American Foulbrood

A newbee vets guide to what you need to know to diagnosis

This awful, horrible, most imaturel bacterial disease of immature brood.

Bees and Humankind

"...A good and spacious land, a land flowing with milk and honey." Exodus 3:8

Cornell Collection

Hunting for honey is a practice that people have engaged in for at least 9,000 years

Honey Hunters of Africa

- Rich history of honey hunting
  - Honey Guide bird

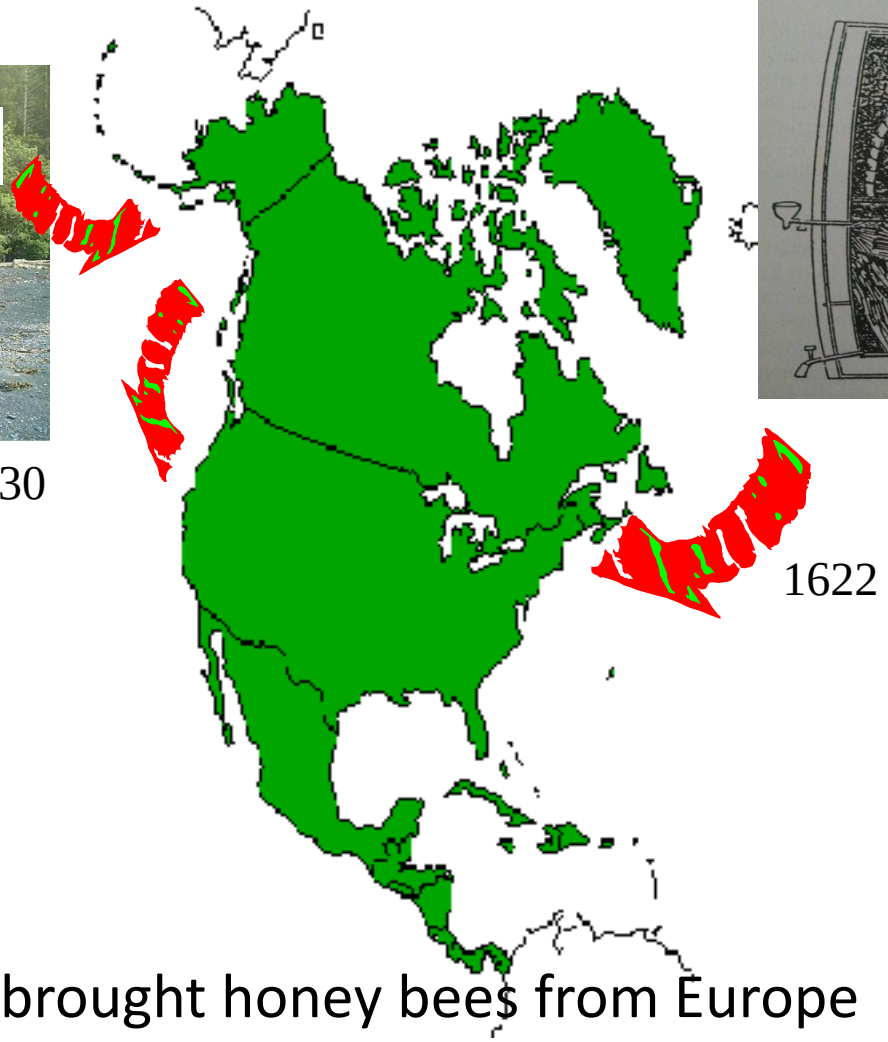
First Real Beekeepers

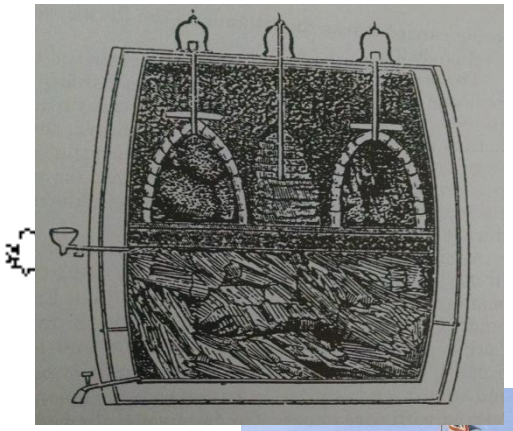
https://www.youtube.com/watch?v=Q8X5GUXRfKc (italian)

Spread of Beekeeping

Beekeeping in North America

1830





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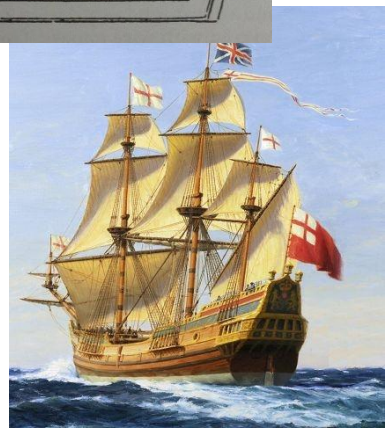


Sea Venture

- Colonists brought honey bees from Europe and Russia

Early American Beekeeping

North America was heavily forested so their was probably a lot of honey hunting done as swarms established in hollow trees

Hives were managed in hollow gum tree trunks
When bees were first introduced a bee gum sold for the price of a cow and a calf

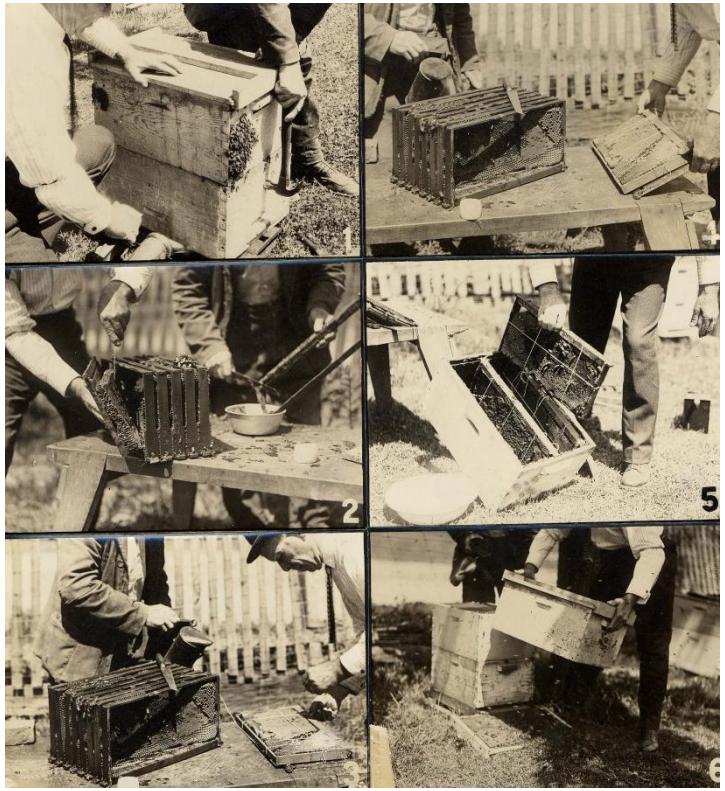
Managing Skep

INTERIOR OF A STRAW SKEP

Note the irregular formation of natural Combs

Movable Frame Hives

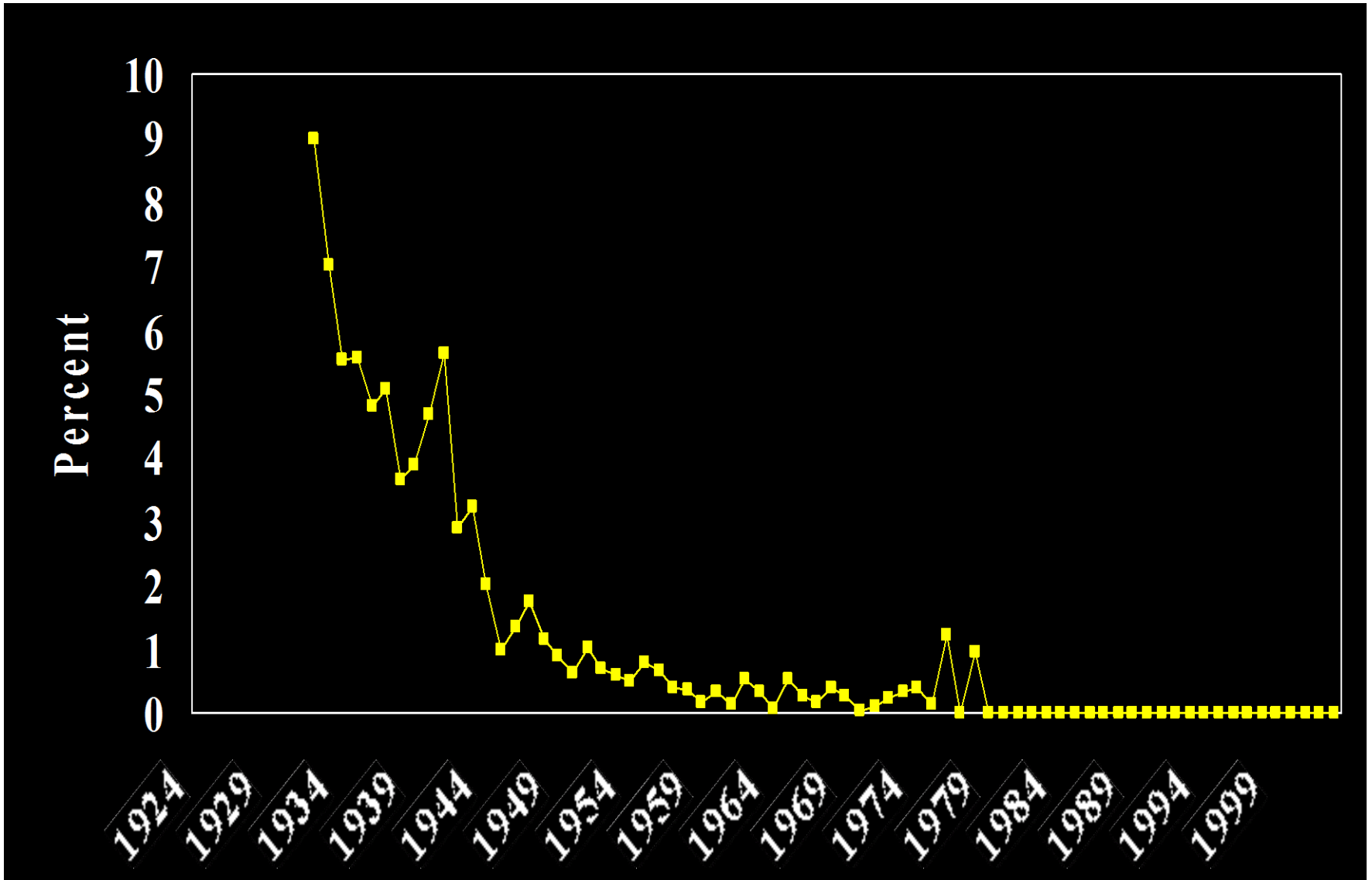
- Permit disease inspection and control
- Moveable frame hives now mandatory



