



## **“Fall-Dwindle Disease”: Investigations into the causes of sudden and alarming colony losses experienced by beekeepers in the fall of 2006.**

### **A Preliminary Report**

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During the months of October, November, and December 2006, an alarming number of honey bee colonies began to die along the East Coast of the United States. West Coast beekeepers (especially those located in California) are also reporting unprecedented losses. This phenomenon without recognizable underlying causes has been termed “Fall Dwindle Disease”, and threatens the pollination industry and production of commercial honey in the United States. This has become a highly significant yet poorly understood problem for beekeepers. States, like Pennsylvania, can ill afford these heavy losses; the number of managed colonies is less than one half of what it was 25 years ago. Many beekeepers are openly wondering if the industry can survive. There are serious concerns that losses are so great that there will not be enough bees to rebuild colony numbers in order service the pollination needs and to maintain economic viability in these beekeeping operations.

This preliminary report consolidates our findings and current thoughts on the symptoms and causes of “Fall Dwindle Disease”. While our investigations continue, the epidemic nature of this disease demands that we share information as it becomes available. It is hoped that, despite its incomplete nature, this report will help to formulate plans of action on how to best tackle this new challenge to the industry. The apicultural industry has proven resilient in the face of past challenges; it is our firm belief that it will do so again.

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## **Survey of Beekeepers Reporting Large Losses Typical of “Fall Dwindle Disease” A Case History Report**

Seven beekeepers (as of 12/15/06) reporting heavy losses in their operations were interviewed by phone. These interviews detailed management practices employed by the beekeepers over the last year. Interviews lasted 45 minutes to 2.5 hours, and were often followed up with a second phone call to clarify certain points. To encourage full disclosure, all beekeepers interviewed were assured that the particulars of each interview would be kept confidential, and the reports resulting from the survey would not disclose their identity. Beekeepers interviewed represented operations based in four States (Florida, Georgia, North Carolina, and Pennsylvania). The colonies managed by these operators were located in these states and moved to and from an additional six States (California, Delaware, Maryland, New York, Maine, North Dakota). The size of the operations varied from 200 to over 3000 colonies. All beekeepers were experienced and knowledgeable. At the time of interview, beekeepers reported losses of 30 to 90 %. One beekeeper, having 1200 colonies, expects 9 to survive the winter.

### Practices and conditions common to interviewed beekeepers.

1. All were migratory beekeepers. All had moved their colonies at least 2 times in the 2006 season, with some colonies being moved as many as five times over the 2006 season
  - Implications:
    - i. Moving colonies is stressful on bees;
      1. Possible reasons: confinement, temperature fluctuations, and possible reduction (or cessation) of egg laying
    - ii. Moving colonies is thought to amplify adult bee disease agent loads,
      1. Possible reasons: increase rate of defecation in the colony, forced mingling of young and older (possibly infected and would otherwise be foraging) adult bees increase chance of disease transmission
    - iii. A remote possibility is the bee colonies are more apt to be exposed to new diseases or pathogens.
2. All experienced a cumulative dead-out rate of at least 30% over the course of the season. It is common that 10% of colonies die after transportation; some beekeepers claim losses of 30% are not uncommon after pollination of crops such as blueberries.
  - Implications
    - i. Beekeepers are constantly “splitting” colonies to make up for losses (see below)
    - ii. The equipment from the dead-out colonies is continually being recycled back into the operation in creation of new splits. Existing food reserves in the dead-outs and comb is provided to the new

colonies; potentially any disease agent or chemical contaminant would be carried over to the new colony.

3. Upon finding a dead-out colony, all interviewed beekeepers placed the dead-out equipment on strong neighboring colonies to facilitate comb care and splitting. When the queen from the strong colony began to lay in the dead out equipment, the dead-out equipment and contained brood were removed (split) from the surviving colony. Some beekeepers then introduced a mated queen or queen cell into the queenless unit while other allowed the unit to rear a new queen naturally.
  - Implications
    - i. Continual reuse of dead out brood comb
      1. Reuse is a known way to transfer disease agents and possibly other chemical contaminants (e.g. Miticide buildup in colonies)
      2. Reuse can potentially amplify the presence of disease agents on comb
    - ii. Large-scale spitting of colonies is stressful on bees and can amplify disease agent populations
      1. The age profile of the worker population is altered by splitting
        - a. Older bees are forced to act as nurse bees; these bees are not as efficient in broods provisioning and may be more likely to be infested with diseases affecting adult bees
4. All producers experienced some form of extraordinary “Stress” at least 2 months prior to the first incidence of “die off” associated with “Fall dwindle disease”. The nature of this stress was variable but included nutritional stress (apiary overcrowding, pollination of crops with little nutritional value), dramatic pollen and nectar dearth, or varroa mite pressure. Due to drought in some areas, the bees may have had limited water resources or contaminated water supplies.
  - Implications
    - i. Stress compromises the immune system of bees, making them more susceptible to infection by opportunistic microbes.

