5)	2nd general sampling trip (n=4)	3rd 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 sampling plan, please reach out to Survey Coordinator Rachel Fahey by email: faheybrl@umd.edu.

References

[1]Ryabov, E. V., A. K. Childers, Y. Chen, S. Madella, A. Nessa, D. vanEngelsdorp and J. D. Evans (2017). "Recent spread of Varroa destructor virus-1, a honey bee pathogen, in the United States." Scientific Reports **7**(1): 17447.

[2]Ryabov, E.V., Nearman, A.J., Nessa, A., Grubbs, K., Sallmann, B., Fahey, R., Wilson, M.E., Rennich, K.D., Steinhauer, N., Fauvel, A.M., Chen, Y., Evans, J., vanEngelsdorp, D., 2023. Apis mellifera Solinvivirus-1, a Novel Honey Bee Virus That Remained Undetected for over a Decade, Is Widespread in the USA. Viruses 2023, 15, 1597. https://doi.org/10.3390/v15071597

[3] Hernández-Rodríguez, C.S., Moreno-Martí, S., Almecija, G., Christmon, K., Johnson, J., Ventelon, M., vanEngelsdorp, D., Cook, S., González-Cabrera, J. Resistance to amitraz in the parasitic honey bee mite Varroa destructor is associated with mutations in the β-adrenergic-like octopamine receptor. J Pest Sci (2021). https://doi.org/10.1007/s10340-021-01471-3

[4] Millán-Leiva, A., Marín, Ó., De La Rúa, P., Muñoz, I., Tsagkarakou, A., Eversole, H., Christmon, K., vanEngelsdorp, D., González-Cabrera, J., 2021. Mutations associated with pyrethroid resistance in the honey bee parasite Varroa destructor evolved as a series of parallel and sequential events. Journal of Pest Science. doi:10.1007/s10340-020-01321-8

[5]Traynor, K.S., Tosi, S., Rennich, K., Steinhauer, N., Forsgren, E., Rose, R., Kunkel, G., Madella, S., Lopez, D., Eversole, H., Fahey, R., Pettis, J., Evans, J.D., D. vanEngelsdorp, 2021. Pesticides in Honey Bee Colonies: establishing a baseline for real world exposure over seven years in the USA. Environmental Pollution 116566.. doi:10.1016/j.envpol.2021.116566

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