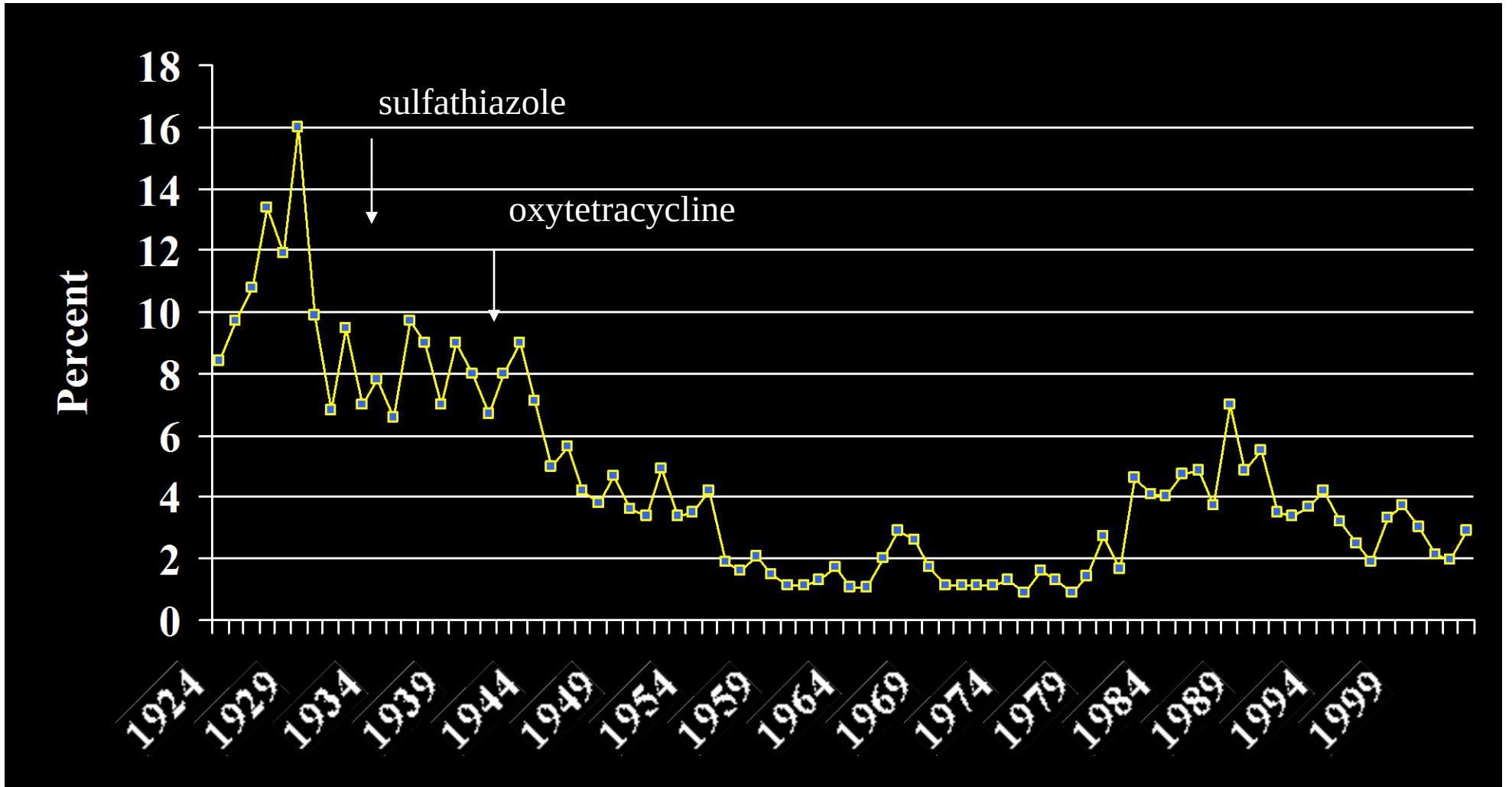
Advantages of the Movable Frame Hive

Inspection for brood diseases possible

Incidence of ‘illegal’ hives



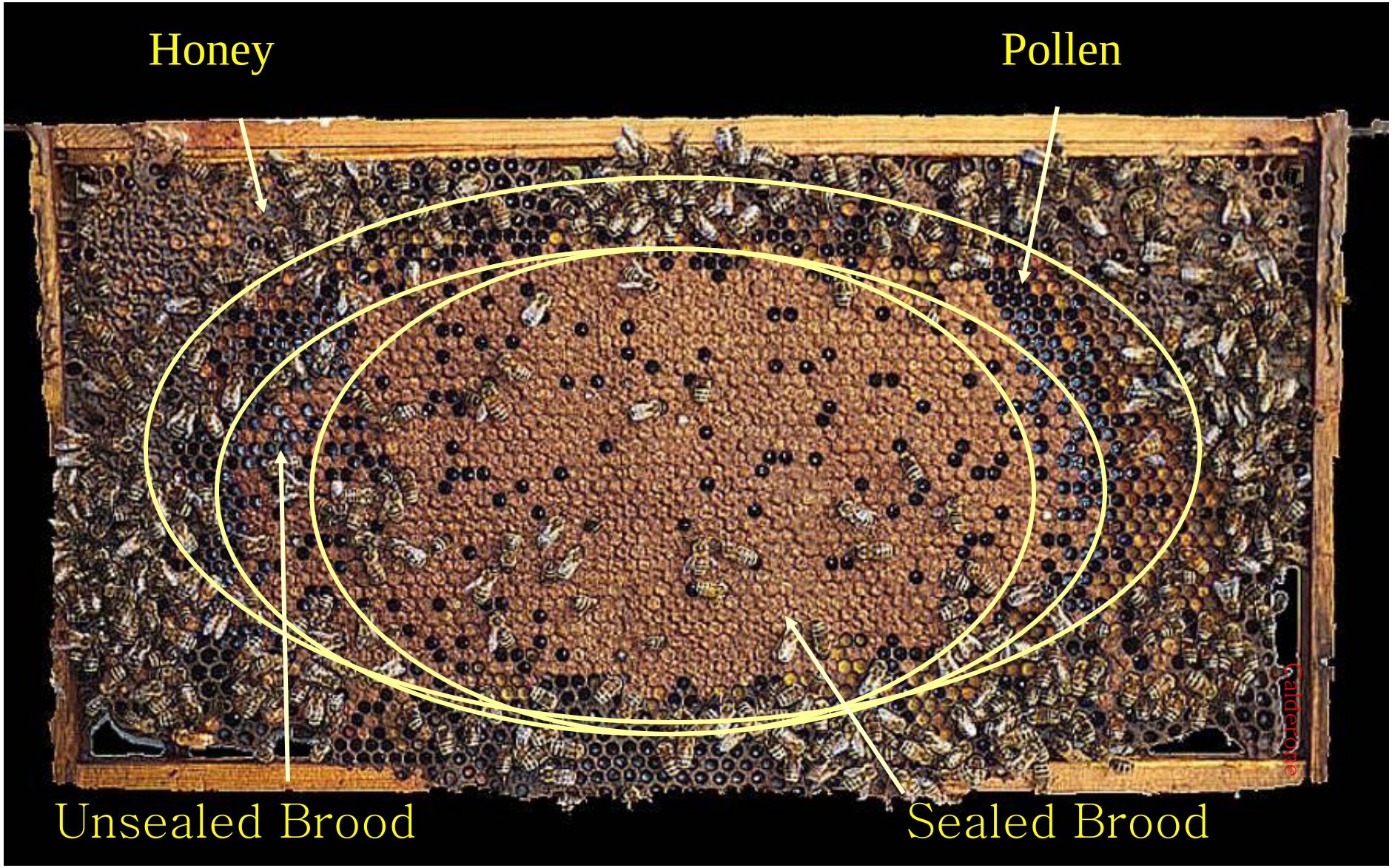
Incidence of AFB in PA
% Colonies Infected



