National Honey Bee Pests and Diseases Survey Wax Sampling Protocol

Please read this protocol carefully and watch the training video (https://www.youtube.com/watch?v=Znv2Bs9ZrjM) prior to sampling. For additional information or questions concerning sampling methods email Rachel Fahey at nhbs@umd.edu.

List of Equipment:

- ✓ Latex gloves
- ✓ Bee brush
- ✓ Clean hive tool
- ✓ Permanent marker
- ✓ 50-mL Falcon tube
- ✓ Aluminum foil and/or black plastic bag
- ✓ Bleach solution

Overview:

Honey bees come into contact with pesticides outside the hive while foraging for nectar and pollen, and inside the hive while feeding, contacting beekeeper applied miticides, or contaminated comb wax. In previous years, the APHIS National Honey Bee Pests and Diseases Survey collected pollen from select beekeepers for pesticide analysis; however, pollen only provided a glimpse of the pesticide exposure of a colony over a short time. Current studies indicate that wax may provide a more comprehensive measure of the total number of pesticide residues in the hive. Wax is also shown to be a better predictor of colony and queen mortality.

For the 2024 National Honey Bee Disease Survey, wax samples will be collected for pesticide analysis from 5 beekeepers participating in the longitudinal monitoring study. 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. Put on a new pair of latex gloves prior to sampling each set of 8 colonies to prevent cross-contamination between samples.
2. Select a frame from the brood chamber that has drawn wax but little to no brood, nectar, or pollen. Sampling from frames that have dislodged the bees is preferred. Find an area of the frame that has drawn wax but little to no brood, nectar, or pollen (Figure 1).

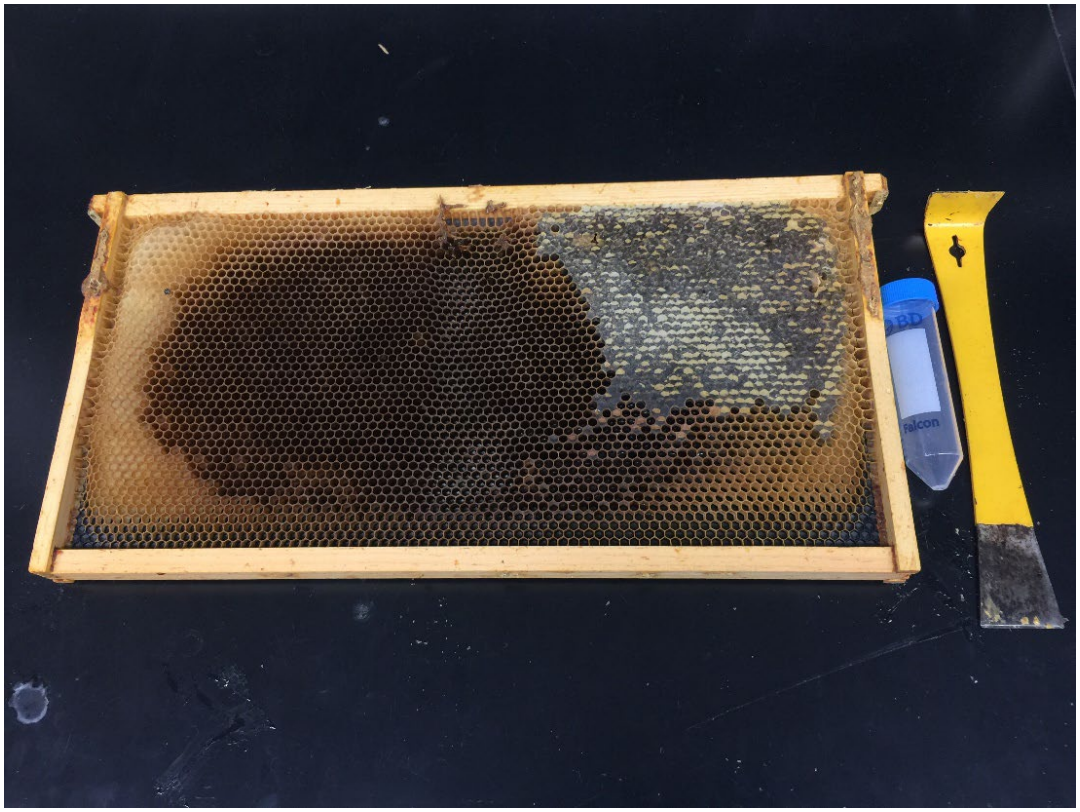

Figure 1: Hive tool, Falcon tube, and brood frame with wax.