Practices and conditions *not* common to interviewed beekeepers.

1. Feeding: The practice of feeding was common to most of the interviewed beekeepers. The reason for feeding varied. Some fed to help encourage build up, while other fed to hold off starvation in the summer during particularly severe drought.
  - a. Carbohydrates: some did not feed, some feed HFC, other sucrose. They used frame feeders, top hive feeders, and barrel feeders. Some added mineral salts to the feed, some added antibiotics, none used Fumagillan.
  - b. Protein: most did not feed, some used pre-made protein supplement.

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2. Chemical use:
  - a. Antibiotic use: While all used antibiotics, the type, frequency of application, and method of application varied.
  - b. Miticide use: all but one beekeeper had applied a miticide treatment over the course of 2006. The products used, method of application, varied.
3. Major income:
  - a. Some reported that their major purpose was the production of honey, while others received most of their income from pollination contracts. Some used both.
4. Source of Queens:
  - a. All purchased at least some queens throughout the year. One beekeeper reared a majority of his own cells, but most bought either mated queens or queen cells. Queens were bought from at least 5 different states (Florida, California, Texas, Georgia, Hawaii) and 2 foreign countries (Canada and Australia)

Continuing Activity:

1. Interviews of beekeepers will continue, especially in other regions.
  - a. As of 12/15/06, at least seven other beekeepers were known to have the large losses.
2. Beekeepers not experiencing problems will be interviewed to determine if there are other factors that are not shared in common with those experiencing the losses. This may help pin-point critical factors triggering the colony collapses.
  - a. Anecdotal second hand reporting suggests
    - i. that non-migratory operations are experiencing this phenomena only in split colonies and not parent colonies
    - ii. migratory beekeepers not experiencing this problem either do not have high losses though out the year or have aggressive comb management/replacement procedures in place

Suggested future action:

1. Monitoring colonies year round to look for evidence of stress and disease agent build up. Little is known about the normal levels of some microbes/pathogens associated with bees, such as fungi causing stonebrood, flagellates, or amoebae. There is also a potential of other viruses infecting bees to be present, which have not yet been fully characterized or have developed methods of detection. New fungal pathogen strains with increased virulence are being reported in other countries and may have been introduced to the U.S.

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## **Examination of submitted samples**

### **Sample collection**

A beekeeper complaining of heavy losses to colonies approached Dr. Diana Cox-Foster of Penn State University. In response, Diana accompanied by Dr. Nancy Ostiguy, visited the beekeeper's home yard and collected samples of 1) bee bread in dead-out colonies, 2) honey from dead-out colonies, 3) frames of dead brood (no dead bees were found by the beekeeper at the original location or within the dead-out colonies) and 4) four living nucleus colonies which were transferred to Penn State and stored in a secure building until live bees could be collected. Live worker bees and queens were placed in individual vials and stored at  $-80^{\circ}\text{C}$ . An additional ~50 bees were placed in alcohol. Samples of bee bread and honey were also collected. The bees in alcohol were sent to Dennis vanEngelsdorp (who is affiliated with both the Pennsylvania Department of Agriculture (PDA) and Penn State University), and he analyzed the samples for Varroa mite abundance, HBTM infection, amoeba disease, noseema disease, and digestive tract abnormalities. Diana Cox-Foster participated in the examination of the samples, given her expertise in bee immunity, physiology, and pathology. Fungal isolations and characterizations were made by Dr. David Geiser at Penn State at the request of Diana Cox-Foster. Dr. Geiser is an expert in fungal taxonomy and molecular characterization, particularly in the group of fungi causing stonebrood disease.

A second beekeeper located in GA, also complaining of high losses, contacted Jerry Hayes, with the Florida Department of Agriculture. Samples of comb from near dead colonies were sent to the PDA. Samples of bees from those same near dead colonies were collected and shipped in rubbing alcohol. Honey and bee bread from the combs were sent to Dr. Diana Cox-Foster for viral analysis. A sample of dead immature bees (imago stage) pulled from beneath the capping were also sent. Samples were stored at room temperature until they were transported to Penn State. These samples were also transferred to David Geiser, when recognizable fungal growth was observed on these bees.

Maryanne Frazier received wax samples from the combs collected from the PA beekeeper. These samples are undergoing pesticide residue analysis.

### **Examination of the bees, honey, and bee-bread**

Varroa mite abundance was examined for those bees received in alcohol (Table 1). While abundance levels were high, these numbers may be artificially inflated considering that the bees tested were the last in collapsing colonies and apiaries.

None of the samples examined had evidence of HBTM (n= 25 per sample). However, when preparing samples for HBTM analysis, morphological peculiarities were found (Figure 1). Crystal-like formations were observed in the thorax where muscles are located. Similar structures have been described in some viral infections; however, it is not clear if these are the same type of structures.

