Alcohol Wash

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Introduction

The honey bee parasitic mite *Varroa destructor* (varroa), is present in almost all colonies in North America. It is essential to monitor varroa populations as this pest can quickly reach detrimental levels within a few months. Visual inspection alone is not sufficient to determine varroa risk, as mites tend to hide themselves in between the abdominal scales on the underside of the bee and are almost impossible to visualize during normal inspection. An alcohol wash is a quick and easy field test to determine the percent infestation of varroa on adult bees.

SUPPLIES NEEDED

A cup for taking samples. A half cup measuring cup will work, but it may be easier to find something that is flat-sided. Examples include a juice box holder with ½ cup marked with a sharpie, or a flat sided measuring cup. Optional alternative: Mark ½ cup on your final container (e.g., quart jar with screened lid), and sample directly into the container. Optional: A dishwashing tub, 5-gallon bucket, or similar container for shaking a frame of bees into.

A strainer device. There are many options dependent on personal preference: double honey strainer, Varroa Easy Check, two jar sampling device, jar with size 8 mesh lid. Note: Many people prefer to sample while each hive is open and perform all of the tests after inspections, and they will therefore use one sampling container for each hive. If you will be doing frequent inspections, you may want to purchase multiple containers.

A liquid to euthanize the bees and dislodge mites: Diluted isopropyl alcohol – you can dilute with water, with final concentration at least 70%. While winter windshield wiper fluid is sometimes recommended, it may not have sufficient alcohol to quickly kill the bees, and the dyes may make visualization of the mites more difficult. A dishwashing soap and water solution can be used in the lab but is difficult to use in the field. The soap solution must be concentrated enough to kill the bees, but this will create excessive sudsing which may obscure the mites and require large quantities of water to rinse.

Water for additional rinsing

Rag / paper towels for cleaning

Filtration for removing mites from liquid for reuse. Can be a very fine wire mesh filter, coffee filters, or a fine cloth

Wide mouth container (like a quart jar) for filtering into

Marking pens for labeling jars if using multiple jars or performing tests in the lab

One minute timer

Magnifying glass (Optional)

Indications

All colonies in North America are at risk from varroa mite damage, so regular monitoring (e.g., every 3 weeks) is recommended for all honey bee colonies when feasible. The greatest risk is seen after periods of high brood rearing (i.e., a large colony at the end of the season), so more frequent monitoring may be needed at that time. Sample at least 8 colonies per yard to capture between-hive variability. If fewer than 8 colonies are present in a yard, then sample all of the colonies. Because varroa mites are so deadly and ubiquitous, varroa mite monitoring should be considered as validation that the current varroa mite management strategy is sufficient – not as a diagnostic tool to determine whether or not a colony will require control methods for varroa.

Conducting an Alcohol Wash

Sample the bees

Your goal is to collect 300 nurse bees (½ cup – lightly packed).

- Fill your jar with enough alcohol to fully cover ½ cup of bees and set nearby.
- Open the hive using a minimal amount of smoke.
- Select a frame from the brood nest ideally containing a mix of brood (open larvae and capped pupae). If no brood is present, select a frame of pollen.
- Check the frame for the queen and remove her to safety or select another frame if found.
- Tap your sample jar on a hard surface to make the bees fall to the bottom so you will have a full sample. *Note: It is not a problem if bees fly away while you are sampling, as these are likely older bees, and your target is younger nurse bees. You can sample from multiple frames if you do not have enough bees from a single frame.*

There are two methods for easily sampling a ½ cup of bees:

Gently run a flat-sided cup along the comb, tripping the bees into the cup (Figure 1). Most people find that gently running the cup downwards, gently over the back of the bees is easiest, though you can also run the cup upwards, tripping the bees' feet.

Figure 1: Sampling bees from a frame. Photo by Geena Hill, UF/IFAS Honey Bee Research and Extension Laboratory.

Dislodge the bees into a tub (Figure 2) with a strong shake or thump on the bottom of the tub, and scoop from the tub. This method allows the unwanted older foragers to fly away and gives time for an extra queen check to ensure she is not sampled.