3. Use the flat end of a clean hive tool to cut into the wax (for information on how to clean your hive tool, see step 8). Then, leaving one corner of the tool in place, pivot 90° (clockwise or counterclockwise) to cut out a quarter of a circle's worth of wax (Figures 2 and 3).

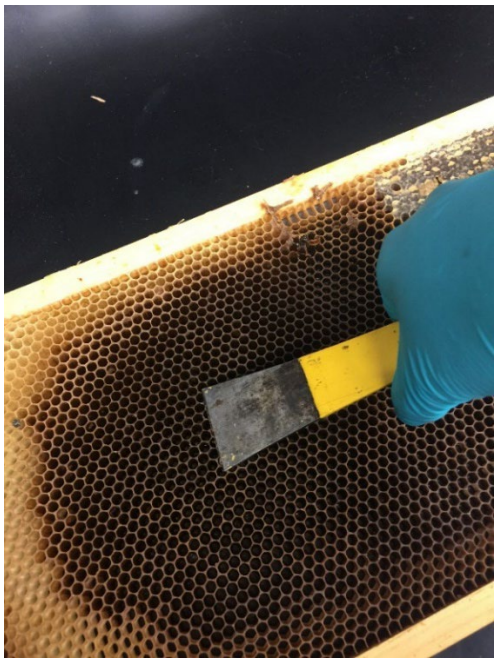

Figure 3: Using a hive tool to cut the wax.

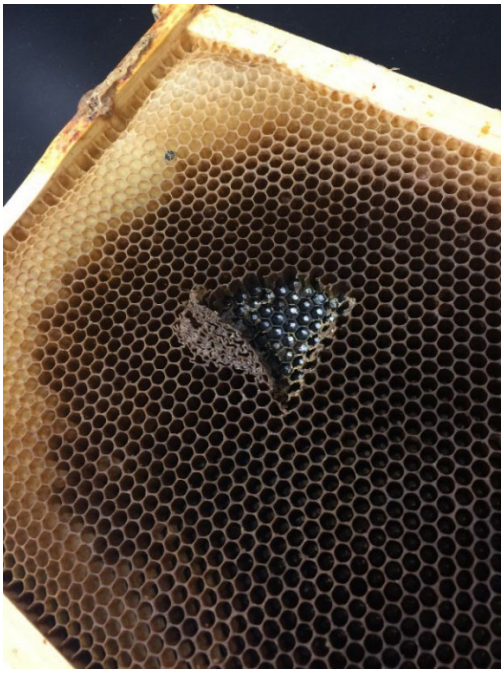

Figure 4: A quarter circle of wax.

4. Using the hive tool and a gloved finger, pick up the scraped piece of wax and place it into the 50-mL Falcon tube (Figures 4, 5 and 6).

Figure 4: Pick up the scraped wax using the hive tool and a gloved hand.

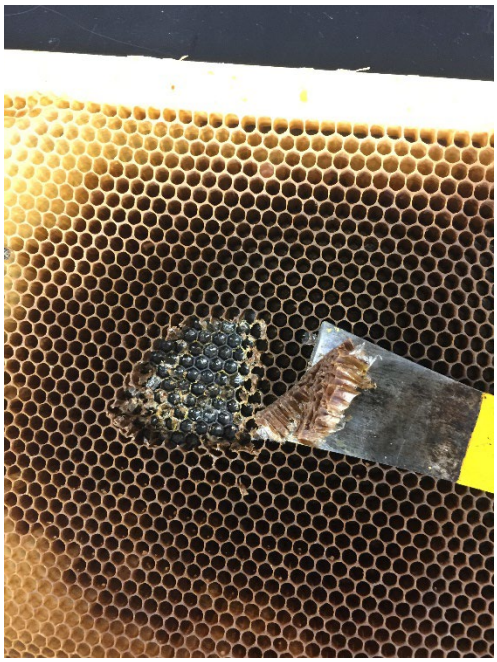

Figure 5: Removed piece of wax.