A set of tweezers was used to grab the poster end of the abdomen and pull the gastro intestinal tract out of the bees abdomen. Along with the intestinal tract, the venom sac and sting gland were often removed (Figure 2). The Malpighian tubules (the bee's "kidneys") were examined for the presence of Amoeba disease. Only the occasional amoeba cyst was found in tubules, but never at levels that would seem pathogenic (Figure 3). However, the tubules were found to be swollen and discolored in many bees, a condition not normally observed. Pylorus scarring was evident in between 0% to 45% of the samples examined (Figure 4; Table 1). In research done in the early 1950's, this discoloration or scarring has been attributed to the infection of the bee by small single-cell organisms known as flagellates. Both flagellates and amoeba have been claimed to be non-pathogenic in bees; however, little or no information is readily available to document these protozoa.

The contents of the rectums of PA and GA bees differed (Figure 5). In the PA bees, cursory examination of the gut contents revealed many pollen grains of unknown origin. The pollen grains seemed largely intact and many did not appear digested (which is abnormal). All PA samples were found to have nosema spores in their rectal contents. The sting gland of many examined bees were obviously scarred with distinct black "marks" (Figure 6); this type of pin-point melanization or darkening is indicative of an immune response to some sort of pathogen. We could find no previous reports of damage to honey bee sting glands, and so this finding was surprising.

In several samples, there were distinct debris clumps in the tracheal network examined in the abdomen of bees (Figure 7). In at least one case what appeared to be fungal mycelium was observed growing from a tracheal branch into a larger tracheal trunk. In several live bees from PA, other potential fungal mycelium was observed in other tissues such as the sting gland, the body wall, etc. Potentially these bees have a low-level fungal infection. Of note, there was few or no dead brood in the colonies exhibiting overt signs of any type of common brood disease. In particular, there was no indication of Chalkbrood mummies.

The dead brood from GA were late-pupae or adults that were about to emerge. The fungal growth on these bees was composed of at least two-different types of fungi. One was chalkbrood and the other was the species of fungi causing stonebrood. Neither is

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known to infect this stage of bee. These fungi are being characterized as to exact species/strain by David Geiser.

The examination of the honey and bee-bread from PA did not reveal significant viral infections in the dead-out colonies. The samples collected came from 10 dead-out colonies and the four living nucleus colonies. The detection of viruses in the honey and bee-bread in combs has previously been demonstrated to reflect the viral infections present in the colony (Cox-Foster and Ostiguy). In the 14 PA colonies samples, only one had detectable deformed wing virus, four had sacbrood virus, and one had a virus similar to Kashmir bee virus. The identity of the Kashmir bee virus needs to be confirmed since the detected viral was abnormal. No acute bee-paralysis virus, blackened queen cell virus, or cloudy wing- virus were detected. Additional analyses are currently being performed on the queens and workers from the living colonies from the PA operation and the GA honey/bee-bread samples. In addition, no chalkbrood spores, AFB, or EFB were detected in the honey/bee bread samples from the PA operation.

#### Ongoing activities:

Additional observation and a more careful review of the literature regarding gut contents will be initiated.

Attempts to positively identify any microbes infecting the bees have been initiated and continue. These analyses include the detection and characterization of fungal pathogens in the bees. Additional attempts will be made to determine if other viruses are present in these bees. These detections of other microbes will not be definitive in determining the culprit underlying the collapses, but will allow for future research to determine if these microbes are the cause.

In addition, a researcher working on characterization of either new or emerging pathogens in humans has agreed to help determine if other pathogens are present in the bees. This researcher has developed a method that may allow any type of pathogen to be identified and provide the means to further characterize these microbes and determine if they underlie the collapse. This researcher is an international expert, who is recognized as a leader in this area by many branches of the U.S. government and by international health organizations. The cost of supplies for this analysis is expensive (approx. \$350 per sample); but this expense is justified for a small number of samples, since this may be the only viable means to find in a timely fashion undescribed or new disease agents that may be associated with the collapse of bee colonies. Of particular note, the researcher having this technology is willing to donate the time and expertise needed to perform these analyses, which represents a significant contribution.

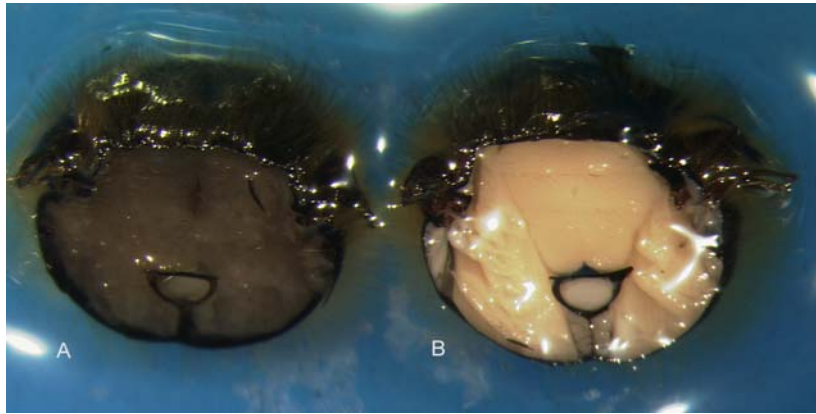
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Chemical analysis of wax:

Maryanne Frazier has initiated the analysis of wax samples for the presence of pesticides, miticides and other potential contaminants.

