# Honey Bee Diseases: American Foulbrood

American foulbrood only attacks honey bee larvae, weakening the colony and quickly leading to its death.



Infected larva ropes out when poked with a toothpick

# General description

American foulbrood (AFB) is a bacterial brood disease that results from the infection of honey bee larvae with *Paenibacillus larvae*. While it only attacks larvae, AFB weakens the colony and can quickly lead to its death in only three weeks. AFB is most commonly transmitted through spores of the bacteria, which can be dormant in the colonies or used equipment for 70 or more years. When nurse bees feed larvae with food contaminated with spores, the spores turn into a vegetative stage that replicates in the larval tissue leading to its death. Larvae killed by these bacteria have a unique "foul" odor that gives this brood disease its name.

New spores form after the larva dies. Death typically occurs after the cell is capped, during the last two days of the larval stage or first two days of the pupal stage. When the larva remains, and bacterial spores dry out, they form a scale that is glued to the cell, and it is typical of this disease (image 4). This is one of the conditions that can be used to diagnose this disease. There is no cure for AFB, and beekeepers must act when the disease is found.

## **Symptoms**

• Spotty, irregular brood pattern (Image 1)

- Sunken, dark, greasy, perforated cappings (Image 2 4)
- Pupal mass under cappings is brown and has a ropey consistency (lasts about three weeks after death) (Image 5)
- Dark, hard scales that cannot be removed are found in late stages (after about one month of infection) containing perhaps 100 million spores (Image 6)
- Pupal tongue sticking up from the remains
- Foul odor (in later stages)

## **Early Stages of Infection**

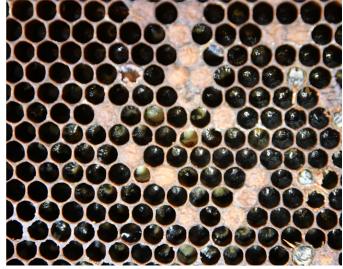


Image 1. Spotty brood pattern. Image by Stephen J Repasky

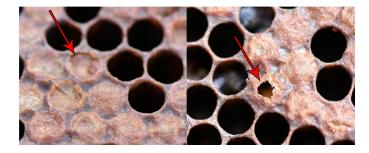


Image 2. (Left) Sunken capping and (Right) perforated capping. Images by Stephen J Repasky

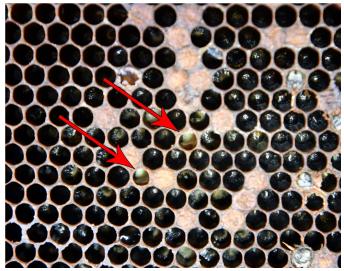


Image 3. Sunken larvae. Image by Stephen J Repasky

# **Later Stages of Infection**

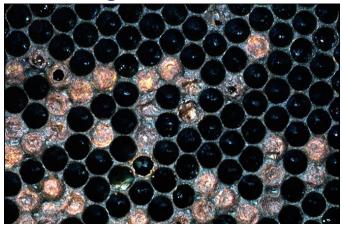


Image 4. Greasy cappings, spotty brood pattern, perforated cappings, and scales. Image by Maryann Frazier

# **Diagnostic tests**

#### Ropiness test

- With a toothpick, match stick, the stem of a leaf, or small twig, poke the brown pupal mass.
- Pull the toothpick out of the cell slowly
- A positive test will result when the brown, snotty mass ropes out of the cell ½ inch or more (Image 5).
- A negative test will result when the pupal mass releases from the toothpick.



Image 5. Ropiness test showing a positive result. Image by Margarita M. López-Uribe

#### Blacklight visualization

- AFB scales glow under a black light.
- Shine the light onto the frame. Scales will glow with a greenish-bluish color (Image 6B).

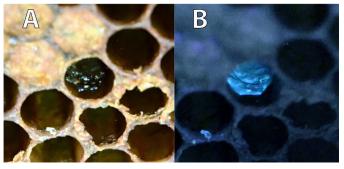


Image 6. Hard scale using normal light (A) and a black light (B). Images by Stephen J Repasky

#### Holst milk test

- Make a weak milk solution containing 1 tsp powdered milk and 100 ml water (a little less than ½ cup).
- When the powder is dissolved, divide the mixture into two small glass containers.
- Gather as much brown pupal mass material as possible using a toothpick, match stick, or leaf stem.

- Add the pupal mass material to one of the containers with the milk solution.
- Put both containers in a warm place for one hour (without adding anything to the second container).
- A positive test will result from the liquid turning clear brown. The control tube (without the pupal mass material) should show a whitish liquid (Image 7).