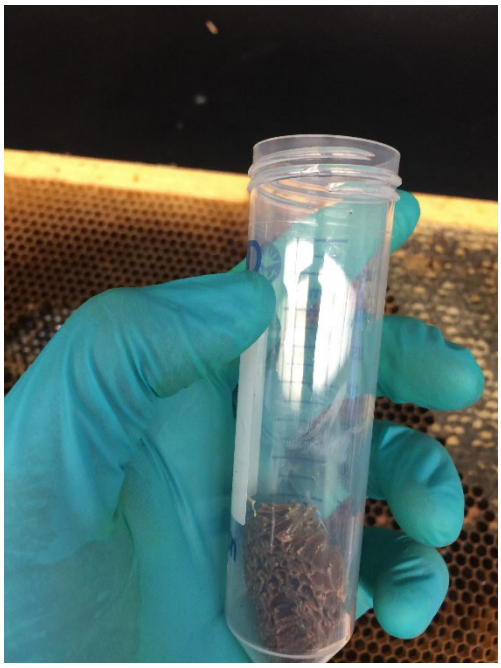

Figure 6: Piece of wax is placed into the Falcon tube.

5. Repeat steps 2-4 for all 8 colonies, adding each piece to the same 50-mL tube. Use your gloved finger, if necessary, to push down the wax so all 8 pieces will fit (Figures 7 and 8). You may use the same hive tool and keep the latex gloves on during the rest of your inspections within the apiary, only replacing them if they rip or become contaminated.

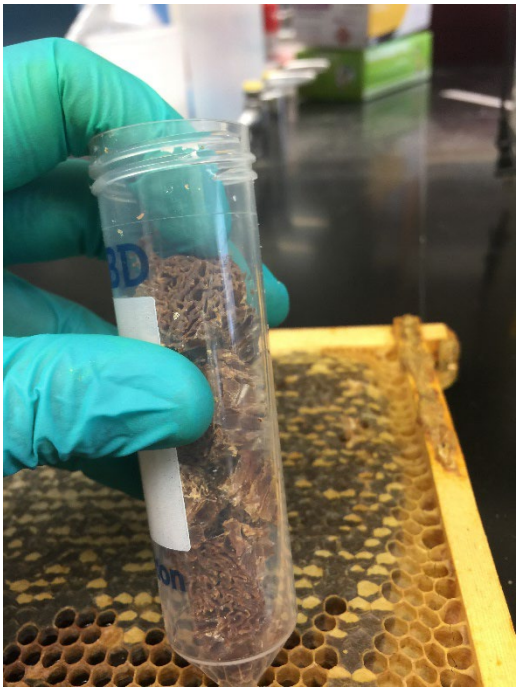

Figure 7: Place all 8 wax pieces in the same 50-mL Falcon tube.

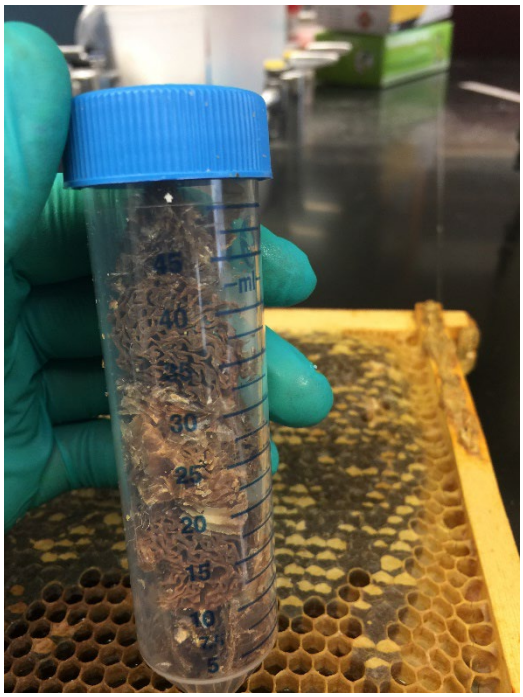

Figure 8: All 8 wax pieces in the Falcon tube.

6. Once wax samples are taken from all 8 colonies, label the tube appropriately with the state, sample number and survey year by using a permanent marker (for example: MD-01-2024) and cap it tightly.
7. After each set of 8 colonies, discard the latex gloves and clean the hive tool with bleach solution to prevent cross-contamination between apiaries.
8. Cover the tube with aluminum foil or put it in a black plastic bag to prevent sunlight from degrading any pesticides that could be contained within.

Shipping:

Once all the hives in the yard have been sampled for pests and disease, place the labeled tube of wax into a refrigerator or freezer for storage. Once all 10 wax samples from all apiaries have been collected, please ship them with an ice pack to:

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