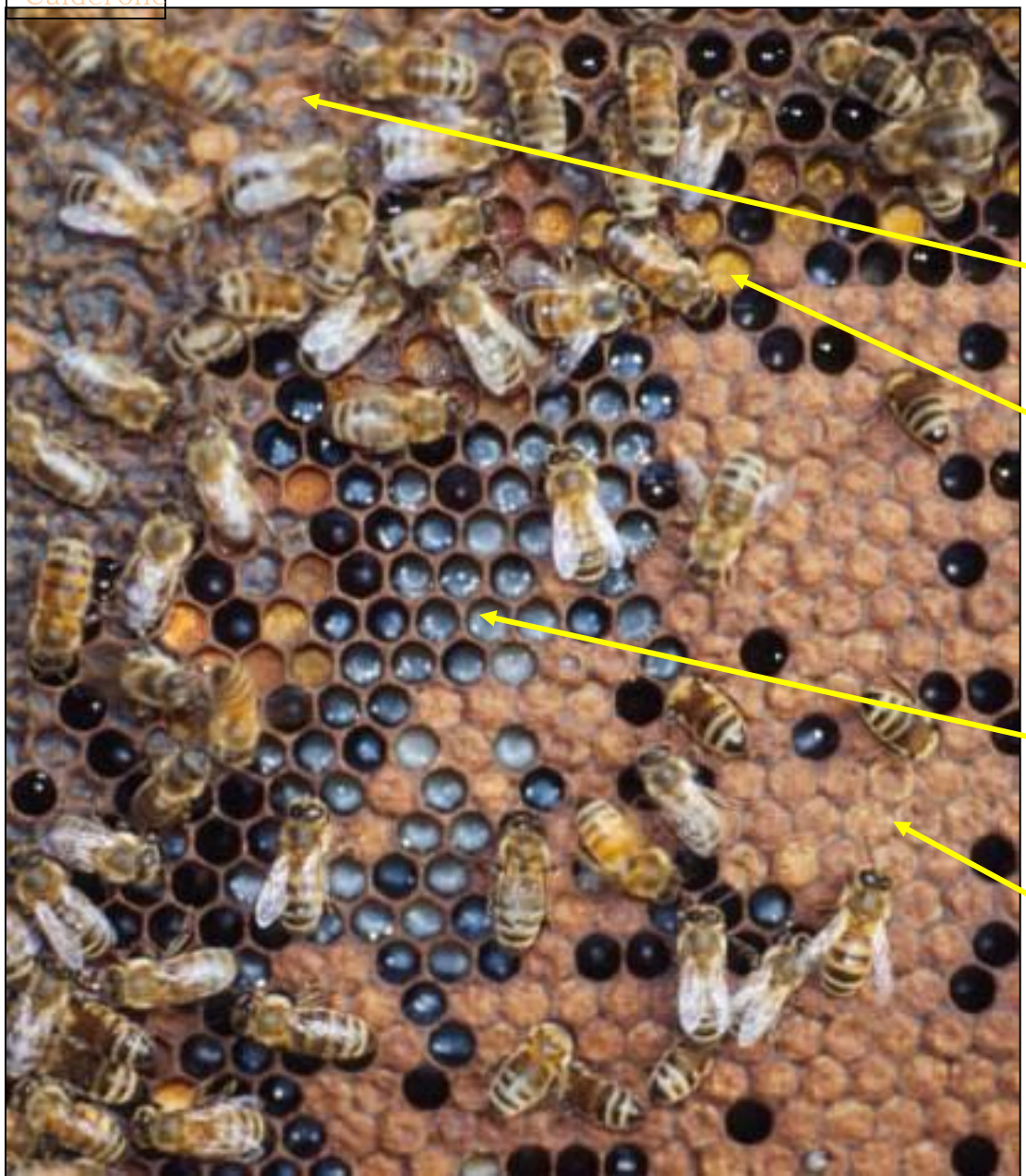
Step 1

- Know a healthy hive

A Healthy Frame of Brood



Calderone



What's on a Frame

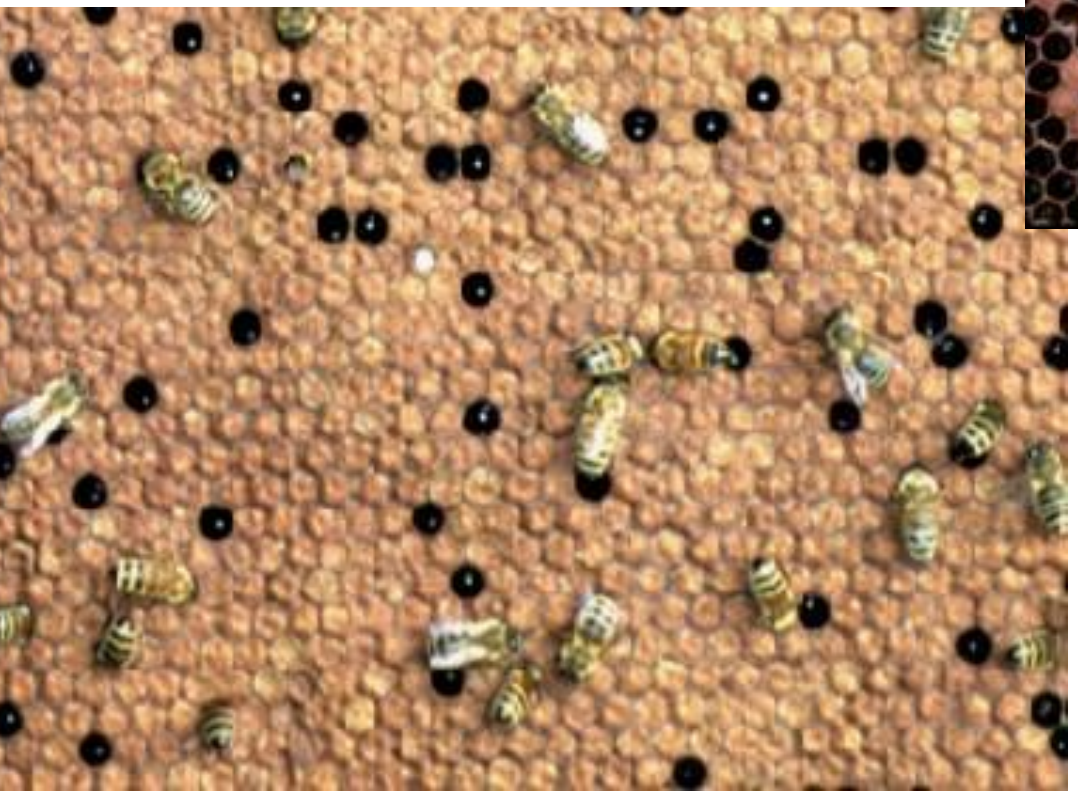
Capped Honey

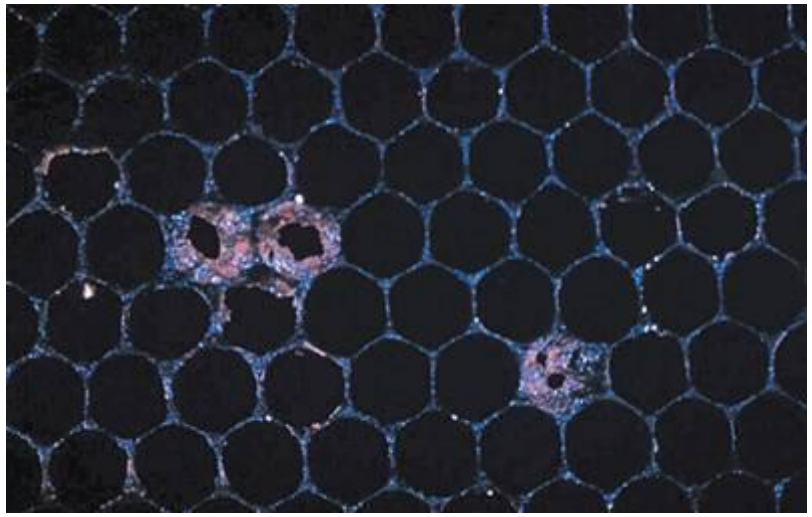
Pollen
(Bee Bread)

Unsealed
Brood

Sealed Brood

Different Brood Cells



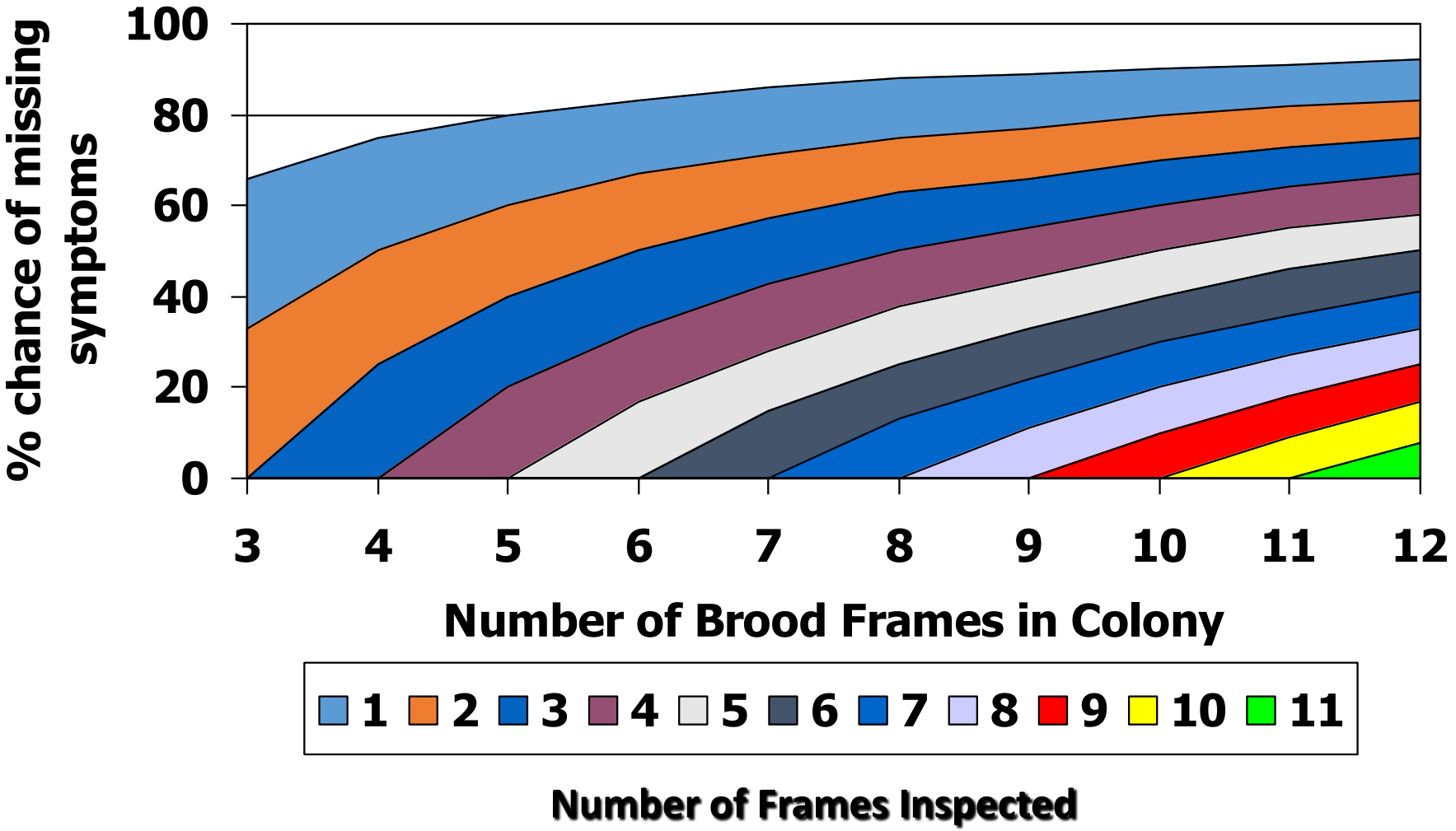


Economic Significance

- Causes over $5 million worth of damage in the U.S. annually
- Most recent statistics (1992) suggest that less than 1% of managed bee colonies in the USA have active AFB infections
  - Incidence may be increasing, due to resistance to Terramycin (TM-25)
  - Infection rate does not include colonies with AFB infections that is suppressed by the continual use of TM-25

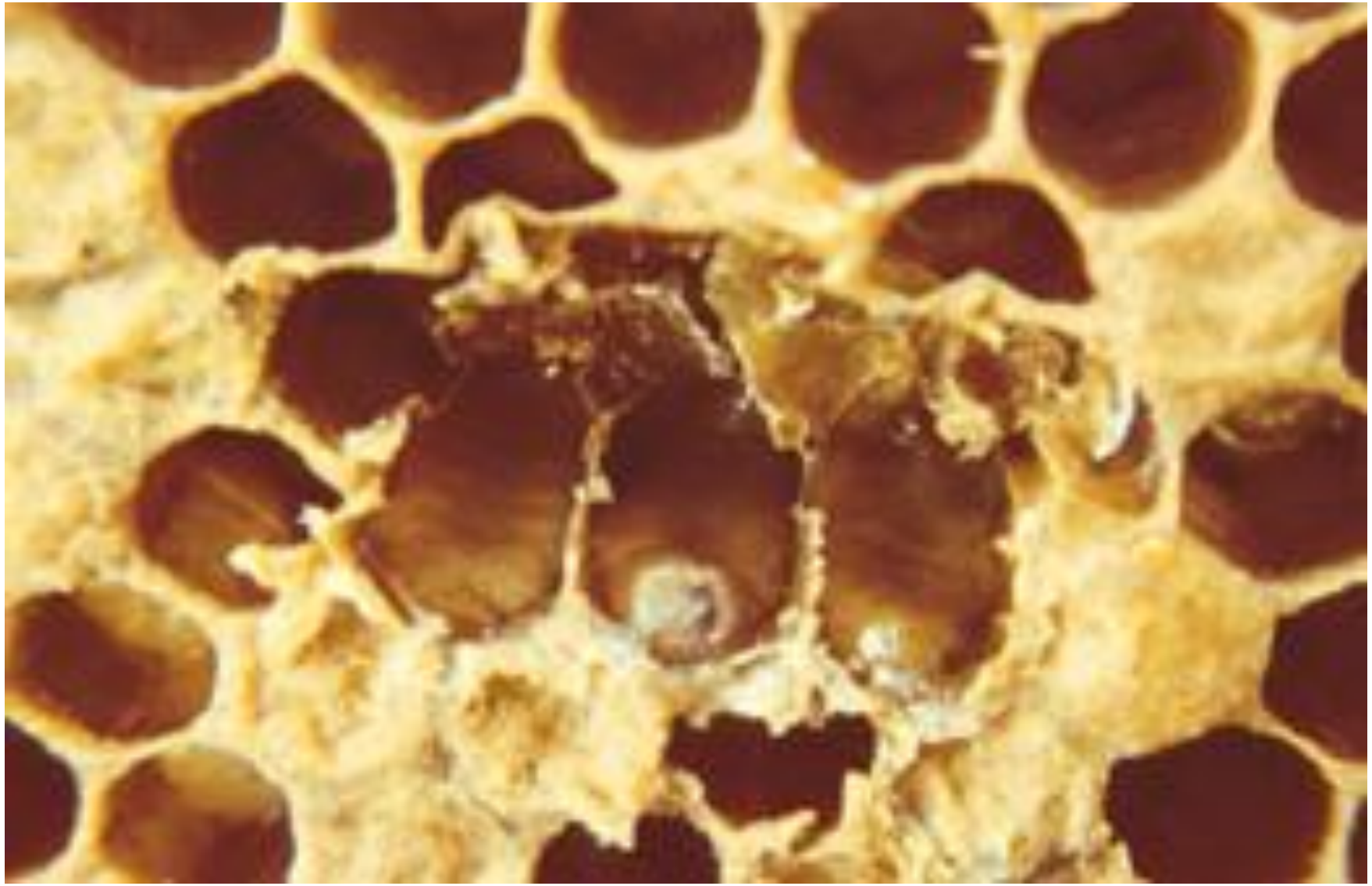
https://www.youtube.com/watch?v=lXEhL5l644o

Percent Chance of Missing Disease Found Only on One Brood Frame

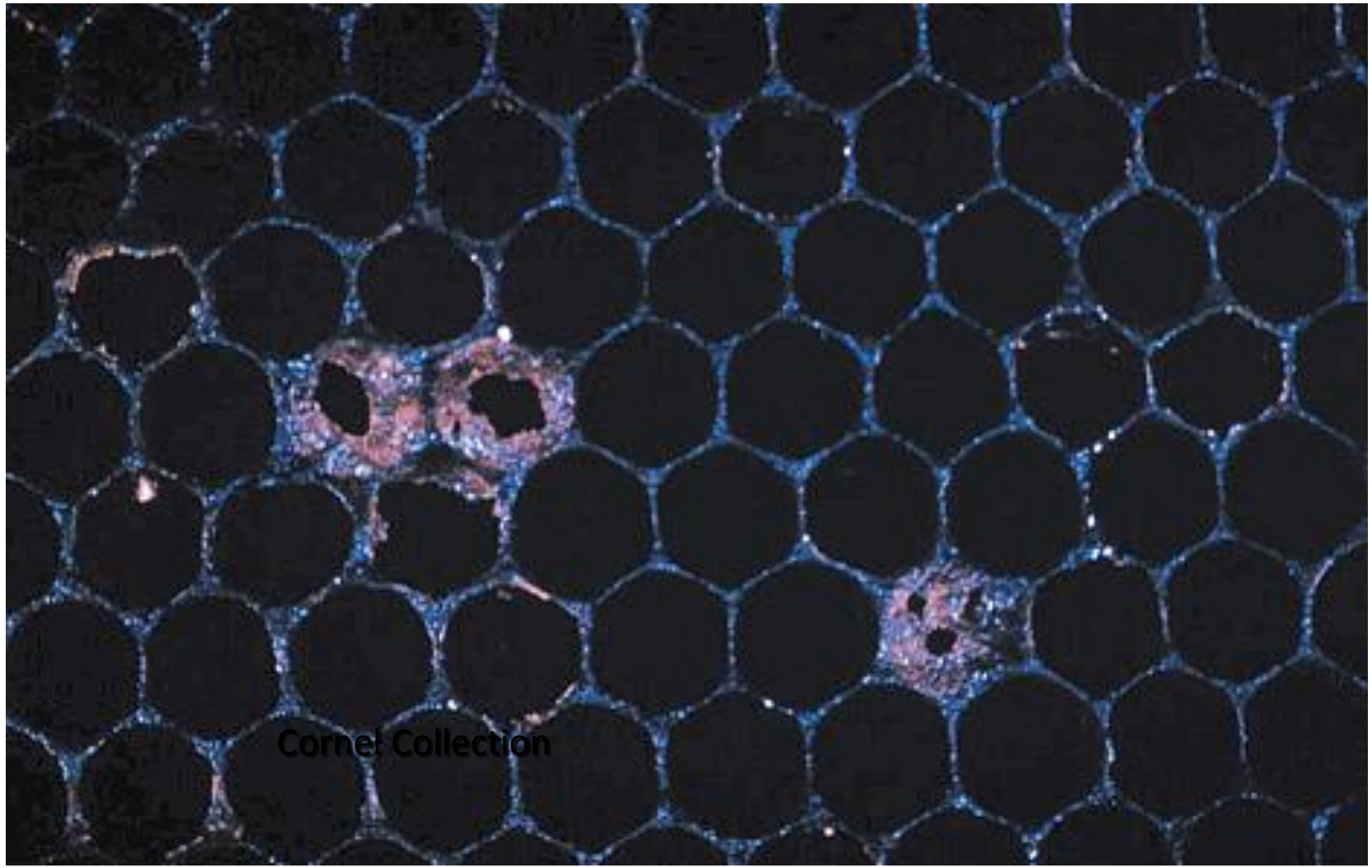


AFB Symptoms

- Usually only infects worker larvae
- Larvae die in upright position (pre- or post-pupal stage)
- Infected brood are dull white, yellow, or coffee colored
- Symptoms are apparent only after capping

