

## AN EASY DISSECTION TECHNIQUE FOR FINDING THE TRACHEAL MITE, *ACARAPIS WOODI* (RENNIE) (ACARI: TARSONEMIDAE), IN HONEY BEES, WITH VIDEO LINK\*

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**ABSTRACT** - Since the introduction of varroa mites (*Varroa destructor* Anderson and Trueman, 2000), the impact of tracheal mites on bees has been largely overshadowed or ignored. Tracheal mites are still present in bees, and may be responsible for some unexplained colony losses. If they cause bee mortality, it is important to be able to identify their presence and at what levels. This paper illustrates a quick and easy technique for dissecting bees for tracheal mites. Hopefully, the video link will be a useful training tool for researchers, beekeepers or regulatory personnel who need to test bees for the mite's presence. Tracheal mites are still present in some areas and can be introduced through the sale of bee packages or purchased queens. The presence or absence of these mites can also help determine or eliminate the cause of unexplained colony losses, especially in overwintered colonies.

**Key words** - Acari, Tarsonemidae, *Acarapis woodi* (Rennie), bee mite, tracheal mite, video link.

### INTRODUCTION

Because of their small size, mites that dwell in tracheal systems of insects are generally overlooked and so far have been reported only in the insect orders Orthoptera, Coleoptera and Hymenoptera (Sammataro, 1995). The most well-known is the honey bee tracheal mite, *Acarapis woodi* (Rennie), a tarsonemid mite of honey bees, *Apis mellifera* L. First identified in dying bee colonies on the Isle of Wight in the early 1900s, these mites were a major cause of honey bee mortality in Europe. With this discovery, the importation of honey bees into the United States was banned by the U.S. Department of Agriculture in 1922.

Reported in Texas in 1984, the honey bee tracheal mites were later reported in seven states that same year. This fast spread was greatly facilitated by the extensive trucking of bee colonies from southern states northward for pollination and for sale as package bees and queens. There were significant colony losses throughout North America after the initial invasion, with some losses of