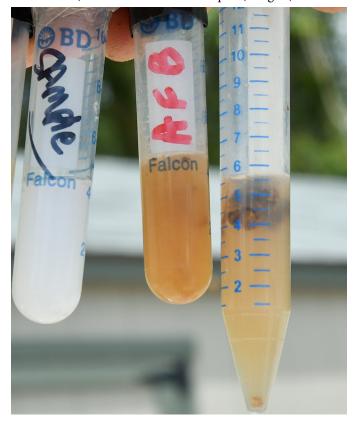


Image 7. Holst milk test with the control (sample) and two test vials that are positive for AFB. Note that the positive samples do not look milky like the control. Image by Steve J. Repasky and Bonnie Hall

## Diagnostic test kit

- An AFB test kit can be purchased from a beekeeping supply company (Image 8A).
- Gather brown pupal mass material using the spatula provided with the kit.
- Place the material into the extraction bottle provided.
- Close the lid of the extraction bottle and shake the liquid for 20 sec.
- Use the provided pipette to add two drops of the solution into the sample well of the test device.
- When the liquid appears in the viewing window, and the control line appears, the test can be read
- A line under the letter "C" and another under the letter "T" indicates a positive result. A line only under the letter "C" indicates a negative result (Image 8).

• How To Use the AFB kit



Image 8. Diagnostic test kit package and (right) the tests. The top test, with two lines, is positive for AFB and the bottom test, with a single line, is negative. Images by Steve J. Repasky and Bonnie Hall

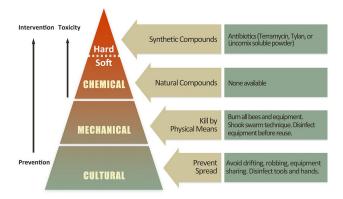
#### **Laboratory Test**

- Brood and comb samples can be sent to the USDA for analysis. Bee Research Laboratory: Beltsville, Maryland
- A comb sample should be at least 2 x 2 inches and contain as much of the dead or discolored brood as possible.
- No Honey Should Be Present in the Sample!
- The comb can be sent in a paper bag or loosely wrapped in a paper towel, newspaper, etc. and sent in a heavy cardboard box. AVOID wrappings such as plastic, aluminum foil, waxed paper, tin, glass, etc. because they promote decomposition and the growth of mold.
- If a comb cannot be sent, the probe used to examine a diseased larva in the cell may contain enough material for tests. The probe can be wrapped in paper and sent to the laboratory in an envelope.
- Send samples to Bee Disease Diagnosis, Bee Research Laboratory, 10300 Baltimore Ave. BARC-East, Bldg. 306 Room 316, Beltsville Agricultural Research Center - East, Beltsville, MD 20705

Contact your regional Pennsylvania Department of Agriculture Apiary Inspector or State Apiarist if you think you have a problem and would like to arrange for an inspection.

## Prevention and control

To prevent the spread and facilitate the most effective control of AFB, it is important to use an integrated pest management approach (IPM) (Image 9). Overall, IPM applies a holistic approach to manage diseases and pests first using cultural, mechanical, and physical approaches before using chemical control. There is currently no chemical control for the spores of AFB. Therefore, the destruction of bees and equipment is the safest way to control the spread of the disease.



#### **IPM for American Foulbrood**

Image 9. The Integrated Pest Management (IPM) pyramid for American Foulbrood. Image by Nick Sloff.

# How to prevent acquiring or spreading AFB

# Prevent drifting and robbing between colonies

- Do not place hives in a straight line, but rather a horseshoe, serpentine, or other haphazard design.
- Keep entrances small when colonies are new or weak.
- Place a robbing screen on weak colonies.

# Avoid sharing equipment between colonies

- Carefully inspect any frames that are being transferred between colonies.
- Avoid sharing, if possible.
- Clean hive tools thoroughly with isopropyl alcohol between colonies and apiaries.
- Avoid wearing leather gloves, as they can harbor spores.
   Instead, wear disposable nitrile gloves or do not wear gloves at all. Nitrile gloves and bare hands can be disinfected using an alcohol-based hand sanitizer.

# Comb culling: remove old combs on a 3-year cycle

• Each year, remove and replace the oldest 3-4 frames in each box. It is helpful to label new frames with the date (year) whenever they are added to the colonies.

#### Buy new, not used, equipment

- Buying new equipment is the safest.
- If receiving or purchasing used equipment, ask the seller for their inspection records.

- Do not purchase equipment from a beekeeper that has had AFB.
- If you buy used equipment, irradiate used equipment prior to use. Many beekeeping clubs or organizations organize irradiation events each year. Used equipment can be irradiated without residual effects.

Irradiation is a sterilization procedure that kills microorganisms present on equipment using gamma radiation powered by Cobalt-60. The radiation penetrates cells and breaks down their DNA. This technology is very safe, has no residual radiation remains, and no chemicals are used. There are irradiation facilities in the Mid-Atlantic region. Contact your state or local beekeeping club to obtain further information about the cost and scheduling of irradiation of hive equipment. If you plan to irradiate equipment that you know is infected with AFB, be sure to store it in a bee-free place to prevent the spread of the disease.

# What to do when a colony is diagnosed with AFB

# **Biological controls**

None

# Physical-Mechanical controls

#### Shook swarm

- If you would like to save the queen and worker bees from an AFB infected colony, purchase new equipment to transfer the bees (old boxes, but not frames, can be kept if all interior surfaces are scraped and scorched with a blowtorch or if it has been irradiated, as described below)
- Add one frame of empty, healthy drawn comb to the new equipment
- Shake bees from the infected colony into the new equipment (be sure to take care with the queen)
- Feed the bees fresh sugar syrup as they build comb
- After 24 hours, shake the bees from the one drawn comb and remove it. Replace it with a new frame
- Treat all infected equipment using the instructions below.
- Note: only use this option if you would like to keep some
  of the bees alive at the risk of not completely controlling
  the spread of the spores.