**Table 1:** Findings in submitted samples

Sample	Varroa abundance	Sticky board	Malpighian tubule damage	Sting gland damage	Pyloris scarring
PA-20	8/80	Na	6/11	2/11	n.a.
PA-21	8/45	Na	10/21	9/20	9/20
PA-23	14/64	Na	7/23	15/22	5/24
PA-24	7/37	na	8/14	11/14	1/14
GA-1	1/21	1	8/16	7/16	4/16
GA-2	1/408	0	19/20	0/20	0/20
GA-3	0/63	88	10/20	na	na



**Figure 1:** When thoracic discs were cut from sample GA-2 the musculature of bees was notably soft and discolored (A) when compared to healthy thoracic cuts (B). This discoloration suggests that the bees were dead upon collection. When questioned the beekeeper confirmed that the bees were alive at the time of collection. Further, the tracheal system of these bees did not show signs of desiccation usually associated with the collection of dead bees. Thoracic discs from this sample, after being placed in KOH for 24 hours, revealed peculiar white nodules (C). When wet mounts were examined they appeared to have crystalline arrays (D) which may be indicative of Cloudy wing virus (CWV) (Bailey et al. 1980). Alternatively these may also be the same “sharp-edged crystalloids” observed in degrading bee muscle tissue by Willie (1967)(as reported by (Bailey 1981)). Another possibility is that these are small tyrosine nodules, which have been reported in the Gasters of bees (Erickson et al. 1997) and more recently observed by FDA (E – Photo courtesy of Jerry Hayes and David Barnes).

