



Ruth O'Neill, Research Associate Wanner Entomology Extension Lab

## HEALTHY BROOD COMB



- Clean polished out cells,
  healthy white
  brood or eggs;
  clean, nonmoldy bee
  bread; capped
  brood cells have
  intact caps
- Laying pattern in concentric circles

### "Spotty Brood" Comb

• Random, ragged brood pattern

Causes? Inspect frames closely

- Problem with queen (old, sick, poorly mated, genetic issue)
- Problem with brood death due to disease, insects, or mites
- Normal concentric pattern breaks down late in the year



#### Other problems:

- Dead bees at the bottom or outside of the hive
- Slimy or webbed-up frames
- Insects in or around hive
- Empty hive workers have vanished

#### OUTLINE

- 1. HONEY BEE DECLINE
- 2. ARTHROPODS
- PARASITIC MITES
- INSECT ENEMIES
- 3. DISEASES
- BACTERIA
- FUNGI
- VIRUSES

## **1. HONEY BEE DECLINE** Some ideas that have been put forward:



- Past research strongly links toxic viral cocktails to CCD, but linking specific viruses has been elusive
- HBs are known to transmit Tobacco Ringspot Virus (TRSV) plant-to-plant during pollination
- TRSV has many plant hosts but not previously known to infect animals
- New study published January 2014, summary:
  - HBs are infected by TRSV via pollen; the infection becomes widespread in their bodies, with unknown health effects on individual bees
  - TRSV has been linked to hives that suffer CCD and fail
  - However, because it is always present in combination with many other viruses, a direct link has not been established



# **2. PARASITIC MITES**



Varroa Mite, Varroa destructor

- External parasites that feeds on hemolymph ("blood") (shown here feeding on an adult HB)
- Brood and adults
- Also occur on bumble bees, scarab beetles, and hover flies; but they can only reproduce on honey bees
- Enormous economic harm to beekeeping industry

Vector pathogens directly through feeding punctures; open wounds also vulnerable to fungi, bacteria etc.



Worker honey bee with "deformed wing virus" vectored by *Varroa* 



#### VARROA LIFE CYCLE:

- Mated female mite enters cell 15-20 h before capping, hides in brood food
- When cell is capped, bloodfeeding begins (HB's day 9-10)
- Cell eventually contains several (~5) mites; female plus offspring (one male offspring mates with sisters)
- When new bee emerges, mites can hitch a ride to new cells

- VARROA PREFER **DRONE** (MALE) CELLS
- In the fall, when drone-rearing naturally ceases, mites switch to worker larvae
- Late-season population crash and hive death often follows

Hygienic behavior: Workers recycle a drone pupa infested with Varroa

Image: Eric Tourneret

... where toxicants won't penetrate, so new mites keep emerging HARD TO KILL ~70% to 85% are concealed in brood cells

### Monitor for Varroa before you're overrun

HOW TO MONITOR BROOD:

- Pull caps off ~50-100 cells (can use an uncapping fork)
- Mites often run to the capped end; larvae can also be pulled out for a more accurate count





#### WHEN TO MONITOR:

- Monitor once in spring, once in fall
- But, monitor every month when mites are a known problem

### Monitoring with drop counts



#### HOW:

- Install for 24 hours
- Count bodies
- Use counts in combination with your cell inspections to determine if treatment needed

#### Monitoring with the sugar roll test

#### "widemouth" with 8-gauge mesh

1 or 2 tbsp powdered sugar + ½ c. brood workers\* (~400)

nt not the queen!

- Mark jar for bee level; put sugar in
- Shake workers from a brood frame into jar Screw mesh over top
- Gently shake and roll jar, coating bees
- Let jar sit 1-2 min; bees actively groom
- Shake inverted jar over receptacle (white tray or white pail of water). Mites + sugar fall through mesh, bees stay in
- Count mites
- Pour bees on ground near hive when done



### How do you interpret the sugar roll test?

- U. Minnesota: <u>http://www.beelab.umn.edu/prod/groups/cfans/@pub/@cfans/@bees/</u> documents/asset/cfans\_asset\_317466.pdf
- 1. Calculate the number of mites per 100 bees; if brood is present, double that number
- 2. 10 mites per 100 is the treatment threshold
- 3. If the colony has over 10 mites per 100 bees, consider control

WHAT KINDS OF CONTROL ARE AVAILABLE?

