Methodology

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Mites aggregate on adult drones. Unfortunately, the alcohol wash was not designed to sample drones. This presents a predicament for beekeepers who want a reliable method to detect mites early in the season. Here we are asking beekeepers in the state of Virginia to help us validate a new sampling technique that maybe better than the alcohol wash at detecting *Varroa* early in the season. With early detections beekeepers can be empowered to take control of mites before the mites devastate their colonies. Thank you for joining us this year. This document serves as a methods paper for your reference.

Figure 1: Mites aggregated on an adult drone.

Methods

Sampling – a brief overview

Two sampling methods will be performed once a month, over the course of 5 months on each colony in the trial. An alcohol wash is performed using standard methods of collecting ½ cup of bees from the center of the brood nest and placing them into alcohol. For consistency across the trial, bees are sampled from a "typical" frame from the brood nest. This frame has worker brood of multiple stages (open and capped). Frames which have emerging brood are avoided. Bees are collected by quickly shaking the adult bees from the frame into a steel pan (large hotel pan), knocking the bees quickly to one corner of the pan, and then scooping ½ cup directly into alcohol.

Next, the targeted sampling method is carried out using a brood frame adjacent to the one removed for the alcohol wash. This frame is set horizontally across a spare telescopic cover that served as a table in the shade. Researchers collect drones individually by hand from the surface of the frame, and toss the bee into alcohol. *Varroa* are recorded as the number of *Varroa* on an individual host. A maximum of 40 drones are sampled from each colony. This sample was procured from the two adjacent frames in the brood nest next to the frame used for the alcohol wash. The data is recorded, along with information asked in the field sheet. Sampling is complete when all the data is submitted via an online form.

Sampling continued and fully explained

Note: Remember to label the colony before taking your first sample. You need to identify and return to this colony each month. Do not skip this step. I highly recommend writing directly onto the hive with black magic marker.

Performing the alcohol wash

1. Remove the honey supers from the colony you will sample.

2. Remove a frame directly from the center of the brood nest. The ideal frame will have a mix of capped and open larva on it, *see figure 3*.

3. Quickly shake the bees from this frame into a container. A small rubbermade, plastic dish pan or metal hotel pan work great. Set the frame aside. It will be fine to sit out by itself for a few minutes.

4. Quickly knock all the bees into one corner of the pan, and using a ½ c measuring cup, scoop approximately 300 bees into an open container of alcohol. Isopropyl alcohol is excellent for this. I prefer to use pint deli containers. Cover the sample immediately so the bees do not crawl out as they die. Set the container aside and proceed to step 5: targeted sampling of drones.

Figure 2: A beekeeper removed the honey supers from a colony, and then removed frames directly from the brood nest. Stacking the honey supers in this way prevents excessive robbing while sampling.