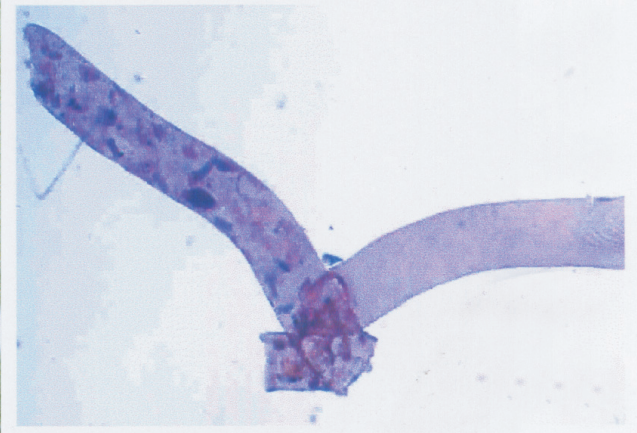
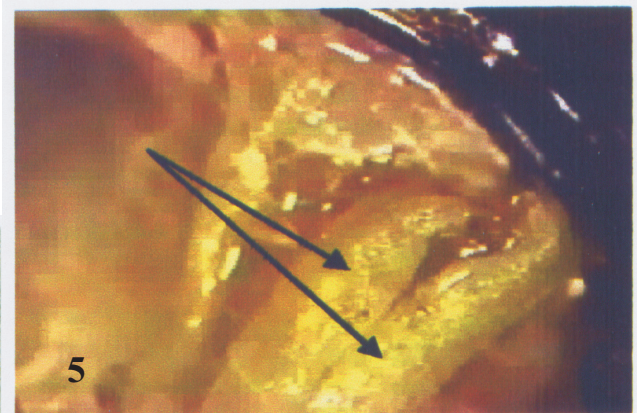
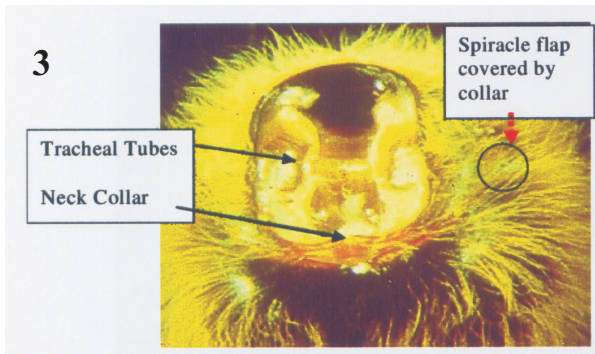
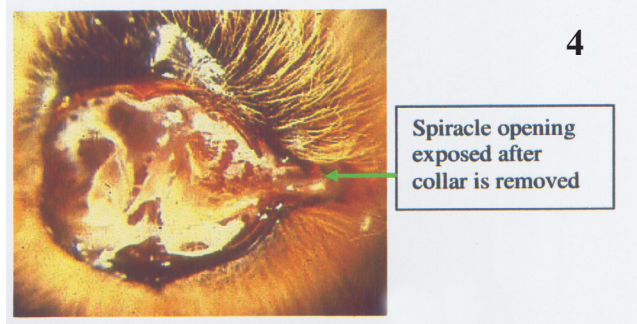
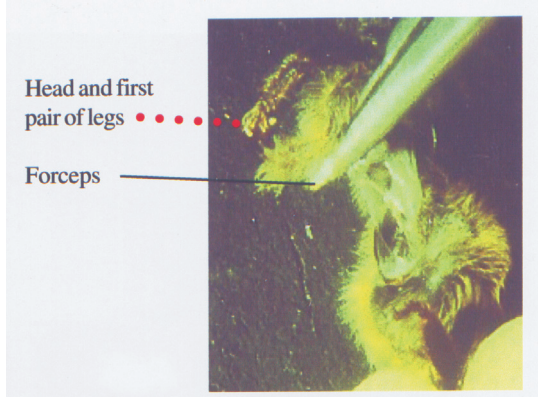
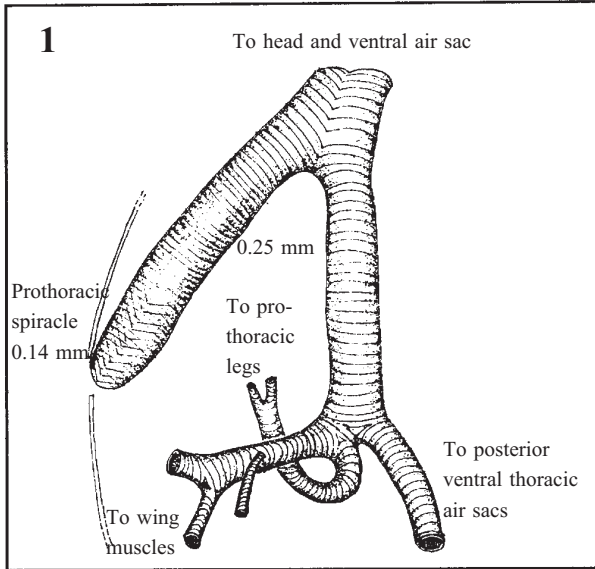
90% recorded two years after initial discovery (Sammataro, 1995). The infestation of these mites affect bee colonies in diverse ways, including diminished brood area, smaller bee populations, looser winter clusters, increased honey consumption, lower honey yields (Bailey and Ball, 1991; Eischen, 1987; Morse and Nowogrodzki, 1990; Otis and Scott-Dupree, 1992; Royce and Rossignol, 1989), and, ultimately, colony demise.

Mite populations in temperate climates increase during winter months when the bees are in the winter cluster and confined to the hive proper. In the summer the opposite is true, and when bee populations are highest (Sammataro *et al.*, 1994), tracheal mites are more widely dispersed and therefore difficult to find. In warmer climates, symptoms of honey bee tracheal mite (HBTM) infestations may be overlooked or not recognized since bees do not normally form winter clusters.

Unfortunately, since the introduction of varroa mites, the impact HBTM has on bees has been overshadowed or ignored. Varroa (*Varroa destructor* Anderson and Trueman, 2000) was identified in 1987 in the United

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When paper was submitted for publication to IJA, author came to know that Dr. James Amrine Jr., Morgantown, West Virginia, was using and teaching same technique to his Apiculture students and that he had the same technique on his web site (<http://www.wvu.edu/~agexten/varroa/TrachealMites.pdf>) since early part of 2006.



