USDA APHIS Honey Bee Pests and Diseases Survey Project Plan for 2020

Background

A national survey of honey bee pests and diseases has been funded annually since 2009 by the USDA Animal Plant Health Inspection Service (APHIS) and conducted in collaboration with the University of Maryland (UMD), USDA Agricultural Research Service (ARS) and the Apiary Inspectors of America. This national survey is being conducted 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*).

The viability of beekeeping operations and, importantly, the production of crops dependent on bees for pollination as well as honey production are at risk from 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 2.5 million colonies of bees in the U.S., the almond crop in California alone requires approximately 2 million colonies, and this need is projected to increase substantially over the next few years. Growers depend increasingly on beekeepers from other states to transport honey bee colonies across the country to meet the pollination demand (a practice known as migratory beekeeping).

Over the past 10 years, this nation-wide survey has been the most comprehensive honey bee pest and health survey, and provides the incidence and distribution of diseases and pest loads in the U.S. This survey has also demonstrated the absence of Slow Bee Paralysis Virus (SBPV) in the U.S. To maximize the information gained from this survey effort, collected samples are analyzed for other honey bee diseases and parasites known to be present in the U.S. Previous samples from the survey helped identify the presence and distribution of Deformed Wing Virus B (DWV-B), also known as Varroa Destructor Virus 1 (VDV) in the U.S[1].

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 on the Bee Informed Partnership (BIP) web site (https://bip2.beeinformed.org/state_reports/). Past survey results have also been published[2] and annual national-level 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 2020. However, due to travel and work restrictions, **longitudinal sampling will not be a requirement this year.** 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 [3]. 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, 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* species 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*, and 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 as well suited to migratory beekeeping for pollination as compared to *Apis 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 Survey over the last 10 years 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 provides additional benefit through informing and guiding the direction of honey bee parasite, disease, and pest research and informing recommendations to the U.S. apiculture industry. All of the data collected from this survey are included in the nationwide BIP database (programmatic details here: https://beeinformed.org/aphis/, diagnostic data provided here: https://bip2.beeinformed.org/state_reports/ and viral data provided here: https://bip2.beeinformed.org/state_reports/viruses/). BIP is a non-profit 501(c)(3) previously funded as a Coordinated Agricultural Products (CAP) grant by USDA National Institute of Food and Agriculture (NIFA). As part of its core mission, BIP 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 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 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 variation. These seasonal trends 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.

States that volunteer to participate in longitudinal monitoring will sample a sub set of beekeepers (n=5) twice–once in the spring (before or at the start of the honey flow), and again 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), as well as annual colony mortality rates by committing to taking the Colony Loss and Management Survey conducted annually in April. 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 2020 National Honey Bee Survey has three goals: 1) early detection of potentially invasive pests such as the exotic mite *Tropilaelaps* and problematic *Apis* species 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* and *A. cerana*, and viral results from the molecular analysis in the sampled apiary and pesticide residue detections, where applicable, within 6 months of sample collection and/or receipt of complete samples for diagnostics. Although report turn-around time is not designed to provide actionable results for beekeepers, processing and reporting for *Varroa* and Nosema are usually sent within 1 month of receipt. However, viral diagnostics and pesticide analysis often are 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 and a report with the national-level results will be published on the APHIS honey bee website. All data collected will be handled by UMD and then stored and maintained at the BIP database which adheres to strict security protocols.

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

The live bees taken from the apiary should be immediately mailed to the UMD Honey Bee Lab. There, the honey bees will be frozen at -80C and transported to the USDA ARS BRL where molecular and visual analyses will be conducted. The molecular analyses will 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 (formerly known as Varroa destructor virus) (DWV-B)
6. Kashmir bee virus (KBV)
7. Israelí acute paralysis virus (IAPV)
8. Slow bee paralysis virus (SBPV)
9. Moku Virus (MV)
10. *Nosema ceranae*

Additionally, ~3 grams of bee bread will be collected from brood frames that will be tested for 199 known pesticides (full list in Appendix). Bee bread will be collected from the 5 apiaries undergoing the longitudinal survey sampling and sent to the USDA Agricultural Marketing Service (AMS) in Gastonia, NC for analysis. Every state is required to take one bee bread sample from each of the 5 beekeepers participating in the longitudinal portion of the survey in the spring, and once again in the fall, for a total of 10 bee bread samples per state. **Note:** If a state is unable to take longitudinal samples, then they may select 10 random beekeepers to sample for pesticides analysis.