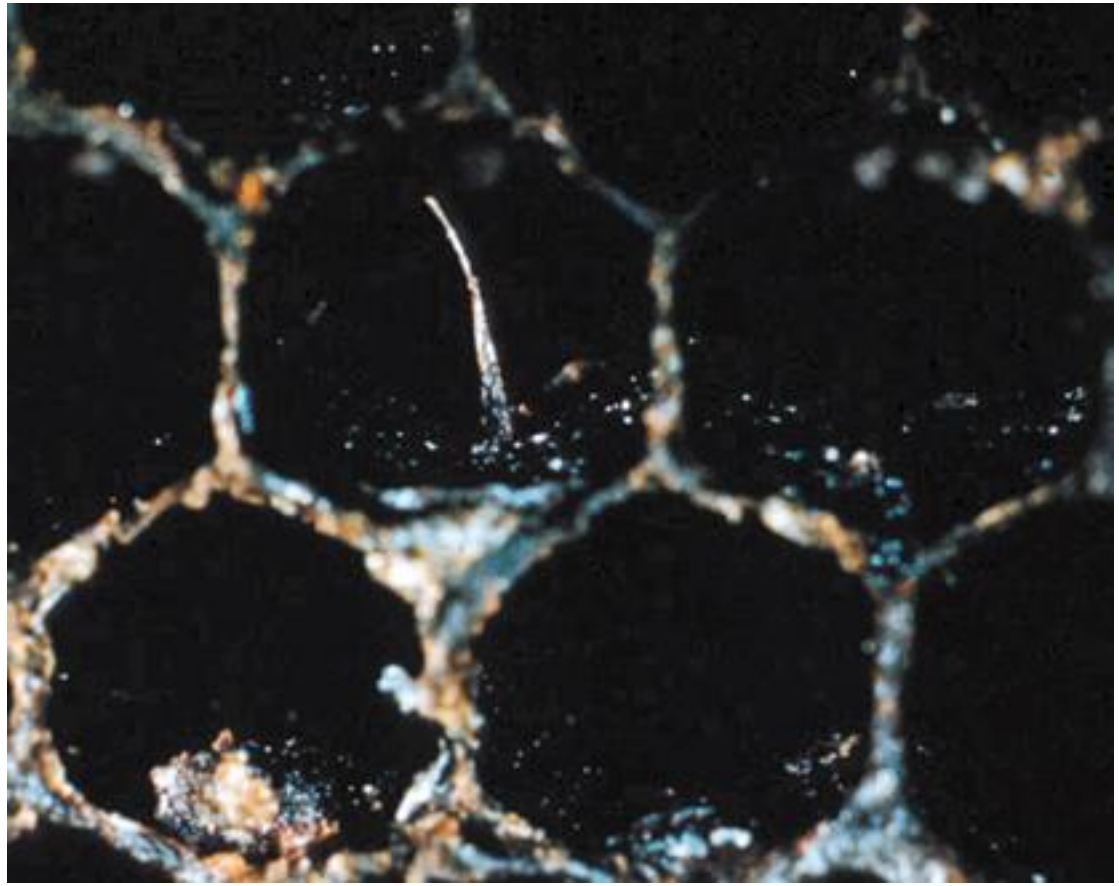


Identification of AFB: Colony Symptoms



- Cappings greasy in appearance
- Scattered brood with sunken and punctured cappings
- Distinctive smell

AFB Scales and Pupal Tongue

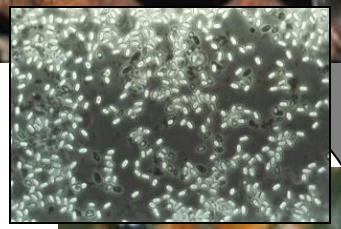


- Pupae that die in their capped cells often die with their proboscis sticking up
- Brood killed by AFB and eventually dry into hard-to-remove scales on the bottom side of the cell
- These scales are a major source of infection

Life Cycle of AFB

House cleaning bees pick up AFB spores

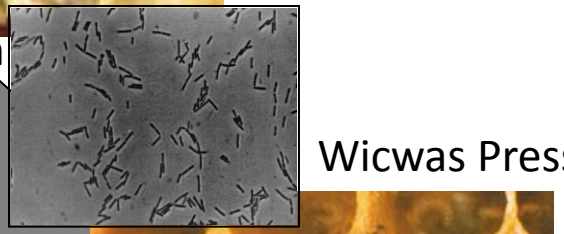
Wicwas Press



Wicwas Press

House cleaning bees transfer AFB spores to nurse bees

Nurse bees feed spore-infected brood food to young larvae



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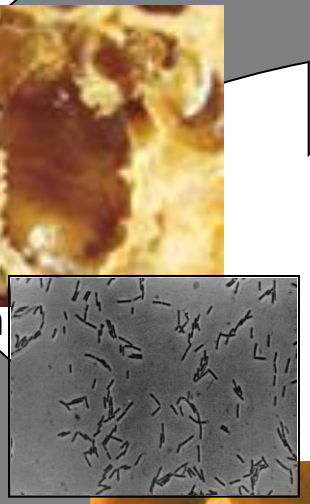

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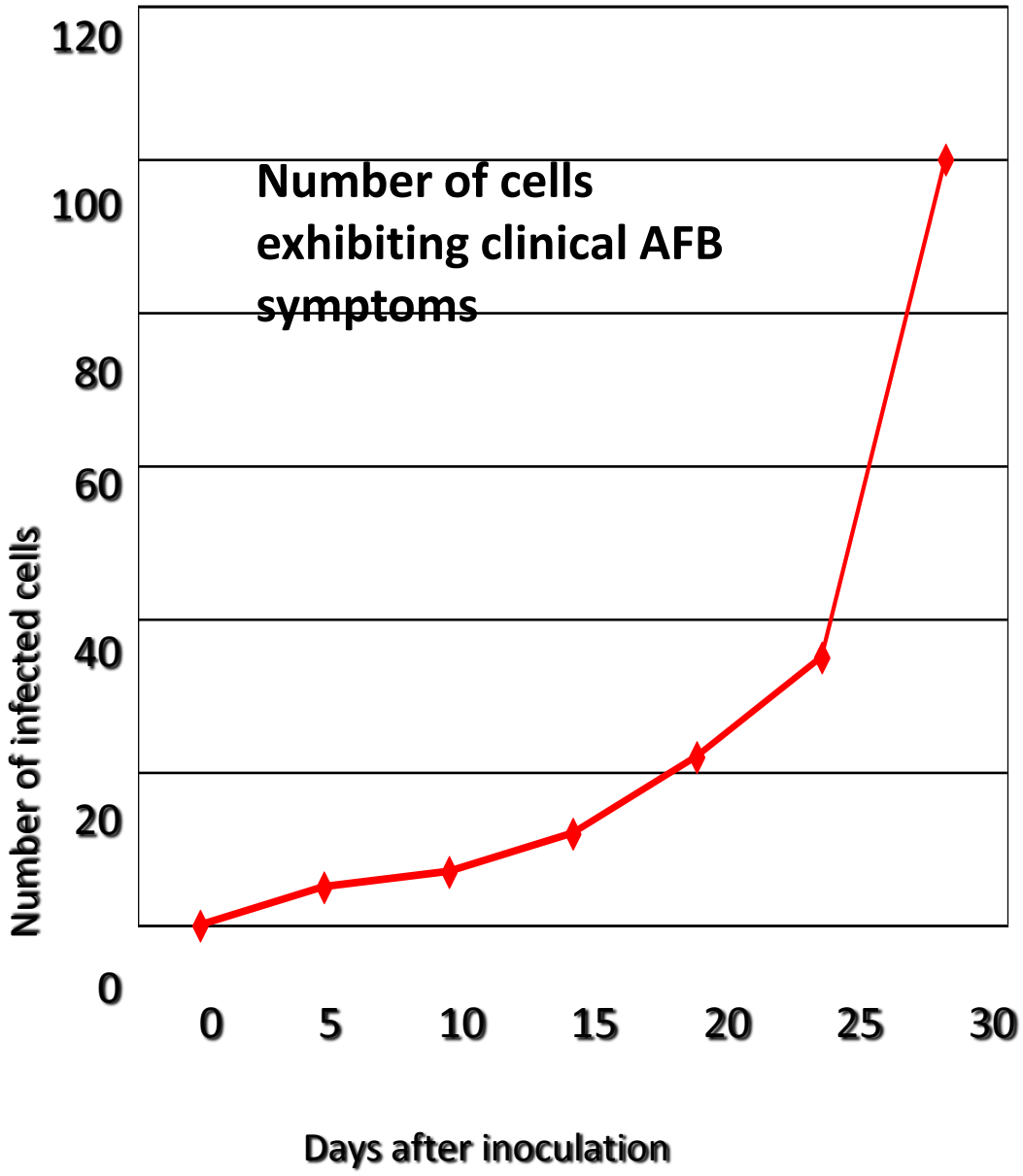
AFB bacteria consume pupae, eventually forming scales

Progression of AFB in Honey Bee Colonies

- One study intentionally introduced number of infected brood cells into established honey bee colonies
- Colonies were monitored for presence of infected larvae
- Three distinctly different disease progressions were noted:
  - Quick disease progression
  - Initially quick progression, to apparent long term suppression
  - No initial progression, followed by quick progression

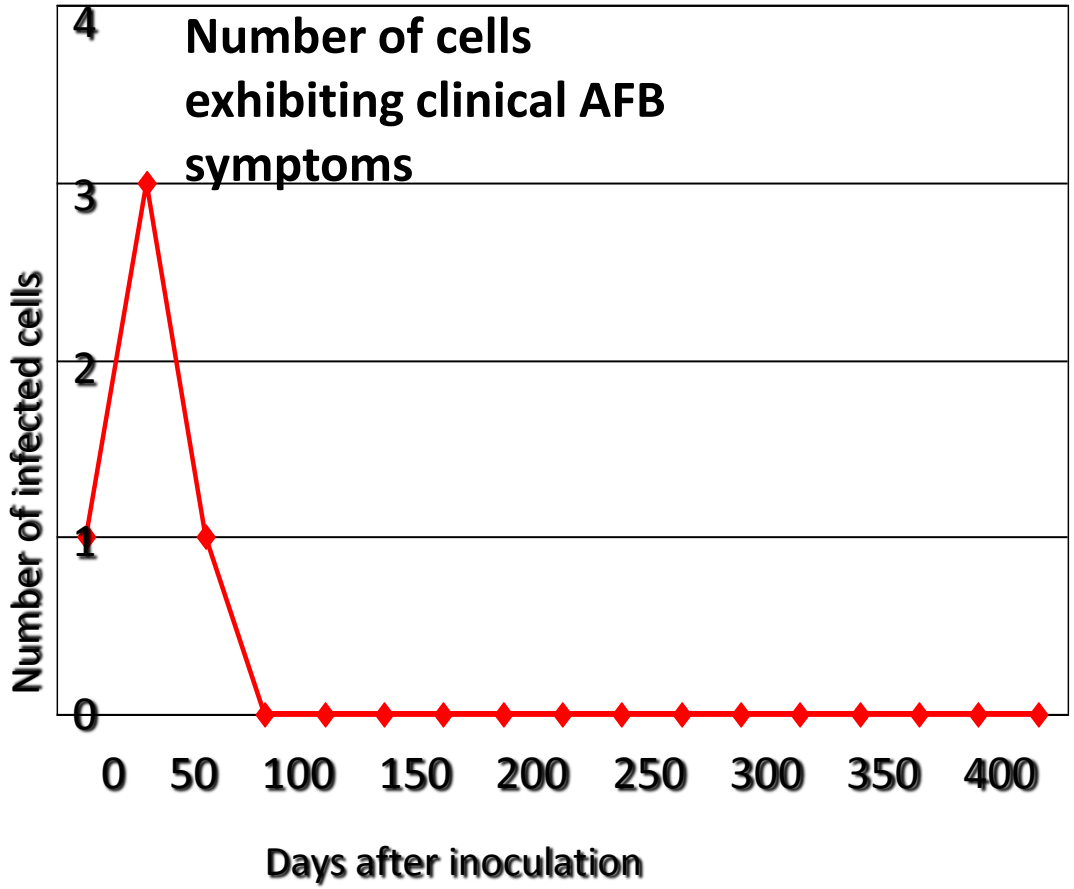
Progression of AFB Disease: Quick Progression