- 1. Mechanical controls
- 2. Chemical controls

### *Varroa* suppression: Try using only screened bottom board (remove wood panel)



### Varroa suppression: Powdered sugar on frames encourages grooming



### Varroa suppression: Drone brood sacrifice



- Drone brood foundation has larger cells than worker brood foundation
- Per hive, insert one or two drone frames (or empty frames)
- Best to place drone frames toward the outside of the brood chamber
- Queen lays drone eggs on special frames; Varroa concentrate attacks there
- Rotate drone frames out and freeze them; don't clean them out; the bees will do this when the frame is rotated back in
- Must be rotated out before any drones emerge (average emergence 24 days)
- Cut any drone cells on other frames but you should see few or none
- Bees recycle nutrients by cannibalizing dead larvae and eggs

## Varroa control: Thymol strips



- Thymol strip (ApiLife-VAR, Apiguard)
- SLOW: Only kills exposed mites, so must be in hive for 3-4 weeks (1 brood cycle)
- Must not be used when honey supers are in place









# Tracheal Mite, Acarapis woodi

A bee trachea with mites



- Detected in the U.S. in 1984
- 125 to 175 microns long (period is ~ 500 microns)
- Eyeless, internal parasite: Clog the respiratory tubes (tracheae) of bees; feed on blood
- Two-three weeks from egg to egg

- Tracheal mites do not cause acute disease; may shorten life span slightly
- May be visible with magnification around the **spiracles** (the external openings of the tracheae, locations shown in red here)

Spiracle 1: Main infestation site

#### The main thoracic tracheae are exposed in these dissected bees



LEFT: In an uninfested bee, the tracheal tubes are clear and unscarred



RIGHT: When tracheal mites are present, the tracheal tubes is blackened with scar tissue / fecal matter

### Tracheal mite (and Varroa) suppression: Grease patties

- Bees feed on patties; hairs become lightly coated with oil
- Disrupts the tracheal mites' host-seeking behavior; tracheal mites are reluctant to accept oily hairs
- Questing behavior is prolonged and more host-switching occurs (Sammataro et al., 1994)
- Many mites dessicate and die

Sugar and oil



Female must find a young (<4 days old) worker within 2-3 hours, or she will dry out

### Tracheal mite (and Varroa) control: Grease patties



2 lbs. vegetable shortening



3 lbs. white granulated sugar



1 <sup>1</sup>/<sub>2</sub> cups highfructose corn syrup (or CLEAN honey)







3 tbsp. wintergreen oil (optional)

1/3 cup pulverized salt

# **3. INSECT ENEMIES**

#### An internal parasites of adult honey bees

#### OPEN O ACCESS Freely available online



# A New Threat to Honey Bees, the Parasitic Phorid Fly *Apocephalus borealis*

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#### Abstract

Honey bee colonies are subject to numerous pathogens and parasites. Interaction among multiple pathogens and parasites is the proposed cause for Colony Collapse Disorder (CCD), a syndrome characterized by worker bees abandoning their hive. Here we provide the first documentation that the phorid fly *Apocephalus borealis*, previously known to parasitize bumble bees, also infects and eventually kills honey bees and may pose an emerging threat to North American apiculture. Parasitized honey bees show hive abandonment behavior, leaving their hives at night and dying shortly thereafter. On average, seven days later up to 13 phorid larvae emerge from each dead bee and pupate away from the bee. Using DNA barcoding, we confirmed that phorids that emerged from honey bees and bumble bees were the same species. Microarray analyses of honey bees from infected hives revealed that these bees are often infected with deformed wing virus and *Nosema ceranae*. Larvae and adult phorids also tested positive for these pathogens, implicating the fly as a potential vector or reservoir of these honey bee pathogens. Phorid parasitism may affect hive viability since 77% of sites sampled in the San Francisco Bay Area were infected by the fly and microarray analyses detected phorids in commercial hives in South Dakota and California's Central Valley. Understanding details of phorid infection may shed light on similar hive abandonment behaviors seen in CCD.

