

How to Dissect Honey Bees (*Apis mellifera* L.) to Detect Tracheal Mites (*Acarapis woodi* Rennie)¹

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Introduction

Tracheal mites, *Acarapis woodi* Rennie (http://entnemdept.ufl.edu/creatures/misc/bees/tracheal_mite.htm), are parasites of the western honey bee, *Apis mellifera* L. (http://entnemdept.ufl.edu/creatures/MISC/BEES/euro_honey_bee.htm) Tracheal mites live within the tracheal system of honey bees and are not visible to the naked eye (Fig. 1). However, even minor tracheal mite infestations can impact honey bee health and potentially decrease colony strength, honey production, and pollination efficacy.



Figure 1. Magnified images of an adult tracheal mite *Acarapis woodi*.
Credits: Pest and Diseases Image Library, Bugwood.org

Multiple symptoms can indicate that a colony may be suffering from a tracheal mite infestation. First, bees infested with tracheal mites may appear disorganized and fail to cluster properly during cool periods. Disrupted clustering can affect the colony's ability to survive the winter. Second, tracheal mites can cause the bees' wings to become disjointed and resemble the letter "K" (Fig. 2). This symptom is referred to as "K-wing." Honey bees suffering from K-wing are unable to fly efficiently and are often seen crawling on the ground near the hive entrance. Third, adult bees infested with tracheal mites may appear physically normal but may be seen crawling on the ground near the hive during the winter and early spring.

Unfortunately, the symptoms described above are not exclusive to tracheal mite infestations and may be associated with other colony pests and pathogens. Proper diagnosis of tracheal mite presence is important in order to evaluate the level of infestation within the colony and determine if treatment is warranted. In order to properly diagnose a tracheal mite infestation, adult bees must be collected and then dissected to expose the bees' tracheae (where the mites live).

Refer to: <http://entnemdept.ifas.ufl.edu/honeybee/extension/TrachaelMites.shtml> for more information regarding tracheal mites and how to minimize their impact on honey bee colonies.

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Figure 2. Adult worker honey bee exhibiting K-wing. Note how the hind wing is disjoined from the forewing, causing the hind wing to stick out from the thorax at a 90° angle that gives the wings and body a distinctive “K” appearance.

Credits: Honey Bee Research and Education Laboratory (honeybee@ifas.ufl.edu), University of Florida

Sample Collection and Preparation

Necessary Equipment

- Screw-cap containers (e.g. half-pint mason jar or baby-food jar)—one per colony to be sampled
- Isopropyl alcohol (i.e. rubbing alcohol)
- Insect net (collection method A)
- Disposable baking pan or plastic tub (approximately 22 × 33 cm or 9 × 11 inch; collection method B).

Overview

Adult bees should be collected in early spring or late winter when the bee population is at its lowest and the tracheal mite infestation is at its highest.

Diagnosis of an infestation is easier in older bees because they are more likely to host high tracheal mite populations than are younger bees. Drones generally host more mites than do workers, possibly due to the increased size of drones' tracheal trunks compared to those of workers. However, workers are more abundant within the colony, especially during times of the year that tracheal mite populations are greatest. Foragers are the preferred worker bees to collect and dissect because they are the oldest bees within the colony and thus have had the most time for mite populations to increase within their tracheae.

Detailed Instructions

1. Prepare collection jars by filling each jar about halfway with isopropyl alcohol.
2. Collect 30–50 older workers or drones per colony into a jar. Only collect live bees, not dead bees from the bottom board or entrance. There are three principle ways that one can collect the adult bees.
 - *Collect adult bees returning to the colony*—Block the colony entrance and allow a cloud of bees to accumulate in front of the colony. Use an insect net to catch the bees hovering around the colony entrance and carefully transfer the bees from the net into the collection container.
 - *Collect adult bees from within the colony by shaking them from a frame*—Shake bees from the outer frames of the uppermost box (if possible, avoid using frames that contain brood) or the hive lid into a disposable plastic bin or baking pan. Gently shake the pan to collect all of the bees into one corner and pour the bees into the collection container. Note: do not shake a frame that the queen is on.
 - *Collect adult bees from within the colony by scooping bees directly from a frame*—Bees can be scooped carefully from the surface of the comb on the outer frames of the uppermost box or the hive lid directly into the prepared collection container; ideally, avoid using frames that contain brood, and of course use caution to avoid collecting the queen.
3. Label the jar to correspond with the colony from which the sample was taken. Also record the collection date on the label.
4. Samples collected in alcohol can be frozen or stored at room temperature. Note: bees that have been preserved in alcohol for more than 90 days will have darker tracheae, and it may be harder to see the tracheal mites.