- AFB infection develops rapidly
- Number of diseased honey bee cells increase exponentially



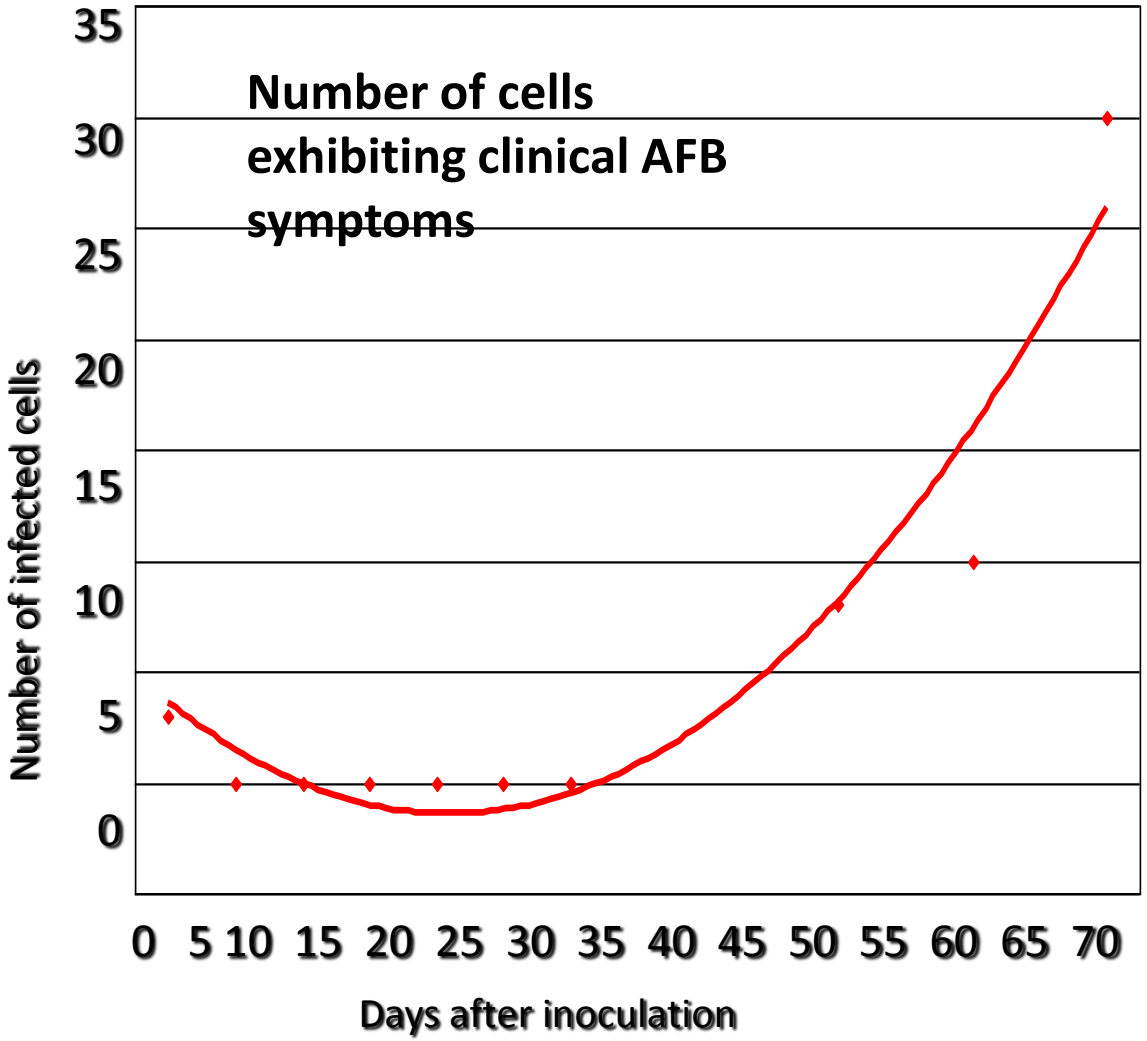
Progression of AFB Disease: Apparent Disappearance

- AFB infection decreases then becomes undetectable for more than 4 years
- <u>Implications:</u> AFB spores may be present in honey bee colonies without clinical signs

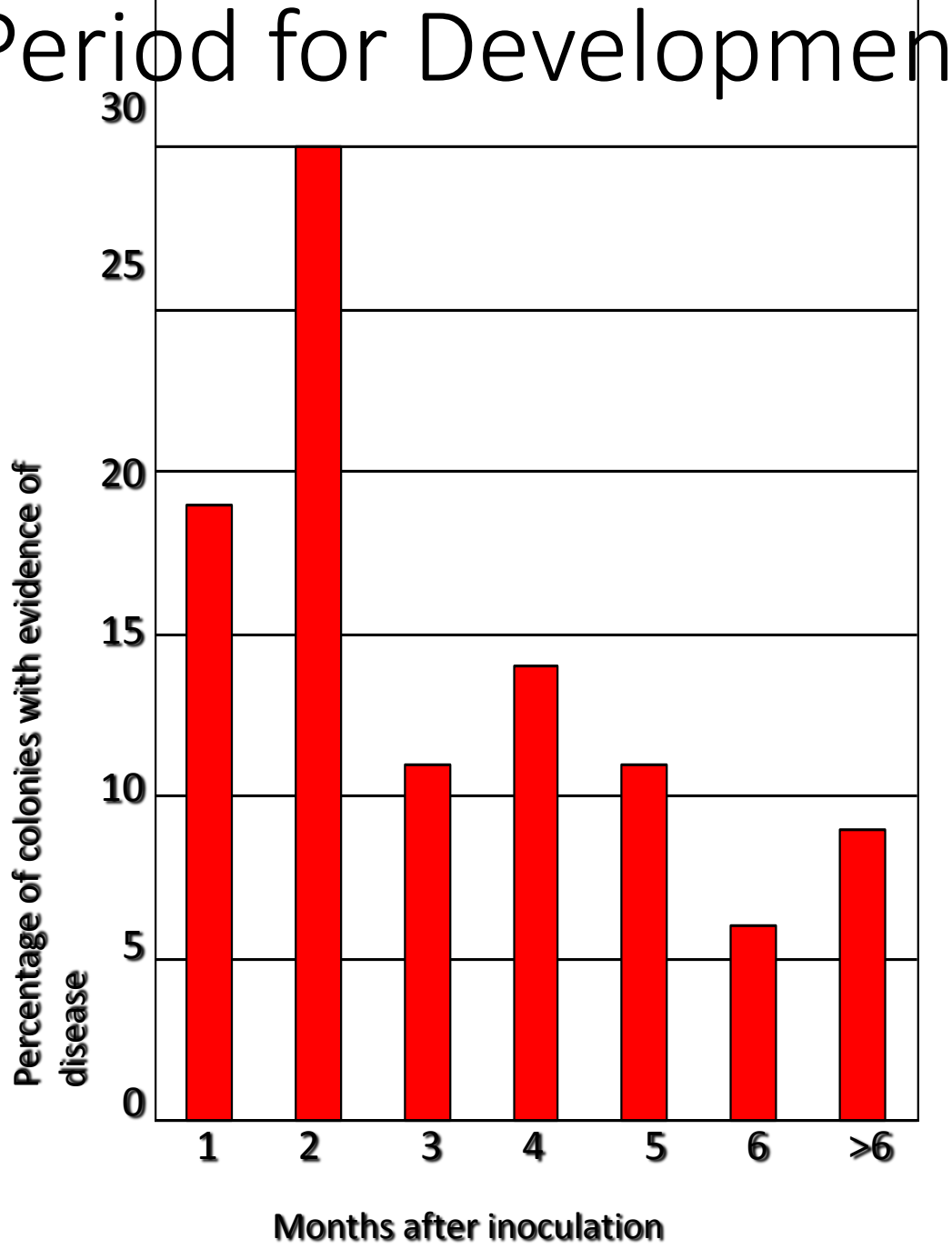


Progression of AFB Disease: Delayed Progression

- AFB infection is not apparent for several weeks; then, followed by rapid growth phase
- Implications: Once infected, honey bee colonies may not show immediate symptoms of AFB

