

Bee Bread Sampling Protocol for the National Honey Bee Disease Survey

Please read this protocol carefully and watch the training video (https://www.youtube.com/watch?v=i8_gxRbEBOQ) prior to sampling (Please note that the video describes how to do pollen sampling, but bee bread sampling is a similar procedure. Please see notes below on how to distinguish between fresh pollen and bee bread). For additional information or questions concerning sampling methods call the University of Maryland Honey Bee Lab at 301-405-7947, or email the UMD Survey Coordinator Rachel Fahey at nhbs@umd.edu.

List of equipment:

- ✓ Nitrile gloves
- ✓ Permanent marker
- ✓ Sample vial
- ✓ 10 wooden sampling sticks
- ✓ Aluminum foil

Overview:

Acute and sub-lethal poisoning from pesticides can be a problem for beekeepers. Honey bees come into contact with pesticides outside the colony while foraging for nectar and pollen, and inside the colony while feeding, contacting beekeeper applied miticides, or contaminated comb wax. Honey bees gather pollen and nectar from many floral sources in their environment, store it in their colonies as fermented bee bread and feed it to developing brood. Therefore, the pesticides found in sampled bee bread is a snapshot of what pesticides that colony was exposed to in their outside environment at that time.

For the 2020 National Honey Bee Disease Survey, bee bread samples will be collected for pesticides analysis **ONLY** from the 5 beekeepers participating in the **longitudinal monitoring study**. State Apiary Inspectors will select and sample 5 apiaries (1 apiary from each of the 5 beekeepers) twice for pesticides—once in the spring (before or at the start of the honey flow), and again in the fall after honey flow.

Important: Inspectors should sample the same 8 colonies in the fall that were sampled previously in the spring. To achieve this, inspectors need to locate the colonies marked with the USDA APHIS survey sticker. If any of the original 8 colonies are dead outs, you may take a sample from other colonies in the apiary to bring the total back up to 8 colonies sampled.

Procedure:

1. Reminder: Put on a new pair of nitrile gloves prior to sampling each set of 8 colonies as this prevents cross-contamination between apiaries.



Figure 1: Bee Bread. Bee Bread is a mixture of pollen and nectar or honey. It can be differentiated from fresh pollen by the appearance of a glossy, moist or wet sheen. Fresh pollen appears very dry and crumbly.

2. After you have taken the honey bee sample from the brood area for the honey bee health survey, look at the frames you have pulled to see if there are any cells with bee bread in them. If so, take one end of a sampling stick and insert it all the way to the bottom of the cell and rotate the stick all the way around the cell scraping the bee bread as you go. You may damage adjacent cells as the stick is slightly larger than a cell (Figure 2) but this is normal.



Figure 2: Scraping Bee Bread out of a cell

3. As you lift the stick from the cell, move slowly as the bee bread, although more wet than fresh pollen, is crumbly and may fall off the stick (Figure 3). Place the bee bread in the plastic container by scraping the stick on the inside mouth of the vial making sure that the bee bread falls into the sampling container. Repeat in at least 4 cells per each of the 8 colonies to gather the minimum of 3 grams of bee bread. Use a new sampling stick per colony.



Figure 3: Sampling Bee Bread

4. If the frame you removed for sampling does not have bee bread in it, set it aside and try to find another frame with bee bread. If you cannot find any bee bread in the colony, move to the next colony for sampling and try to get bee bread from it. If you cannot gather bee bread from a particular colony, it is necessary for you to take extra samples from the remaining colonies to collect the requisite total of 3 grams.
5. The sampling sticks and gloves can be disposed of in the trash and should not be used again.
6. Once all colonies have been sampled, close the vial, and label it with the corresponding sample ID (ex. MD-01-2020). Wrap it in aluminum foil to prevent sun exposure.

Shipping:

Once all of the colonies in the yard have been sampled for pests and disease, place the labeled vials of bee bread into a freezer for storage. Once all 10 samples have been collected, ship them to:

University of Maryland Honey Bee Lab
4291 Fieldhouse Drive
Plant Sciences Building Rm. 4112
College Park, MD 20742

It is preferred that these samples be shipped on dry ice, but if not possible, ship them overnight with cool packs.