States and since then the impact and awareness of the tracheal mites has been largely ignored by beekeepers. However, this mite is still present in bees, and may be responsible for some unexplained colony losses (Hempken, pers. comm.) (For more complete information on *Acarapis*, see Sammataro *et al.*, 2000; Webster and Delaplane, 2001). If tracheal mites are the cause of recent bee mortality, it is important to be able to determine the presence of tracheal mites in bees and at what levels.

Treatments used to control the two bee mites are very different. Treating colonies without knowing the causative agents not only wastes money, resources, and bees, but could contribute to contaminating an otherwise healthy hive with pesticides and other unnecessary chemicals. In addition, supposed tracheal mite symptoms may in fact be caused by other treatable pathogens, such as Nosema disease. Thus, positive identification of these mites is necessary to select the proper control measures. Unlike varroa mites, tracheal mites are microscopic. Visual symptoms of infestation, such as bees crawling on the ground, K-winged bees or empty hives in the spring (called ‘disappearing’ disease), are either unreliable or could be an indication of other bee diseases.

There are at least seven methods described for detecting HBTM (Camazine *et al.*, 1998; Lorenzen and Gary, 1986; Royce, 1990; Smith *et al.*, 1987; Shimanuki and Knox, 2000). These include: 1) cutting thoracic disks and soaking them in heated potassium hydroxide (KOH), 2) a two-part method which includes utilizing the KOH method then staining cleared tubes with methylene blue (requiring special chemicals and equipment), and 3) the Enzyme-Linked Immunosorbent Assay (ELISA), only useful in laboratory settings.

Most beekeepers do not have access to the chemicals or equipment needed to detect HBTM, using previously reported methods. However, beekeepers are able to obtain and use a dissection microscope. The purpose of this paper and video is to demonstrate an easier ‘tracheal

pull’ technique that will give an instant yes-or-no answer to the presence/absence of tracheal mites. This is a fast and accurate method that takes the minimum of special instruments and sample preparation. Because the method is difficult to learn without visual aides, I have added photos and attached a video to explain and illustrate this technique.

## MATERIALS AND METHODS

A good binocular dissecting microscope of at least 50X magnification is required as well as a pair of jeweler’s forceps that have very fine tips (such as needlepoint biological or watch maker forceps #3 or #5). In collecting bees for dissection, older worker bees or drones are best. Collect foraging bees as they return to the hive’s entrance. Alternatively, bees can also be collected inside a hive from a honey frame or inner cover (not from a brood frame). The best sampling time is in early spring or fall, not during the summer months, as summer bees will have fewer mites. Bees from the over-wintering cluster collected in early spring will have the highest mite populations.

At least 50 bees from each colony or a composite sample of 1000 bees from each apiary should be examined. Sample as many hives as possible per apiary (Frazier *et al.*, 2000). Old queens (when requeening colonies) and drones can also be collected and examined for the presence of HBTM. Do not use the dried, dead bees from a winter-killed colony, since mites will not be easily revealed.

Place the live bees in a plastic bag or in a glass jar/vial. If a large number of samples are collected at one time, place the bags on ice in a cooler, until they can be frozen. Alcohol preservation is not recommended, as the tissues can become too dark for easy mite detection when stored for longer than 90 days.

Explanation of figures (see left page):

Fig. 1. Prothoracic tracheal trunk of left side of honey bee thorax. This, or the right trunk, is the easiest place to locate mites. The spiracular opening is the interface of the trachea to the outside of the bee, located on the thorax right behind the wings (Drawing by D. Sammataro).

Fig. 2. Step one - Hold the bee by the thorax and use fine forceps to pull off the abdomen, then the head and first pair of legs of the bee (Photo by L. Royce).

Fig. 3. Step two - Once the head is removed, the neck or cervical collar now must be pulled free to reveal the tubes lying underneath. This is the view into the anterior foramen of the thorax with head removed. Tracheal tubes and neck collar are clearly visible. All tubes are clear of mites in this photo (Photo by L. Royce).

Fig. 4. Step three - The prothoracic trachea are clearly visible (see below) once the collar has been removed (from previous figure). All tubes are clear of mites in this photo (Photo by L. Royce).