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Adult female A. borealis phorid fly

- A native fly, normally found on bumble bees (probably their main hosts), as well as other hosts (e.g., yellowjackets)
- None in MSU collection, but assuredly occurs here
- Very recent host shift to honey bees (HB is a nonnative old-world species)



- Female phorid flies chase workers and land on abdomen; insert ovipositor in abdomen, lay eggs
- One or more larvae hatch out and feed internally
- Infested bees disappear from hives
- Infested bees collect in light fixtures. Manipulation of host's behavior? (i.e., "zombie bees"?)



- In ~7 days, mature phorid larvae emerge from between thorax and head
- Emerged larvae move away from the bee to pupate
- Adult flies emerge in ~ 28 days
- In CA, infestations first appeared in June, peaked in July, remained high through December
- In Montana, season would be shorter

Two final instar phorid fly larvae exit a honey bee worker between the head and thorax (red arrows) Phorid adults and larvae have tested positive for *Nosema ceranae* and deformed wing virus

#### WILL THIS BE A PROBLEM IN MONTANA? WHAT TO WATCH FOR:

- Hive abandonment. Could be phorids, could be some other source of "CCD"
- Dead honey bees collecting in light fixtures near hives
- Dead bees and empty fly pupal casings in the bottoms of hives (indicating some emergence within hives and the potential for within-hive multiplication)

If dead honey bees are collected in light fixtures, put them in clear sealed container 4 - 6 weeks; watch for emerging phorid flies



## "Waxworms"

• Larvae spin silk tunnels on hive surfaces and underneath cell caps; feed on wax, pollen, honey, and shed larval skins

Lesser Wax Moth

> Greater wax moth

Greater Wax Moth

# Greater Wax Moth, Galleria mellonella



# Lesser Wax Moth, Achroia grisella

(these are less common)



- From Asia; now distributed worldwide
- Egg-laying female moths prefer to be active at night
- Primarily infest unused supers, frames etc.
- Will invade active hives with weakened defenses (swarming, loss of queen, diseases or parasites, cold temperatures)
- Workers in strong colonies remove wax moth larvae and repair damage promptly







STORED FRAMES AND SUPERS:

- Scrape propolis off and brush frames; put them back in the supers
- Put supers in plastic bags sprinkled with moth flakes (paradichlorobenzene)

ACTIVE HIVES:

• Healthy hives will successfully clean out moth infestations

# Bee Louse, Braula coeca



- Not actually lice, but flies (notice six legs, not eight)
- Often misidentified as Varroa mites
- "Kleptoparasites"; they steal food from bees; cling to hairs on bees' heads, move to bees' tongues when hungry and feed on regurgitated food
- The larvae tunnel under cell cappings and feed on honey and pollen residues no harm to pupae
- Aesthetic damage only

# Robber Flies, Stenopogon spp.

- Robber flies *Stenopogon inquinatus* and *S. rufibarbis* common around western U.S. apiaries
- Adults can be over an inch long
- Approximately 70 90% of their prey are honey bee workers







### Sick bees display a range of symptoms ...





#### HEALTHY LARVAE / PUPAE:

- Plump, white or cream-colored
- Springy texture

#### DISEASED LARVAE / PUPAE:

- Discoloration (brown, grey, black)
- Dessication possible
- "Melting" possible

### Sick bees display a range of symptoms ...



Image: T. Jadczak

#### DYSENTERY:

- Look for excessive bee excrement on the inner cover, top bars and front of the hive near the entrance(s)
- There are usually a handful of bees remaining that appear bloated and wet



#### OTHER COMMON SYMPTOMS OF ILLNESS:

- Lethargy
- Trembling
- Spasms
- Paralysis
- Withdrawal to remote parts of hive
- Shiny or greasy looking (loss of hairs)

### Are you spreading disease?

- Keep hive tools and smokers clean (carry a bucket with washing soda + water)
- Wear disposable gloves that are discarded after each apiary visit
- Dip boots in washing soda bucket
- Control pest arthropods, especially *Varroa* mites
- Regularly replace brood comb foundations in the spring (move old ones toward the outside progressively)



## **BACTERIAL DISEASES American and European Foulbrood**

# (AFB)

Paenibacillus larvae larvae: A rod-shaped bacterium, microscopic size

### Capped AFB cells are sunken



- Before capping, the larva appears perfectly healthy
- Larvae up to 3 days old ingest spores in food; spores germinate in gut
- First day of life most susceptible
- Each dead larva may contain 100 million spores
- Infected brood succumb after cell is sealed; so, AFB is observed in mature "standing" larvae and pupae



- AFB DIAGNOSIS
- When sunken caps are opened, the brood has often melted
- Color may be dull white, brown, to almost black
- Larvae are sticky / stringy / ropey
- Characteristic odor (sulfur, "chicken house" ...)