Figure 2: Bees collected in a tub. Photo by Hannah Gurland, UF/IFAS Honey Bee Research and Extension Laboratory.

Euthanize the mites and bees

Euthanize the bees and mites by adding a sufficient amount of diluted isopropyl alcohol to cover the sampled bees. You may also choose to have your jar prepped with alcohol ahead of time to euthanize the bees immediately and avoid losing bees while uncapping and capping the jar.

Dislodge the mites

Shake vigorously for one minute to dislodge the mites. If you are doing this in the lab, a stand mixer can be used for this step.

Separate the mites from the bees

You want the liquid and mites to flow through a sieve or strainer that holds back the bees, but not the mites (about size 8 mesh). Multiple tools can be used for this process:

- Use a pre-made mite check tool such as the Varroa Easy Check tool.
- Use a screened lid to pour the mites and alcohol into a tub. You can pour the alcohol through a filter for later re-use.
- Pour the bees onto a nested honey strainer with a large sieve (Figure 3).

Rinse multiple times to ensure that all the mites are separated from the bees.

- This can be done with multiple rinses of water, after you separate out the alcohol for reuse.

Figure 3: Bee sample being poured into a honey sieve. Photo by Geena Hill, UF/IFAS Honey Bee Research and Extension Laboratory.

Interpreting the Results

Count the number of mites in your sample and divide by 3 to determine the percent infestation:

# of mites in sample of 300 bees / 3 = percent infestation.

For example, if you count 9 mites in your sample, you will have an infestation of 3%.

Varroa mite risk is seasonal and depends on the time of year and the amount of brood in the hive. Usually, a percent infestation of < 2% will mean that the beekeeper has discretion to apply a treatment or wait, depending on the length of the season, the beekeepers' season long management plan, and many other factors. In general, varroa mite populations should never be above 3%, so action may be taken while the percent infestation is at a lower level to prevent a peak from occurring. Being conservative with management in the spring can help avoid a level above 3% in summer when the colony is large with lots of sealed brood and treatments may be less effective.

Common Errors to Avoid

Using too much smoke when opening the hive - Smoke will cause the bees to run and will make them much more difficult to sample from the frame.

Under sampling - Most beginners will tend to under sample the number of bees, resulting in an underestimation of the varroa risk. To ensure that you are collecting a sufficient number of bees, take the time to validate by counting the dead bees in your sample after you count the mites.

Insufficient shaking - The goal is to dislodge the mites from under the scales of the bees, so vigorous mixing is required. You cannot simply roll or tilt the jar side-to-side. The vigor of this shake should be like a bartender shaking a cocktail or a painter mixing a can of spray paint. To further improve the separation of varroa from bees, you can instead use a hand/stand mixer.

Sampling the wrong bees - The goal is to sample younger bees for an accurate estimation of in-hive mite levels. Sampling large quantities of older foragers will give inaccurate results.

Comparison with Other Methods

Many other methods are listed online for monitoring the varroa mite.

Powdered sugar roll: The powdered sugar roll is quite similar to the alcohol wash test in that it provides a result of percent infestation. In this method, powdered sugar is used in place of alcohol to dislodge the varroa mites. It is used most frequently when beekeepers are resistant to sample because they do not want to kill the bees in the sample, however, the effect of the test on the bees is not well studied. While the sugar roll can work fine, it is more prone to error. If performed in humid conditions, the sugar may be too wet and stick to the bees. The bees have to sit for many minutes in this method to dislodge the mites, so the process takes longer. Many beekeepers will not shake the bees hard enough with this method to dislodge the mites, so it often results in underestimation. If done well, where the bees are dry the entire time, they are allowed to sit long enough, and they are shaken with sufficient vigor, this test is fine.

Sticky board / mite fall: This method fails to provide a mite count relative to the bee population and is difficult to convert results (mites on a board / length of time) to an actionable result. This method is useful to validate the efficacy of a treatment and for general monitoring but should not be used as the sole tool for monitoring mite populations.

Visualization on drone brood: Checking drone brood is listed as a method for monitoring varroa, but there is no evidence that visualizing mites can be used as a method to estimate varroa risk.

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