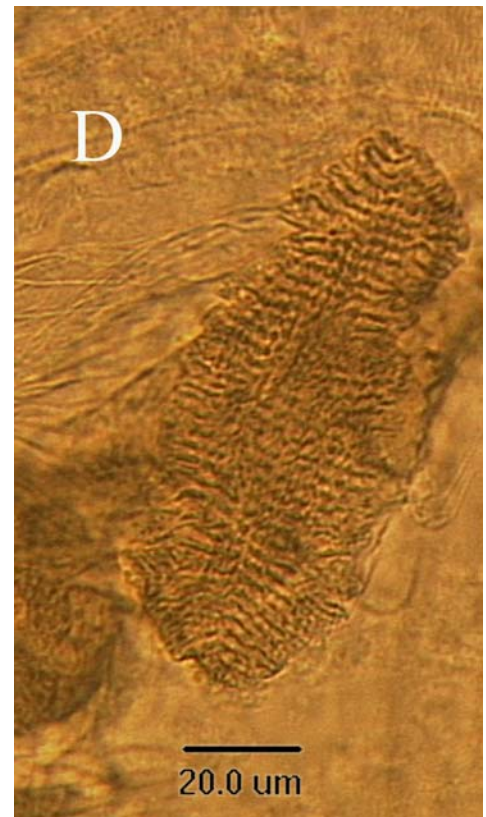
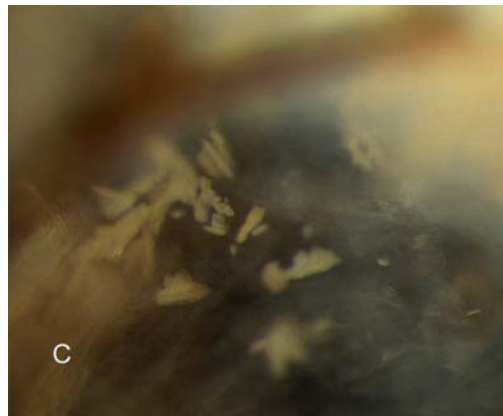
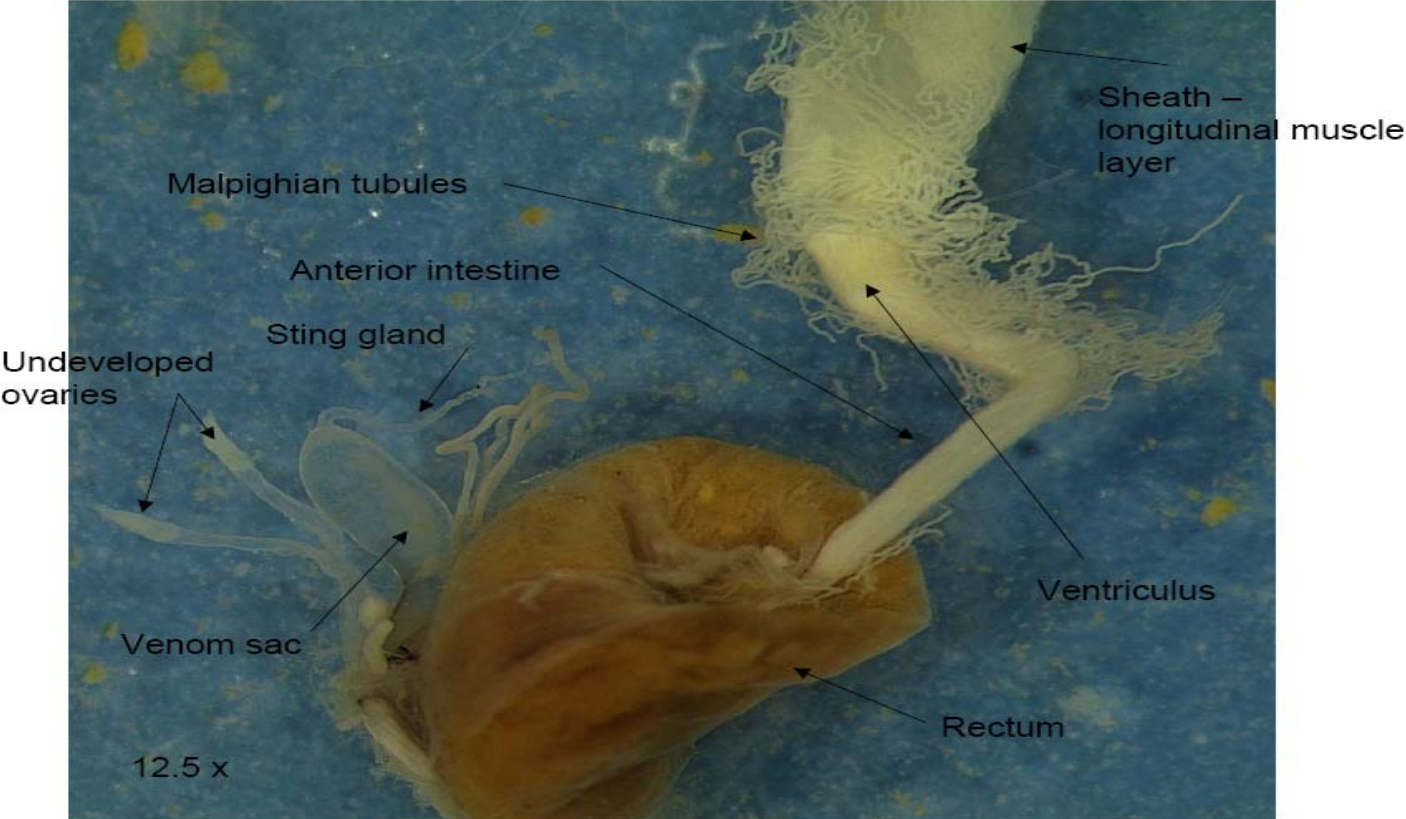


Figure 2: Digestive tract of healthy bee.