#### Matchstick Test →





#### A pupa killed by AFB usually has its tongue pointing away from its body



# Dead AFB larvae dehydrate over time, forming a spore-infested scale stuck to the cell



#### AFB DISEASE SPREAD:

- Highly contagious; not seasonal, can occur at any time
- Spreads when workers clean cadavers out of cells and become coated with spores
- When colony weakens, marauding workers from other hives may take contaminated honey back to their hives
- Spores can survive for 40 years





In Montana, as in many states, AFB-infested hives must be completely burned

EUROPEAN FOULBROOD (EFB) COMPARED TO AFB:

- World-wide distribution for both; named for where investigative work was first done
- EFB and AFB bacteria ingested by young larvae in food ۲
- The bacteria that cause EFB does not produce spores
- EFB not as contagious, not as deadly •
- EFB is odorless or nearly so •
- Evidence that Varroa bites may vector EFB
- EFB-infested brood die before they are capped, usually on day four •



European foulbrood

Streptomycin, penicillin, terramycin can

# FUNGAL DISEASES Nosema



Image: T. Jadczak

- Nosema apis (Na) impairs digestion; causes dysentery (yellow streaks)
- Inability to fly, bloating
- May see many dead workers in / around hive
- More common during times of confinement: winter, spring
- *N. ceranae* (Nc) has now largely replaced Na
- Nc does not cause diarrhea; workers tend to die away from the hive
- Can treat with Fumidil B when honey supers not in place

### Chalkbrood, Ascosphaera apis



- Fungal disease of capped larvae; CB mummies on bottom board, caps with holes
- CB spores ingested with larval food; spores germinate in gut if conditions favorable
- Early infection white fluffy mycelia; later, black spore balls may form
- CB thrives in chilled brood just after capping / before pupation
- Larvae vulnerable chilling / CB at this time; even slight T drop for a few hours
- Chilling most prevalent in early summer when colonies are growing
- Excess moisture / insufficient ventilation are also culprits
- Keep hive warm; strengthen weak colonies with more workers; enlarge entrance for ventilation; new comb

# Stonebrood, Aspergillus spp.



- Fungal disease of larvae; causes mummification
- Can kill all larval stages
- Black spore growth often forms bands or blotches
- Infected larvae quickly harden; not spongy like CB-infested larvae
- Usually appears in colonies seriously weakened by other factors

## VIRAL DISEASES

- Viruses are pieces of genetic material that parasitize a host cell, making the cell produce more copies of the virus.
- No vaccines or medications are available for any of the honey bee viruses
- Good sanitation practices are the key to virus suppression



# Family Dicistroviridae PCR Testing Available

Acute Bee Paralysis Virus

- Causes trembling, inability to fly; loss of hair causes greasy appearance
- When paralysis is serious, large numbers of afflicted bees can be found at the colony entrance, crawling up the sides of the hive and blades of grass, and tumbling to the ground
- A common infective agent of bees; associated with colony collapse disorder in hives infested with *Varroa* mites.

Similar to ABPV, with similar symptoms:

- Chronic Bee Paralysis Virus
- Kashmir BeeVirus
- Israeli Acute ParalysisViruses Associated with CCD

Black Queen Cell Virus – only affects the larvae of queen brood

# Family Iflaviridae

Deformed WingVirus (shown)

- Vectored by *Varroa* mites during the pupal stage
- PCR test is available but not usually needed

Kakugo Virus (not shown)

- Newly discovered; occurs here; Asian origin; resides in bees' brains
- Causes aggressive guarding behavior even in non-guard bees

## Sacbrood Virus, Morator aetatulas

- Affected larvae are usually found in capped cells, standing upright
- Heads of larvae are narrow, pointy, darker, may lean forward ("gondola larvae")
- Typically the scales (dried out larvae) are brittle but easy to remove
- Widespread but rarely causes serious losses; should learn to differentiate from foulbrood diseases



# When sacbrood larvae are pulled from their cells, they often look like watery sacks