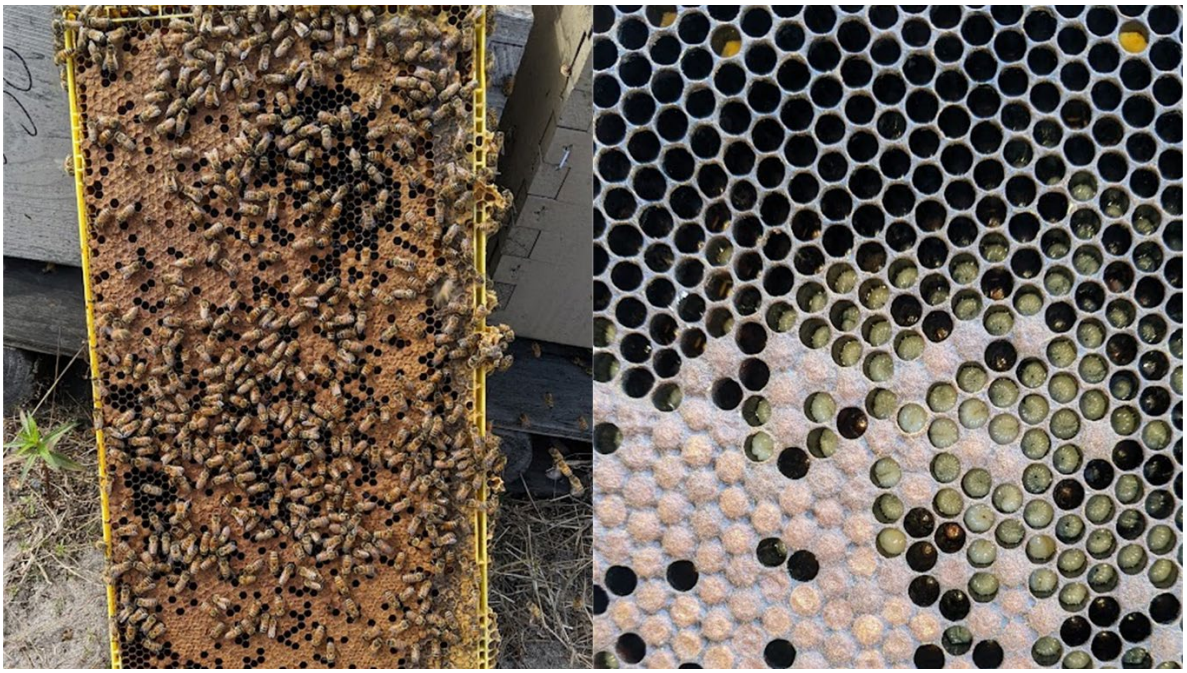

Figure 3: Brood frames from the center of the brood nest are used for the alcohol wash. (Right) a typical brood frame will have a mixed of open and capped brood. If this cannot be found, don't worry. Simply use a capped brood frame.

Figure 4: A beekeeper is ready to shake adult bees off from a brood frame into a large pan. The container of alcohol is ready, and a ½ cup scoop is within arm's reach. Bees are dislodged easiest with a quick downward motion and an abrupt stop.

Step 5: We are now ready for the drone sampling. Go back into the colony and retrieve a new brood frame. Any brood frame from the center of the brood nest will be fine. Be sure to avoid the outside brood frames though. See figure 5.

Figure 5: We will sample adult drones from brood frames. We want to avoid the very outer most brood frames. Avoid these frames (red x). We want frames from where the blue arrow is.

Step 5 (continued) Place the frame and bees horizontally on a surface. The surface should have a lip so that the bees on the frame are not squished. I suggest using an extra cover and turning it upside down. This can be viewed in figure 6. Now that the frame is laid down, pickup about 20 drones from this frame and toss them into an open container of alcohol. When you have completed this, repeat this step on an additional frame for another 20 drones. Cover this container and set it aside.

Step 6. When you have successfully sampled up to 40 drones sampling is complete. Make sure you have answered all the questions on the field worksheet (queen status, colony strength, etc.) Once you have, you are done and may put the colony back together again.

Step 7: Shake the alcohol wash and the drone sample (alcohol wash 3 minutes, drones 1 minute or until no new mites appear). Count the number of bees and mites in each sample. Record the data on the field sheet.

Step 8: Upload the data online using the google form which was sent to you at the beginning of the month.

Figure 6: **(Left)** We don't want anyone to over complicate doing an alcohol wash. After the sample was shake vigorously for 3 minutes, find a convenient way to count out the sample. Some people use EasyCheck shakers. Others may use screens. Here, I just used a white surface with a lip (bucket lid). I placed the lid on a slightly uneven surface and moved the bees out of the alcohol exposing the mite. **Pro tip:** Count out the bees in groups of 10. This makes getting the total number of bees much easier in the end. Remember we want to know how many workers and drones are in the sample!!!

Figure 7: **(right)** Picking up drones are easy. They do not sting. Quickly toss them into alcohol. You are welcome to inspect the drones, but you do not have to. If you find a random mite on your hand, it likely crawled off the drone and onto you when you picked it up. Do not try to pickup drones based on some sort of bias (i.e. likely seeking out newly emerged drones) Simply grab random drones from the brood nest.

Note from authors: Thank you for participating in this study. Although this may look simple, the data you will collect will be instrumental in expanding 3 years of work already done on the subject. Feel free to contact virginiadrones23@gmail.com with any questions!