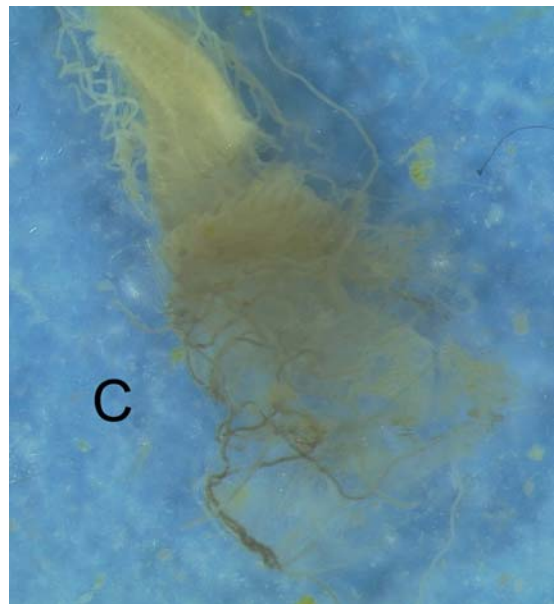
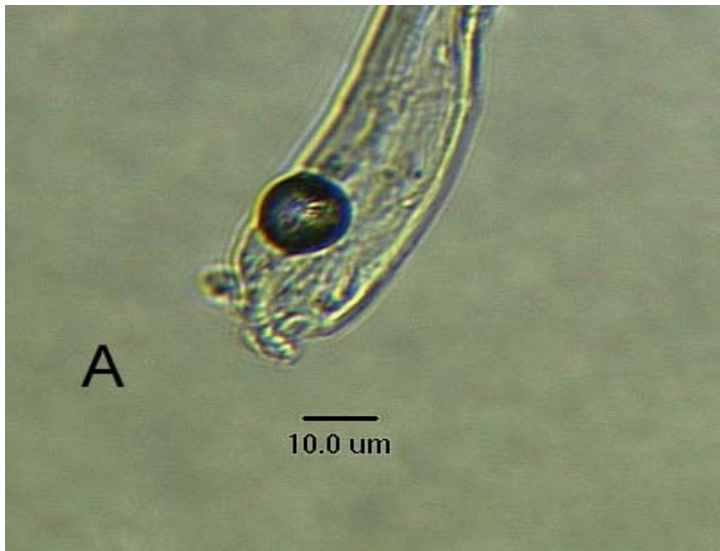
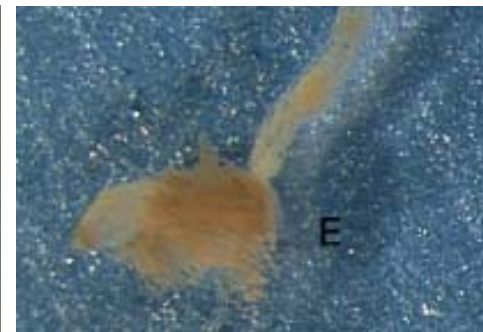
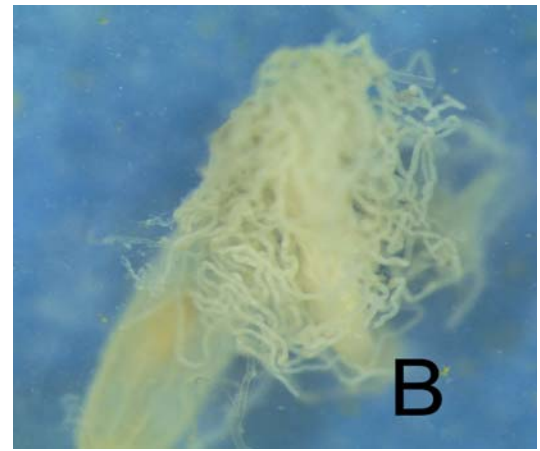


Figure 3: The Malpighian tubules were examined for the presence of Amoeba disease. Only the occasional amoeba cyst was found in tubules, but never at levels that would seem pathogenic (A). When compared to apparently healthy tubules (B), many samples had Malpighian tubules that were obviously discolored (C). Examination of these tubules revealed heavy debris load (D). GA-2 and GA-3 had significantly reduced Malpighian tubules, a condition reported to have an association with nosema disease (E).



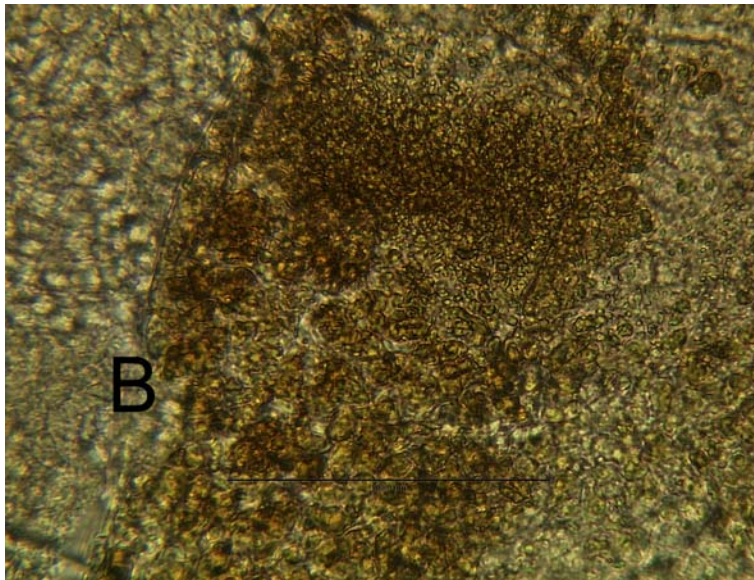
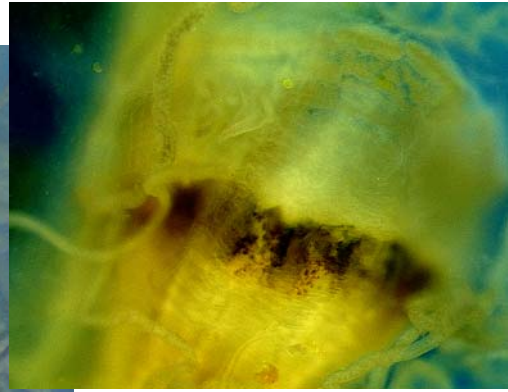
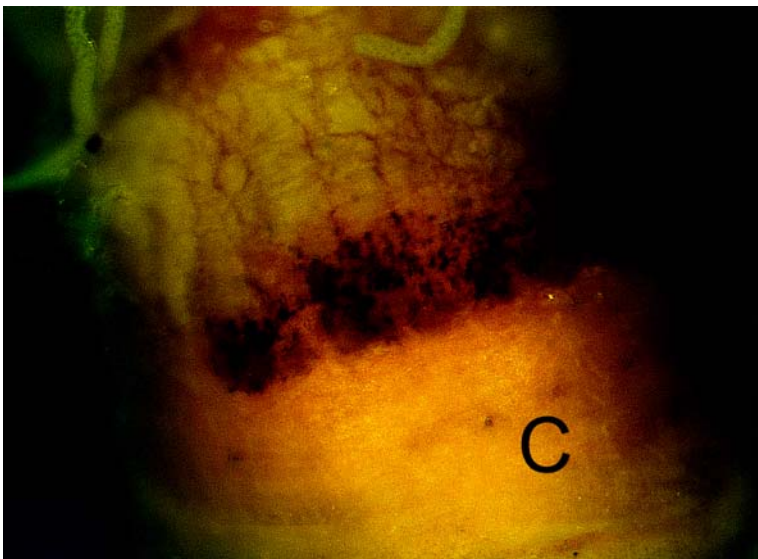