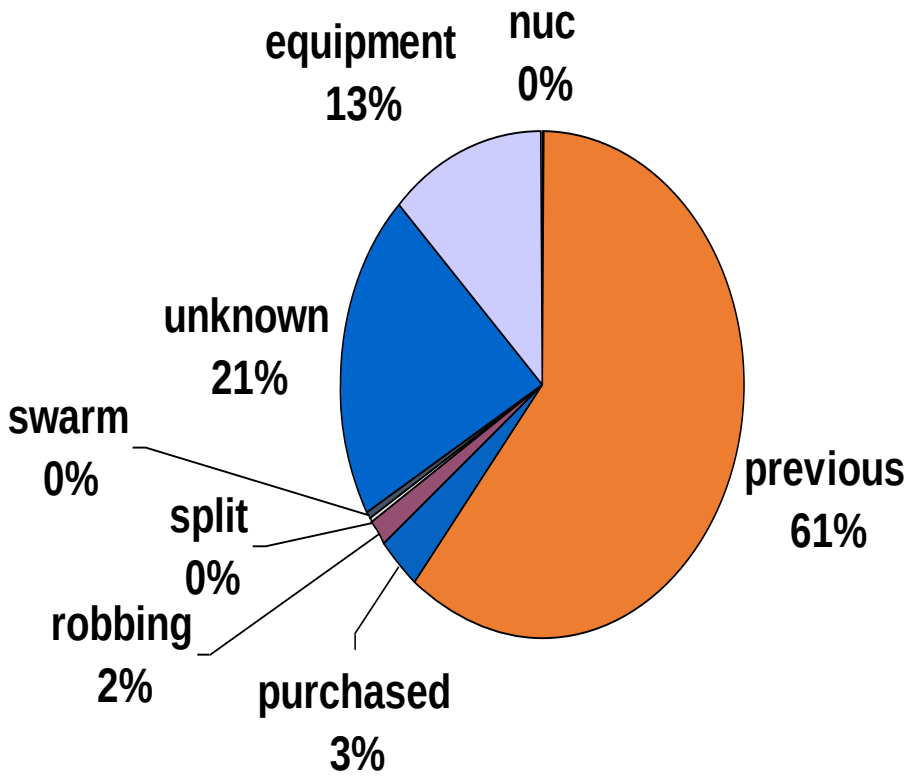


Time Period for Development of Symptoms

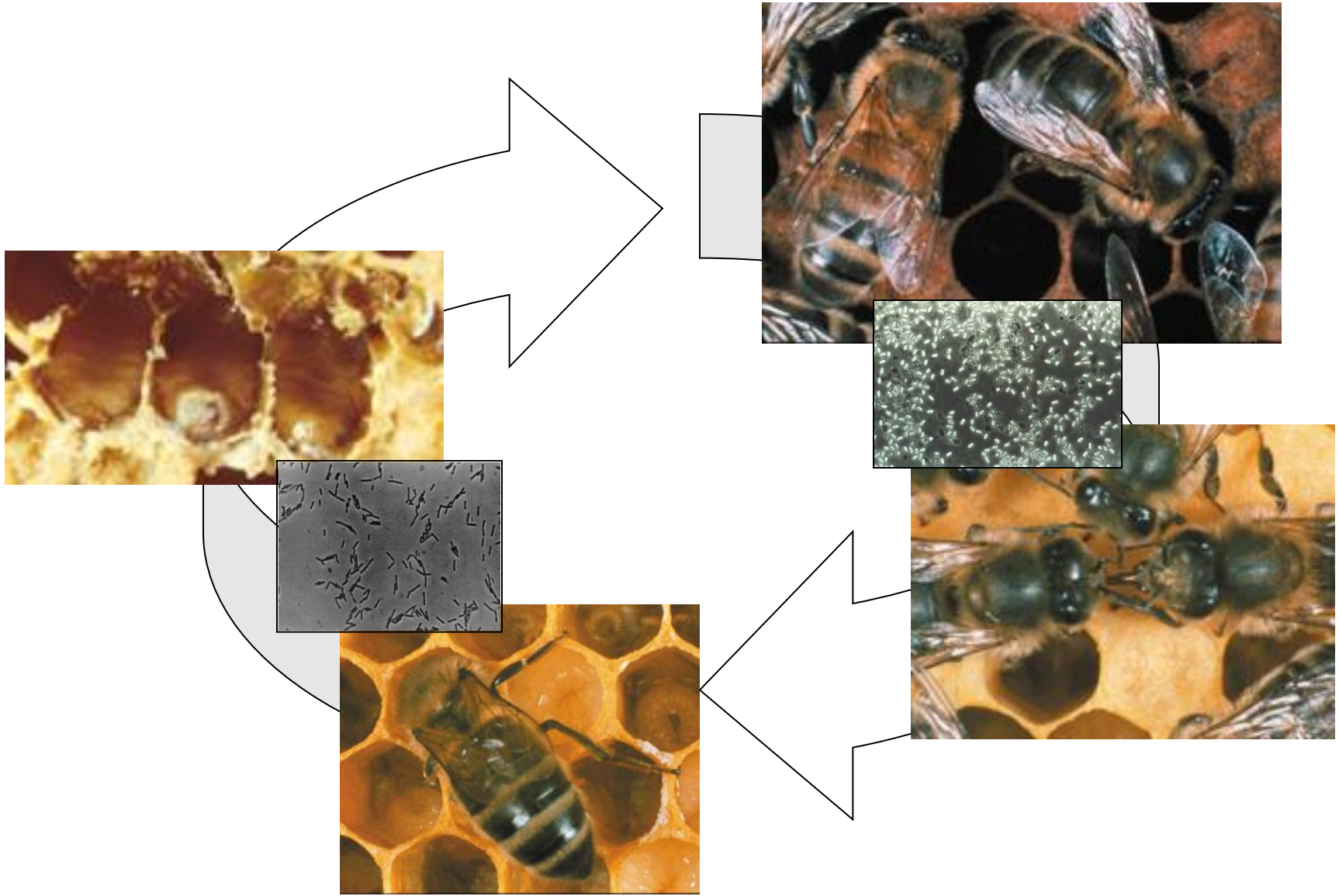


- Average time between honey bee larva being fed AFB spores, and showing clinical signs of disease is 12.5 days
- However, time needed for colonies to display AFB symptoms is considerably longer

Buying used equipment



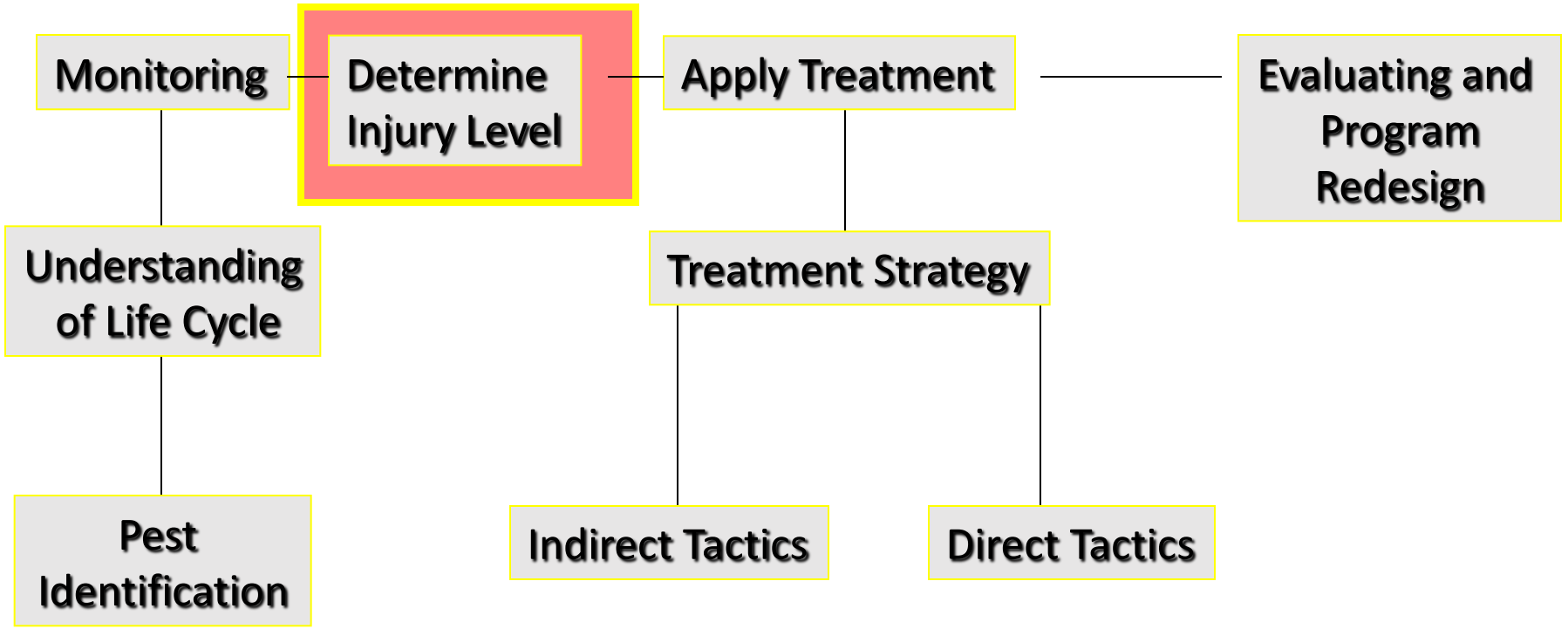
American Foulbrood



Monitoring for AFB

- Honey samples (400 g) can be sent for diagnosis
  - Samples from individual hives which may have been exposed
  - Composite samples from apiaries
    - Samples from settling tank
- Test results may provide insight into problem areas
  - Positive tests do not necessarily mean AFB infection

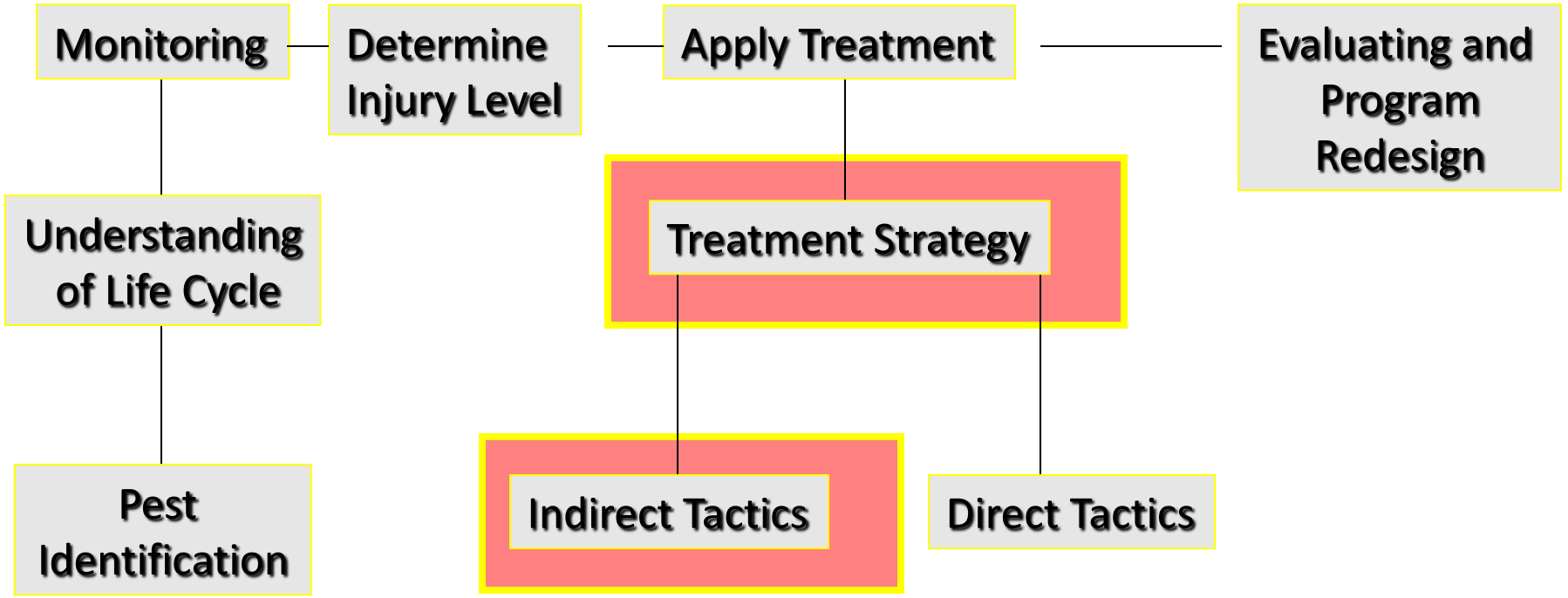
Components of an IPM Program



Thresholds for Brood Diseases

- Low levels of most brood diseases, especially during stress periods, should be expected
  - Address persistent problem hives
- NO AFB should be tolerated

Components of an IPM Program



Indirect Suppression of Brood Diseases

- Modify Environment
  - Breed/use resistant bees
  - Reduce disease spread by robbing and drifting, or careless comb transfer
  - Cull old comb
- Modify human behavior
  - Keep informed

Assay

- Is a way to quickly and quantifiably evaluate a honey bee colony for the expression of a desirable trait
  - Usually faster than full-colony evaluation
  - This may or may not test the mechanism behind colony improvement
    - Some traits are strongly correlated to other traits

Breeding for Increased Honey Production

- A primary breeding objective
- Success often involves selection of other desirable traits:
  - Resistance to disease
  - Overwintering ability
  - Strong spring buildup

Assays for Increased Honey Production

- Measuring one day or one week gain
  - Daily/weekly gains are closely correlated to seasonal gains
- Brood area measurement
  - Larger brood areas are correlated to increased yields

Mechanisms of Disease Resistance

- Three general mechanisms:
  - Physiological
  - Behavioral
  - Anatomical
- More than one mechanism may work against one organism
- One mechanism may help control more than one organism

Breeding for AFB Resistance

- Two classic studies:
  - Park (1937) demonstrated that AFB resistance
    - Is heritable
    - Responds to artificial selection
  - Rothenbhuler (1964) developed resistant and non-resistant lines
    - “Brown” and “Van Scoy” lines
    - Identified genetic mechanism for AFB resistance

Physiological Mechanisms for AFB Resistance

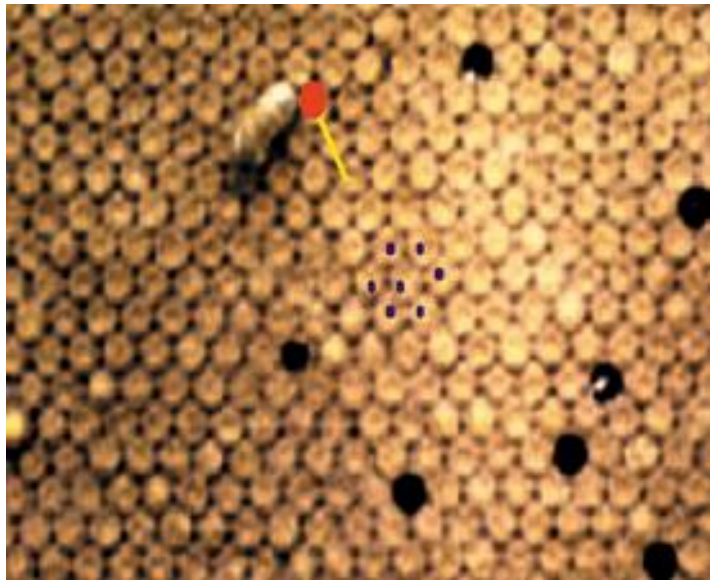
- Speed of larval development
  - Smaller larvae are more susceptible than bigger larvae
- Nurse bees produce brood food with more antibiotic activity