#### Burn all bees and equipment

Burning the bees and all of the equipment is the only sure
way to be absolutely free of this disease. Burning bees and
equipment found to be infected with antibiotic-resistant
AFB is mandatory in some Mid-Atlantic states. This
should be accomplished as soon as possible once AFB is
detected.

- Do not attempt to keep any honey from these colonies, as it contains spores and will contaminate your extraction equipment.
- The bottom board, hive bodies, supers, and inner and outer covers can be disinfected and reused (see below).
   However, there is no guarantee that the equipment can be completely sterilized, and the disease may reappear, so you may opt to burn everything.
- Plastic hive components pose an environmental hazard if they are burned. Thus, these can be double-bagged using heavy plastic bags, then transported to a waste facility, where burial should be witnessed by you. Alternatively, these hive components can be carefully stored in a bee-free place until irradiation is possible. This method is described above in the "buy new, not used, equipment" section. Note that the frames need to be scraped after irradiation because the equipment will appear to still have the symptoms of AFB even though they will not be infective. Collect all scrapings and dispose of them.
- Before burning, diseased colonies should be killed in the
  evening after all foraging activities have ceased. This can
  be done by closing up all hive entrances and drenching the
  bees in the colony with diesel fuel at a rate of 1 cup per
  hive body. Pour it over the cluster, close the lid and wait at
  least 10 min to allow the bees to become immobile.
- Prior to beginning the burning process, determine whether you will need a burn permit. If so, obtain one before you proceed.
- To burn diseased equipment, dig a sloping pit 18 inches deep and wide enough to hold all combs and equipment to be burned (about 3 ft). With water or a fire extinguisher on hand, build a fire in the pit using newspaper or other kindling. Set your unopened hive close to the pit and drop combs and dead bees into the fire, a few at a time. Never leave the fire unattended and allow for 2-3 hours to complete the process. Be extremely careful with this step to avoid flares from fuel residue.
- After everything (every single frame must be burned; other equipment may also be burned) has been completely burned and the area is cleaned of small pieces >of comb or bees, cover the ashes with dirt.

# Lids, bottom boards, and hive bodies can be saved as follows

• Equipment that was saved (bottom boards, hive bodies, and covers) should be scraped to remove all propolis and wax, then scrubbed with a stiff brush and hot, soapy water. Dispose of the wash water and burn the scrapings, so they are not accessible to the bees. After scraping and scrubbing, all equipment should be either fire scorched or completely immersed in a boiling lye solution.

#### Lye Solution

Prepare your lye solution (sodium hydroxide) by mixing 1 pound of lye with 10 gallons of water. Boil the equipment for 20 minutes; wooden parts can be damaged by longer exposure. Weaker solutions may not remove all of the wax and propolis from the equipment. Remember that lye solutions are caustic and can cause severe burns. Before using, read the label carefully and observe all precautions.

#### Fire Scorching

- A blowtorch is suitable for scorching small quantities of equipment. Burn the surface until it is light brown, making sure to include the corners. For large quantities of hive bodies, brush the inside surfaces with kerosene. Stack the hive bodies upside down on top of each other, five to eight supers high, and then ignite the stacks, allowing them to burn long enough to lightly char the wood. Another approach is to fill the stack with wadded sheets of newspaper sprinkled with kerosene. Place an outer cover on top of the stack to smother the fire when you are finished. Be sure to check all surfaces to be sure they were scorched, including all wood, and spot-scorch as needed. A fresh coat of paint on the outside of the hive would also be advised.
- After scorching, the equipment can be sprayed with a 1.5% bleach solution to kill any lingering AFB spores. If using household bleach, make a solution of one part bleach to one part water to obtain the desired concentration. When handling bleach, protective clothing, goggles, and rubber gloves must be used. This step is optional.
- Contaminated equipment can also be irradiated (see above).

### Chemical controls

### Soft (biorational)

None

### Hard (conventional; synthetic)

- Antibiotics [Terramycin (Oxytetracycline), Tylan (Tylosin titrate), and Lincomix soluble powder (lincomycin hydrochloride)]
- Note that as of January 1st, 2017, these antibiotics can only be obtained with a prescription from a veterinarian in the United States.
- Antibiotics do not kill *Paenibaccillus larvae* spores but prevent or delay their growth when present in low concentrations in the food that workers feed to susceptible larvae. While this treatment allows individual larvae to survive, it does absolutely nothing about the virulent spores in the contaminated equipment. Thus, the disease usually reappears once drug feeding stops.

• Increasing numbers of colonies have been reported infected with AFB that is resistant to the antibiotic Terramycin. Thus, treatment by burning or irradiation is recommended and more cost-effective.

Remember to contact your regional Pennsylvania Department of Agriculture Apiary Inspector or State Apiarist if you think one or more of your colonies have AFB.



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