Figure 4: Pylorus scarring was evident in some samples (A). Wet mounts of the scar area showed extensive melanization (B) that may be the result of Morison's cell inclusion. This immune response has been previously reported in association with chronic bee paralysis, accumulation of flagellates, or possibly some other microbe. The net like distribution of this scarring suggests an immune response to a fungal infection (C).



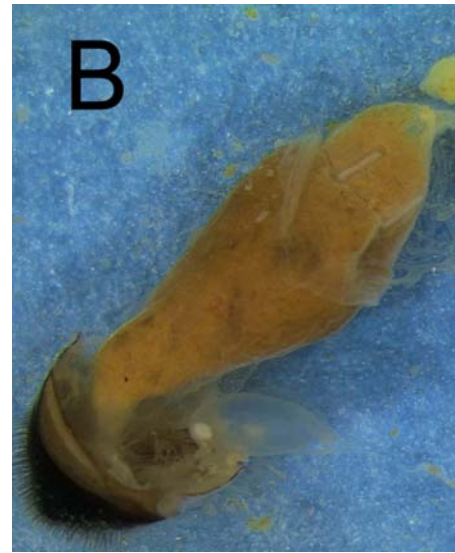
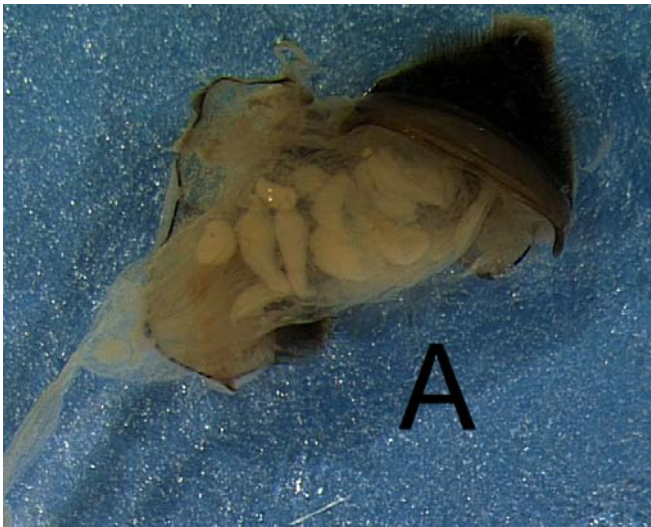
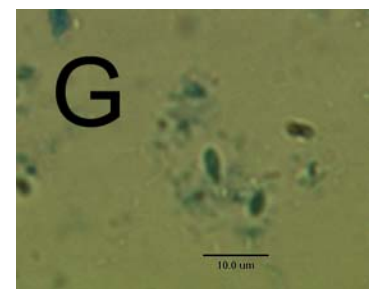
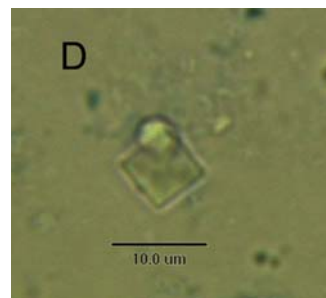


Figure 5: The rectal contents of GA bees (A) were distinctly different than the contents of PA bees (B). The rectal wall of GA bees were notably transparent revealing contents that looked like small stone packets (C). While (Fyg 1964) describes similar stone like contents in poorly laying queens, the stones observed in the GA bees were not attached to the epithelium layer as Fyg (1964) describes. When these packets were ground and mounted, some unidentified floating objects (UFO's) were observed. A cubic particle (D) that resembles the cubic bodies of polyhedrios viruses (this viruses attacks wax-moths) excepting that the cube observed was ~10x too big for a virus particle. There were fragments of pollen grains husks in all samples examined. All PA samples were found to have nosema spores in their rectal contents (E) while none of the GA samples did. In two samples epithelial cells were apparently packed with spores. Amoebae cysts (F) and what appeared to be flagellates (G) were also observed.