Dissections

Necessary Equipment

- Dissecting microscope, up to 50× magnification
- Shallow, open container (e.g. petri dish)
- Beeswax or wax with similar consistency
- Two pairs of microforceps (Fig. 3)
- Pins (insect pins are best; any size will be adequate)
- Isopropyl alcohol or water (optional)



Figure 3. Examples of several pairs of microforceps that could be used for tracheal mite dissections. Although the pairs on the left will be adequate, the pairs on the right will be easier to use because of their more finely pointed tips.

Credits: John Bonkowski (jbonkowski@ufl.edu), University of Florida.

Overview

The collected bees are dissected under a dissecting microscope. Forceps are used to remove the abdomen, head, and first pair of legs. A small piece of exoskeleton below the first pair of legs, called the collar, must be removed by peeling it away from the rest of the thorax (it is important to remove the entire collar). Once the collar has been removed, one can see the mesothoracic tracheae. The mesothoracic tracheae are shaped like an inverted letter “V;” this is where the largest concentration of tracheal mites can be found.

Inspect both the right and left tracheae for scarring and lesions caused by mite feeding. The bee has a healthy trachea and is not likely to be infested with tracheal mites if the tracheae are transparent and lack scarring, discoloration, or lesions.

Detailed Instructions

1. Prepare the dissection dish—pour a layer of molten wax, approximately 6 mm thick, into the bottom of the Petri dish and allow the wax to harden (Fig. 4). This will provide a substrate to which the dissected bee can be pinned. It must be pinned to keep it from shifting during dissection.
2. Set the dissecting scope to 20–30× magnification.
3. Any frozen bee samples must be allowed to thaw completely.

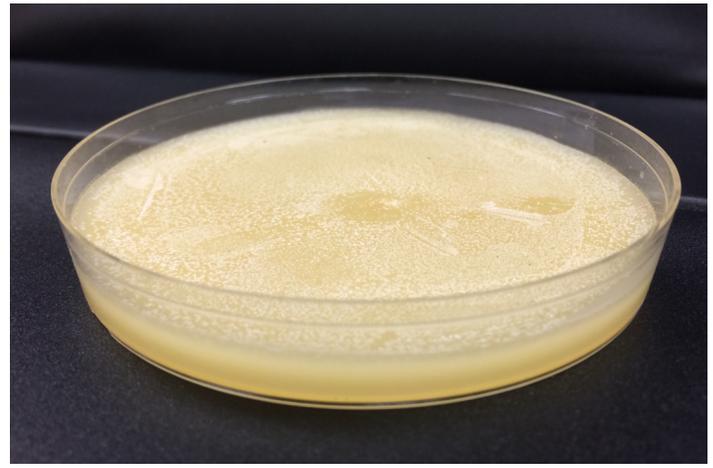


Figure 4. A prepared dissection dish. Note that approximately 6 mm of beeswax has been poured into the dish and allowed to harden. The wax provides a substrate to which a bee can be secured with pins during dissection.

Credits: Ashley Mortensen (mortensena@ufl.edu), University of Florida

4. Put one bee in the prepared petri dish, place the dish under the microscope, and focus the microscope on the bee.
5. Use two pins to secure the bee to the petri dish by placing a pin through both sides of the thorax. It is best if the pins are inserted at approximately a 45° angle facing away from the center of the thorax (Fig. 5).