Behavioral Mechanisms for AFB Resistance

- Hygienic behavior
  - Bees uncap cells and remove dead/diseased pupae
  - Uncapping and removing behavior is controlled by two different genes
    - Both genes are needed for expression of hygienic behavior
    - These genes are recessive

Assay for Hygienic Behavior

- Pin prick test
  - Pin is inserted into cell to kill contained pupae
  - 24 hours later, cells cleaned of dead pupae are counted



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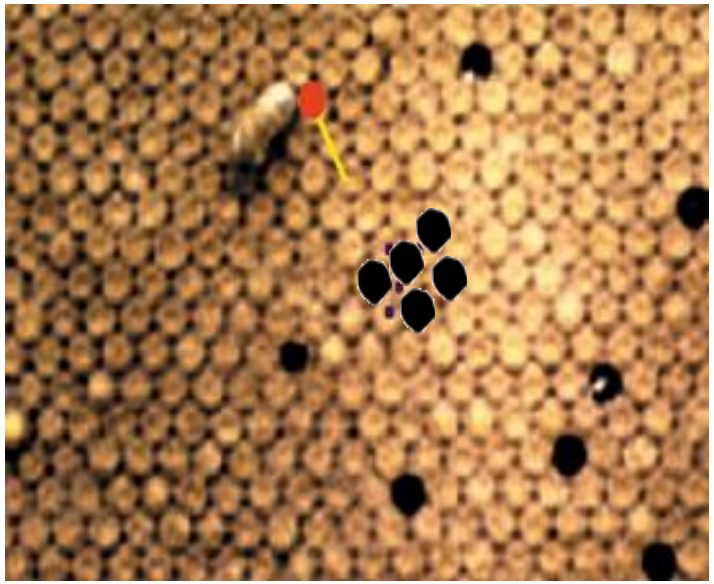
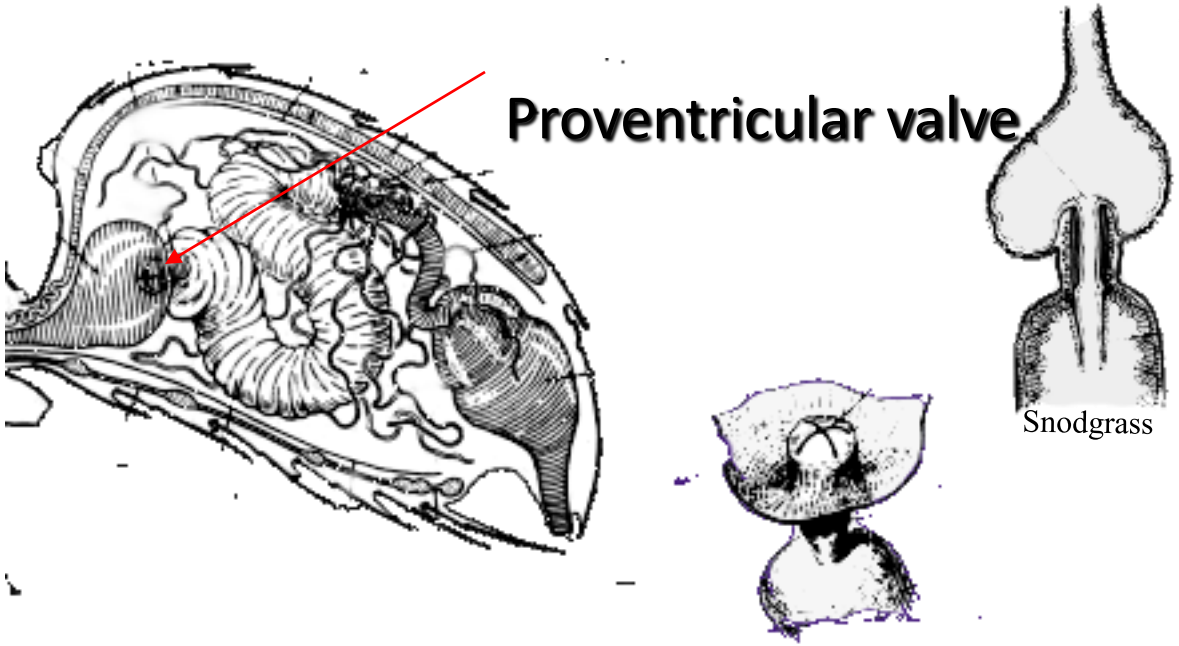


Anatomical Mechanisms for AFB Resistance

- Proventricular Valve
  - Helps adult nurse bees filter AFB spores from food



Reducing Spread of AFB

- Maintain 5 miles between neighboring apiaries
  - Not always possible
- Employ anti–robbing and anti-drifting strategies
- Keep colonies strong

Quarantine

- Larger operations should develop quarantine apiaries for:
  - Recently-purchased used equipment and colonies
  - Colonies that may have come in contact with AFB infected colonies
- Keep colonies/equipment in quarantine for at least 18 months

Reducing Disease Spread by Comb Transfer

- Transferring brood comb is a common management practice for:
  - Increasing or decreasing colony population
  - Making splits
- **All** comb should be thoroughly inspected for disease before transferring

Monitoring Honey Super Use

Calderone

- Smaller operations
  - Number each colony and honey super
  - Place used honey supers on the same colony every year
- Larger operations
  - Exchange honey supers within the same apiary only

Comb Culling

- Systematic culling of old brood comb may reduce incidence of disease
  - Old brood comb has smaller cell size
  - Acts as a sink to disease spores
- Some beekeepers replace each comb in the brood nest every 5 years
  - Each year, 2 outer brood frames are removed, and 2 frames of foundation are added to center of each brood box
- Some beekeepers cull frames on quality of comb
  - Fist size patch of drone brood, or can not see light through comb

Renting Hives for Pollination

- Renting hives for pollination can be especially stressful
  - Melons, squash, cucumbers, and cranberries are low nectar producing crops
  - Low bush blueberries require a lot of colonies in a small area resulting in scant recourses for individual colonies
  - Colonies become vunerable to EFB and Chalkbrood
- Supplemental feeding of pollen patties and sugar syrup may be advisable
- Prophylactic medication is advisable

Decreasing Stress While Moving Hives

- Follow “more than 2 miles, less than a foot” rule
  - Avoids loss of foraging force
- Prevent overheating
  - Use a top moving screen
  - Move at night or on a rainy day
- Avoid crushing bees and queen
  - Load colonies so frames run parallel to the direction of the road

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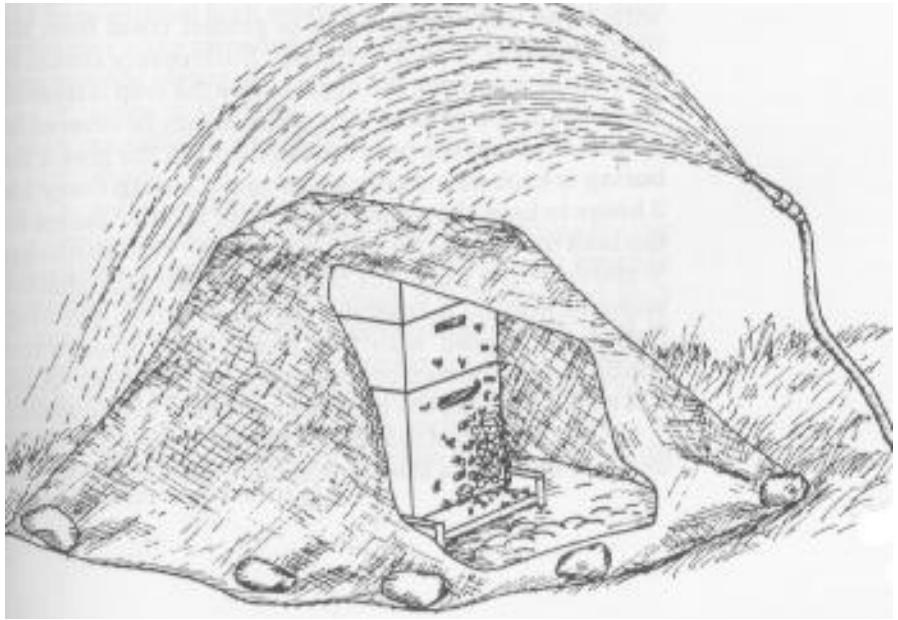
Prevention of Pesticide Kill

- Use low-hazard apiaries
  - 10 foot hedge rows between apiaries and sprayed fields
  - Post signs indicating hive ownership
- Educate growers about appropriate pesticides
  - Extensive index in the Hive and the Honey Bee
- Move bees away before spraying
- Confine bees during spraying of pesticides which break down quickly

Short Term Confinement

- Place burlap over colony and secure edges with weights to ground
- Use sprinkler to keep burlap moist

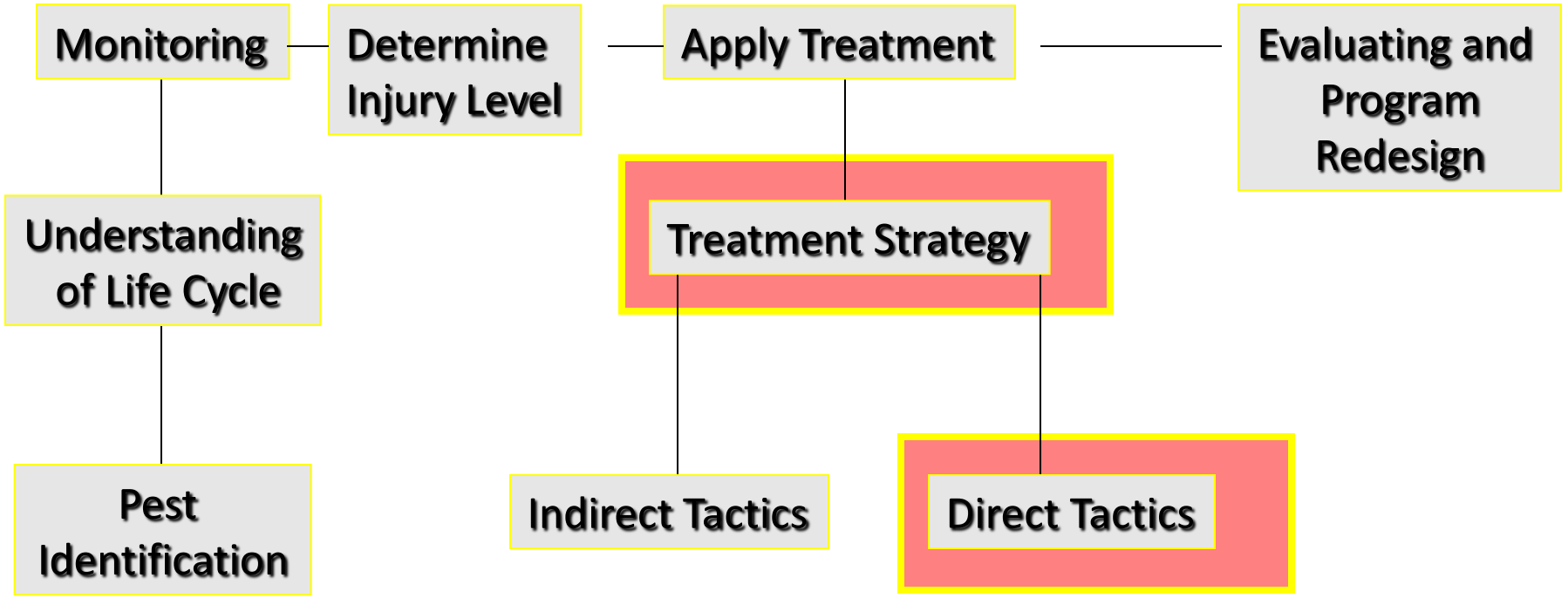


Johansen and Mayer

Keeping Informed

- Regularly attend disease identification workshops
- READ, READ, READ!