Fig. 5. Step four - By increasing the magnification of the tubes, mite presence can be seen more clearly. The shadows and debris in the tracheal tubes are tracheal mites (arrows) (Photo by D. Sammararo, from video).

Fig. 6. Dark field micrograph close-up of a tracheal tube full of mites and eggs (Photo by D. Sammataro).

Fig. 7. Tracheal tubes stained with methylene blue illustrate tracheal trunk infested with HBTM (left) and uninfested (right) tracheae trunk (Photo by D. Sammataro).

## BEE DISSECTION

Honey bee tracheal mite lives and reproduces in the trachea of honey bees. The largest tracheal tube in honey bees is in the thorax (the prothoracic tube), which supplies air for the legs and wings of the bee (Fig. 1). The female mite enters the prothoracic tube by means of the spiracular opening. Once inside, she generally stays near the entrance and lays her eggs. Therefore, it is important to check this area for mites and eggs, as the presence of mites near the spiracle opening will be indicative of the initial infestation.

As mite numbers increase, they move further into the tracheal system, until the tubes become dark brown or completely blackened because of mite-feeding activities and melanization of the tubes.

**Sample preparation** - If live bees are used, put them into the refrigerator or freezer or on an ice pack to cool down before dissection. Pull out the sting or the abdomen to avoid being stung. Bees stored in alcohol can be used as long as samples are not older than 3 months. Alcohol tends to make tissues dark and brittle and can cause the tracheal tubes and mites to become too transparent. If using frozen bees, take bees out of the freezer and carefully place some on a paper towel to warm to room temperature. Avoid shaking the frozen bees in the container, as bee parts are fragile and will break off easily. When thawed, grasp the bee by the legs or thorax; never hold by the abdomen which is full of fecal material and will be easily evacuated when pressed.

**Method to dissect bees** - First, pull off abdomen, then the head and first pair of legs using forceps (Fig. 2). Once the head is removed, hold the thorax straight up so that the neck opening is visible under the microscope and adjust the focus. The tracheal tubes will appear as white thread-like structures on either side (Fig. 3). If the bee is heavily infested, mites will appear as dark spots or stains on the white trachea; the tube may appear discolored and botchy.

In order to see the entire prothoracic tracheae and the mites that may lie near the spiracle opening, the prothoracic collar or neck collar must be removed. Grasp the collar with the forceps on the ventral side at the suture line where the collar joins with the thorax (arrow, Fig. 3). Pull upwards and around, following the ring of the collar. It will come off easily, usually in one piece. Once off, there will be a better view into the thorax and the spiracle opening (Fig. 4). This is the area that is covered by the spiracle flap or lobe, which protects the spiracular opening. In light infestations, female mites will often lay eggs here so it is important to examine the area next to the spiracle.

If mites are present, shadows and dark patches inside the tubes will be visible (Figs. 5 and 6). In order to see the tracheae more clearly, pull them out of the body cavity and place them on a piece of double-sided tape

fixed to a glass microscope slide. The trachea can then be teased apart with a minute insect pin mounted on a wooden skewer or applicator stick. Tubes can also be stained with methylene blue (Peng and Nasr, 1985) or other material to make the mites more visible (Fig. 7). If using live bees, the mites will crawl out onto the slide and the male/female ratio can be determined.

## VIDEO

The video recording (Sammataro, 1994) illustrates this technique using a drone. To view the video, log onto [http://www.ars.usda.gov/main/site\\_main.htm?modecode+53420300](http://www.ars.usda.gov/main/site_main.htm?modecode+53420300). Drone bees are larger in size and easier to handle, but workers and queens are also susceptible to mite infestations. The most important tools are a sharp, straight pair of fine forceps, in order to grasp the tissues cleanly and easily, and a microscope or a high power hand lens.

## CONCLUSION

This paper illustrates a quick and easy technique for dissection and detection of tracheal mites. The video link can be used as a training tool for those researchers, beekeepers or regulatory personnel who need to test their bees for the mites' presence. Beekeepers must not ignore that tracheal mites are still present in some areas and may even be introduced through bee packages or queens purchased from bee breeders from infested areas. The presence or absence of these mites can also help determine or eliminate the cause of unexplained colony losses, especially in the early spring months.

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