Figure 5. The honey bee is secured in the dissection dish with two insect pins that are placed on either side of the thorax at approximately a 45° angle facing away from the center of the thorax. Credits: Lyle Buss (LJBuss@ufl.edu) in Sammataro et al. (2013) (<http://dx.doi.org/10.3896/IBRA.1.52.4.20>), Copyright the International Bee Research

6. Flood the petri dish with alcohol or water so that the bee's entire body is submerged. (This step is optional based on your preferred method for dissecting and examining specimens.)

7. Remove the abdomen. Secure the thorax with one pair of forceps and use the second pair of forceps to grasp the petiole (the constricted area of the abdomen, near the thorax). Gently pull the petiole away from the thorax to remove the abdomen (Fig. 6).



Figure 6. A secured bee with its abdomen removed.
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8. Remove the bee's head and first pair of legs. Use one pair of forceps to secure the thorax below the first pair of legs and the second pair of forceps to grasp between the head and the thorax and gently pull the head away from the thorax. Typically, the first pair of legs will separate from the thorax with the head, but if they do not, remove them.

9. Reposition the bee so that the opening created by removing the head and first pair of legs is facing up and visible through the microscope (Fig. 7).

10. Remove the collar. The collar is a plate found below the head and first pair of legs that covers the opening of the first pair of spiracles (Fig. 7—the spiracles are not shown). Use one pair of forceps to secure the thorax, then grasp the edge of the collar on the ventral side (underside) of the bee with the second pair of forceps. Gently pull the collar away from the body, peeling along the suture line where it connects to the rest of the thorax. Be careful; the collar may be brittle, and pieces may break off while removing it. Be sure to remove the entire collar (Fig. 8).

11. Inspect the exposed tracheae for signs of tracheal mite infestation. Removal of the collar reveals the mesothoracic tracheal tubes (two transparent tubes shaped

like inverted Vs; Fig. 8). Healthy tracheae are creamy white, transparent tubes and do not have any scarring or lesions. Tracheal mites cause scarring in the tracheal system, making the trachea cloudy. Dark lesions also may develop within the trachea when moderate to heavy mite infestations are present. Scarring will start closer to the spiracle (outer edges of the trachea; Fig. 9).



Figure 7. The repositioned thorax of the honey bee after the head and first pair of legs have been removed. The collar completely surrounds the opening and is highlighted by a black outline and arrow.
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Figure 8. The honey bee thorax after the entire collar has been removed. The mesothoracic tracheae are completely visible and are in the shape of an inverted letter "V." The trachea on the right has been outlined.
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Figure 9. A honey bee's infested and healthy mesothoracic tracheae. a) Full view of both the left and right tracheae of a single bee. The red (left) arrow is pointing to a trachea that is infested with mites. The green (right) arrow is pointing to a healthy trachea. b) A close-up view of the scarred tracheal tube infested with mites. Note that dark lesions are present throughout the trachea. c) A close-up view of a healthy tracheal tube (no mite damage). Note its transparency as well as the fat bodies around the outside of the tube. Do not mistake healthy fat bodies for mites or scarring. Several fat bodies have been highlighted in this image with two black arrows.

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12. Record any scarring and/or lesions identified in either of the visible tracheae of the bee, discard the bee, and clean any debris from your dissecting dish.
13. Repeat steps 3–12 for 20 bees per colony sample
14. If you discover that 15% or more of the bees you dissect are infested, consider treating the colony for tracheal mites.

Optional Visualization of Tracheal Mites

Necessary Equipment

- Compound microscope (100–400× magnification)
- Glass microscope slides
- Glass coverslips
- Water

Overview

Tracheal mites are so small that it is difficult to see them using a dissecting microscope. To view the mites directly, remove the tracheae from the body of the bee that has been dissected as described above by gently pulling them with a pair of forceps. Put the tracheae on a microscope slide in a drop of water, cover it with a coverslip, and use a compound microscope to view the tracheal mites under higher magnifications (100–400× magnification; Fig. 10).



Figure 10. Five tracheal mites visible in a dissected honey bee trachea (140× magnification).

Credits: Lilia De Guzman, Bugwood.org

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