Components of an IPM Program



Direct Suppression of Brood Diseases

- Physical and mechanical controls
  - Shaking
  - Burning
  - Sterilization
  - Quarantine
  - Temperature treatment
- Least toxic chemical controls
  - Terramycin®

Stress and Disease

- Most brood diseases will self correct as they are stress related
- Beekeepers should endeavor to:
  - Decrease likelihood of spread
  - Relieve stress
  - Re-queen if disease persistent
- AFB is an exception: NO LEVEL OF INCIDENCE SHOULD BE TOLERATED

"To burn is to cure"

Treatment of Suspect Colony

- If colony is suspected to be infected with AFB:
  - Reduce entrance
  - Confirm infection
  - Destroy colony

“TO BURN IS TO CURE”

Burning Hives

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- At night, seal hive entrance with grass, all other cracks and holes with duct tape
- Kill bees
- Dig a pit
- Burn inner cover, frames, combs, bees, and honey
  - Burn parts individually, rather than all at once -- especially honey laden frames

Burning Alternative

- Some regions do not allow open burning
- Seal equipment in double heavy duty bags
  - Witness it incineration or burial at dump
- Use of plastic frames may make burning an environmental problem

Saving Hive Bodies

- Scorching
  - Scrape interior surfaces and top and bottom edges of hive bodies with hive tool
  - Scorch with a weed burner to a depth of 1/16”
- Wax Dipping
  - Dip cleaned parts in paraffin wax heated to 320^{O}F (160^{O}C) for 10 minutes
    - Very dangerous, and a fire hazard

Saving Frames

- Frames can be saved by scraping all surfaces clean, followed by:
  - Fumigation of frames in ethylene oxide gas chamber
  - Irradiating with gamma radiation
  - Autoclaving at 240^{O} for 45 minutes

DO NOT PROCRASTINATE!

Better to burn than save

Saving Adult Bees: Shaking Method

- Shake adult bees into a clean super containing foundation
- Shake bees at night, away from other colonies, to prevent drift
- Confine bees for two days in cool area
  - As bees draw foundation, they consume the AFB spores in their honey stomach
- Burn infected equipment burned

Cleaning Beekeeper Equipment

- Gloves and smoker
  - Scrape off all wax and propolis
  - Scrub with soapy water
    - Will not kill all AFB spores, but will removes wax/propolis which harbors significant amounts of spores
  - Rubber gloves are much easier to clean than canvas gloves
- Hive tool
  - Scrape off all wax and propolis
  - Scorch with propane torch

Sterilizing Nosema Infected Equipment

- Heat all hive equipment for 24 hours at 120^{O}F (49^{O}C)
  - Frames should not contain honey or pollen
  - Ensure good air circulation
  - Ensure a constant and consistent temperature
    - No hot spots
    - This temperature is just below melting point of wax
  - Warm frames in supers in upright position
  - Allow supers to cool to room temperature before moving

Chemical Control
Terramycin®

- Oxytetracycline HCL discovered as disease control agent in 1951
  - Prevents AFB – BUT does not cure it
  - Controls EFB
- Sold as Terramycin Soluble Powder (TSP) for cattle and bees
  - TSP (25 g active ingredient per pound)
  - TM-50D (50 g active ingredient per pound)
  - TM-100D (100 g active ingredient per pound)

Medicating Colonies

- Several application methods used:
  - Bulk feeding
  - Dusting
  - Extender patties
- Never apply while honey supers are on colony within 45 days of marketable nectar flow

http://maarec.cas.psu.edu/

Bulk Feeding

- TSP (TM-25) is mixed with 1:1 sugar syrup
  - 1 Part TSP: 600 parts sugar syrup
  - Feed 0.5 gal (1.9 L) 3 times at 4 – 5 day intervals
- Considerations:
  - Terramycin breaks down quickly in water
    - Feed during nectar dearth
    - Complete feeding at least 45 days before honey supering
  - Terramycin breaks down quickly in sunlight
    - Do not use Boardman feeder

Dusting

Calderone

- Mix 1 part TSP with 15 parts confectionary sugar
- Apply to top bars on the edge of brood frames
  - Dust will kill exposed brood
- Apply 1 oz (28 g) 3 times at 4 to 5 day intervals
- Complete applications 45 days before supering for honey production

Extender Patties

- Mix TSP with vegetable oil, petroleum jelly, and sugar
- Oil and jelly deter feeding so mixture is consumed over extended period
  - Usually 6 – 8 weeks
- Patties can be purchased pre-mixed
- Patties have not been proven effective for EFB
- MUST be removed 45 days before honey flow
  - Difficult as weak colonies will not consume proper dosage

Storing TM-25

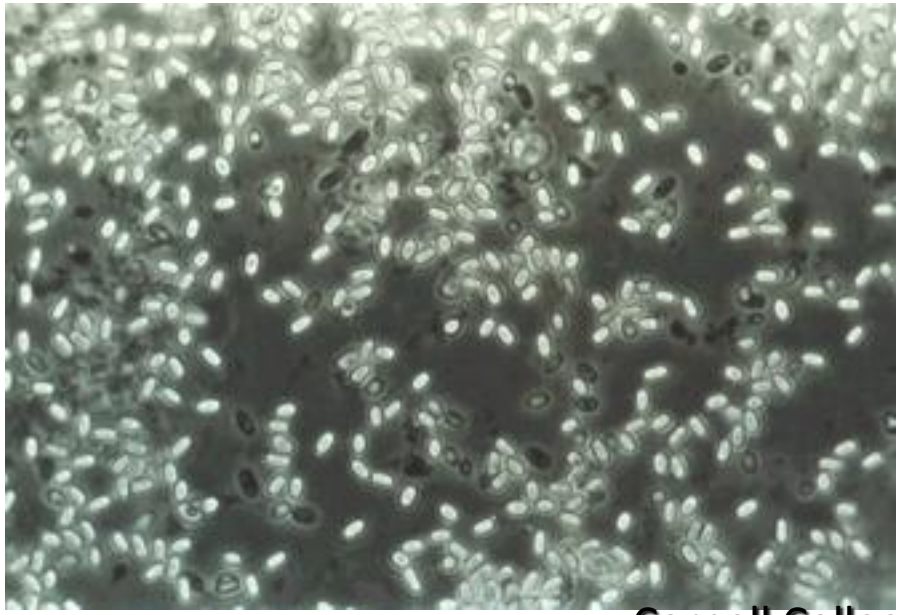
- Store TM-25 in a sealed, dry container
  - Moisture will break down active ingredient
- Store in a cool, dark place

Fumagillin

- Anti-biotic bicyclohexyl-ammonium fumagillin
  - Trade name Fumidil B or Nosem-x
  - Isolated from fungus *Apergillus fumigatus*
- Only effective if fed in sugar syrup
- Thought to disrupt DNA replication in *N. apis*

Diagnosing AFB: Microscopic Examination of AFB Spores

- Observe spores after treatment with the "hanging drop method"
  - Involves staining and fixing the spores
  - Spores are 1.3 by 0.6 μm



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Diagnosing AFB: Ropiness Test

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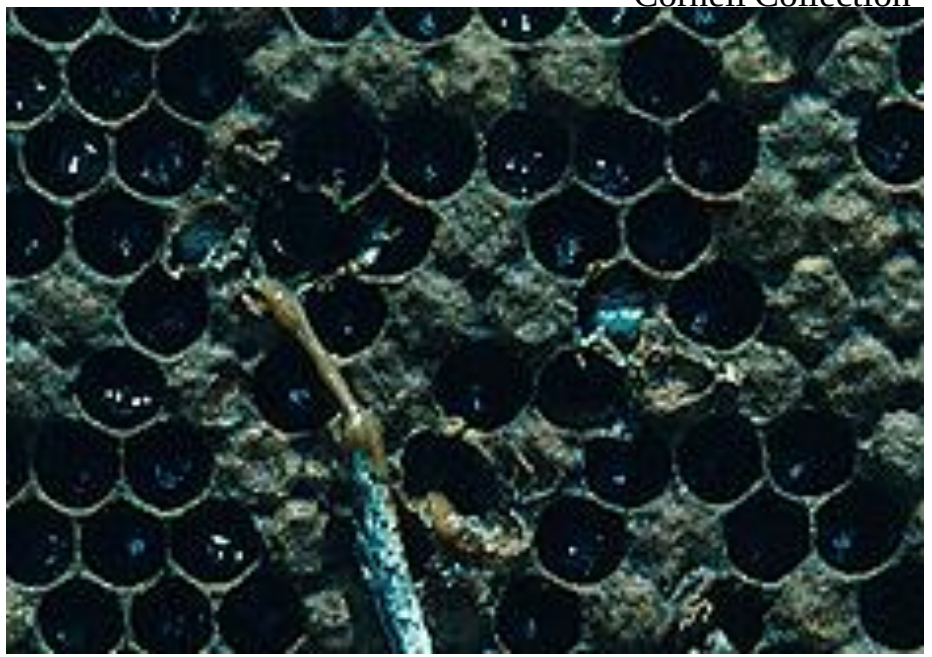


- Place flat, dry toothpick in dead larvae or pupae, stir, and slowly, draw out
- If larval mass stretches to 1 inch, AFB is probably the cause of death
- Works for a limited time on larvae/pupae still infected by the bacteria's vegetative stage

Diagnosising AFB:
Holst Milk Test

- Suspect scale or larvae is placed in 3-4 ml of 1% powdered skimmed milk solution and stored for 10 –20 minutes at 98^{O}F (36^{O}C)
  - If solution clears, AFB is present
  - Not always reliable
- Spore-forming bacteria produce proteolytic acid, which reacts with skimmed milk

Confirming Diagnosis

- Wrap 4” X 4” honey and nectar free sample of suspect comb in newspaper –NOT in foil, plastic wrap, or wax paper
- Pack with absorbent packing in a sturdy cardboard box
- Include a letter with your name, address, phone number, and suspected diagnosis

Where to Send Samples

Mail To:

U.S. Department of Agriculture

Bee Disease Diagnosis

Bee Research Laboratory

Building 476, BARC-E

Beltsville, MD 20705

USDA

Life Cycle of AFB: Spores

- Spores are the dispersal and dormant stage of AFB
  - Spores are covered with a hard outer coat that protects them from most severe conditions
  - Spores are viable for up to 70 years
  - Spores are fed to developing larva by nurse bees

Virulence of AFB Spores

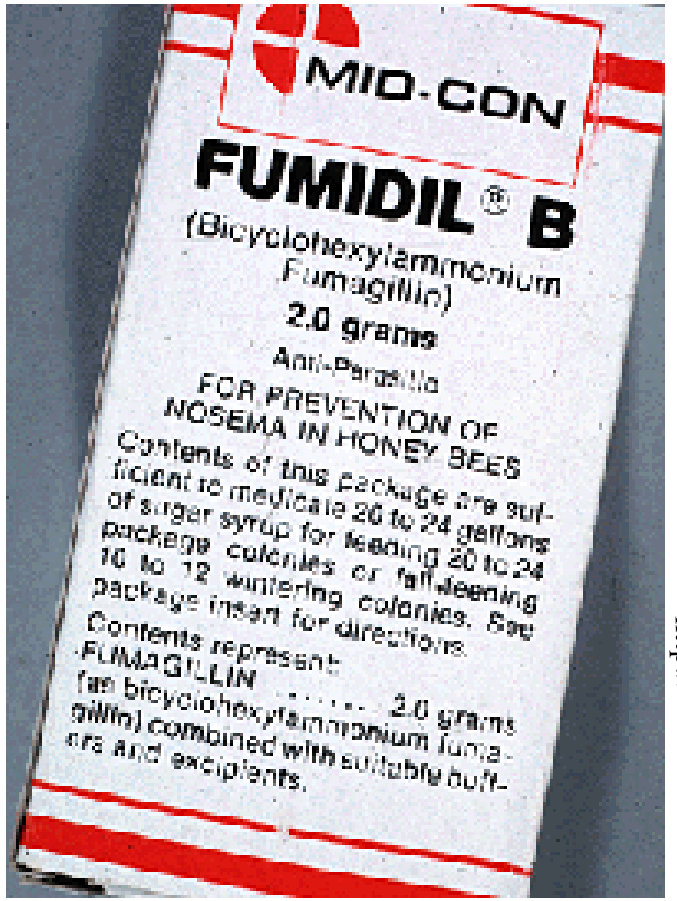
- As honey bee larva age, they become more immune to infection
  - LD_{50} = 35 spores per one-day-old larvae
  - LD_{50} = over 1 million spores per-two-day old larvae
  - Bee larva are immune 53 hours after egg hatching

Life Cycle of AFB: Vegetative Stage

- AFB spores germinate in bee larva's gut
- AFB bacteria penetrate gut wall when bee larva stretches out in cell
  - Bacteria reproduce rapidly
- Death usually occurs in the pre-pupal stage
- When all larval tissue is consumed, bacteria form spores (scale)
  - 2.5 billion spores per bee larva

Application of Fumidil-B

- Wintering colonies
  - 4.5 g (1 level tsp) to 1 gal 2:1 sugar syrup
  - Feed one gallon per colony
  - Treat twice before brood rearing stops



http://doacs.state.fl.us