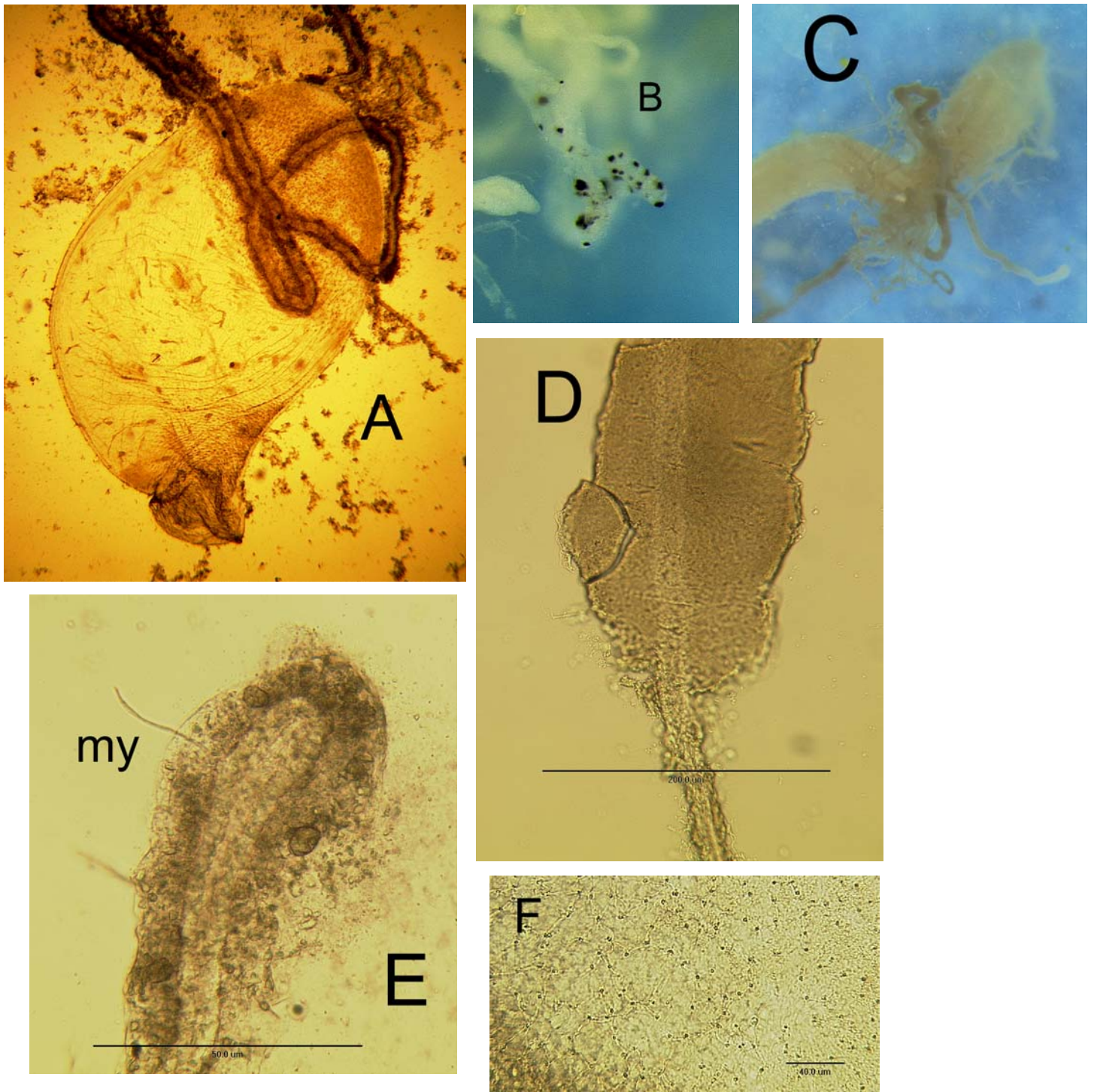


Figure 6: The venom sac and sting gland of bees were examined. In many examined bees (Table1) there were obvious black scaring. In some cases the marks were small specks (A), while in other cases damage was easily visible to the naked eye (B). The sting gland in some bees appeared “swollen” (C). What appeared to be immune defense cells accumulated in thick layers around the sting gland (D). In some cases there appeared evidence of fungal mycelium growing from the sting gland (E, my). Examination of the venom sac also revealed evidence of fungal growth (F).



Figure 7: Distinct debris was observed in the tracheal network associated with the gastrointestinal tract. In some cases what appeared to be fungal mycelium was observed growing from a tracheal branch into a larger tracheal trunk.