The survey includes a visual inspection of the hives before sampling. The presence of the following are 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 included on the reports:

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

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/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 who identify beekeepers whose colonies will be used for sampling. Some of these beekeepers may also participate in conducting the survey. Bee Informed Partnership (BIP) will assist with survey in CA and ND) The 39 states and territories that will be sampled in the 2020 National Survey are:

Alabama	Kansas	Oregon
Alaska	Kentucky	Pennsylvania
Arkansas	Louisiana	Puerto Rico
California	Maine	South Carolina
Delaware	Maryland	South Dakota
District of Columbia	Massachusetts	Tennessee
Florida	Michigan	Texas
Georgia	Minnesota	Utah
Guam	Montana	Vermont
Hawaii	Nebraska	Virginia
Idaho	Nevada	Washington
Illinois	New York	West Virginia
Indiana	North Dakota	Wisconsin

UMD personnel are responsible for the sample kit fabrication and distribution. Mailing labels for returning samples are included with the kits, however, states/territories are responsible for purchasing postage. UMD is the contact for receiving all live bee samples, alcohol bee samples, *Tropilaelaps* samples and apiary data information forms from the field. 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 transported to the USDA ARS BRL for molecular analysis of honey bee viruses and *Nosema ceranae*. Pesticides samples are sent to USDA AMS for processing. All the other sampling materials including alcohol samples are processed at UMD. UMD is responsible for all pest, diseases (including viruses) and exotic

species and subspecies, as well as pesticide reporting to the beekeeper and the apiary contact for the selected states. 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 2020 National Honey Bee Survey sampling in each participating state will be divided into two sections, 1) longitudinal sampling of 5 beekeepers, and 2) 14 general survey surveillance samples split into 3 or more sampling trips throughout the year. Because the longitudinal sampling will be conducted twice for each of the 5 beekeepers, each state should have a total of 24 samples at the end of sampling season.

Longitudinal Sampling	General Sampling
-Select 5 (preferably at least 2 commercial migratory) beekeepers and their respective apiaries to be sampled. The colonies selected should be easy to locate on the next sampling event. **First samples (May or June)** -Regular sampling and pesticide sample -Mark hives with APHIS survey stickers (provided) -Have beekeepers 1)fill out pre-sampling survey and 2)sign a commitment to complete next year's loss and management survey (in April). **Second sampling (September or October)**-- -Locate previously marked colonies (complete sample size in case of dead outs) -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	-Select 14 beekeepers and their respective apiaries to be sampled -Preferentially select beekeepers who have large operations, are queen or package producers -Plan three (for northern states) or four (for southern states) sampling periods: 1) pre-honey flow (May or June), 2) mid-season (July or Aug.), 3) fall (Sep. or Oct.), and for southern states only 4) 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 (~4-5 beekeepers per period in northern states and ~3-4 beekeepers per period in southern states) -Sampled apiaries should include migratory and stationary practices as well as queen breeders. -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

General Requirements for National Honey Bee Survey Sampling

- Apiaries should have at least 10 colonies (8 of which will be sampled, with 2 extra in case inspector encounters dead outs 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 exotics invasion (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 4 quadrants with apiaries randomly chosen from each quadrant.
- When sampling an apiary, it is critical to select colonies at random, which is different than haphazard or regularly spaced. Colonies should under no circumstances 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 be provided with the sampling kit sent to each state.

Example National Honey Bee Survey Sampling Calendar:

May	June	July	Aug.	Sep.	Oct.	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

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]Traynor, K. S., et al. (2016). "Multiyear survey targeting disease incidence in US honey bees." Apidologie: 1-23.

[3]vanEngelsdorp, D. and M. D. Meixner (2010). "A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them." Journal of Invertebrate Pathology 103: S80-S95.

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