

CCD

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Colony Collapse Disorder (CCD)

The following report references Fall Dwindle Disease. This name has been changed to Colony Collapse Disorder

What's in a name?

Symptom's similar to those described for this disorder have been described in the past, and the condition has received many different names. These include autumn collapse, May disease, spring dwindle, disappearing disease, and fall dwindle disease. The CCD working group felt none of these names were appropriate for the current condition. The reasons are as follows:

- 1) References to the season are inappropriate as there is increasing evidence that the condition manifests itself throughout the year
- 2) Dwindle implies a gradual decline of colony population. While the actual rate of adult bee loss in populations have not been recorded, it is clear that otherwise strong colonies can quickly lose their entire workforce in a matter of weeks.
- 3) Disappearing has been used to refer to a host of other conditions that do not necessarily share the same symptoms as those presently being described.
- 4) The term "disease" is commonly associated with a pathogenic agent. While the definition of disease does have a broader meaning (i.e. coronary disease), until (or if) such an agent is found the use of the word disease would be misleading. Should a biological or other agent(s) be found the name of this condition will likely be reconsidered.

Symptoms of CCD

- 1) In collapsed colonies
 - a. The complete absence of adult bees in colonies, with no or little build up of dead bees in the colonies or in front of those colonies.
 - b. The presence of capped brood in colonies.
 - c. The presence of food stores, both honey and bee bread
 - i. which is not immediately robbed by other bees
 - ii. when attacked by hive pests such as wax moth and small hive beetle, the attack is noticeably delayed.
- 2) In cases where the colony appear to be actively collapsing
 - a. An insufficient workforce to maintain the brood that is present
 - b. The workforce seems to be made up of young adult bees
 - c. The queen is present
 - d. The cluster is reluctant to consume provided feed, such as sugar syrup and protein supplement



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

**Preliminary Report:
First Revision**

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December 15, 2006
Revised January 5th, 2006

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 are also beginning to report unprecedented losses. This phenomenon, without a recognizable underlying cause, has been tentatively 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 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 the interview, beekeepers reported losses of 30 to 90 %. One beekeeper, having 1200 colonies, expects 9 to survive the winter.

General description of condition:

Beekeepers report the sudden loss of a colony's population of adult bees. In all cases few, if any, adult bees were found in or near the dead colonies. Capped brood was often found in these colonies. Dead out colonies often contained food reserves that had not been robbed out, despite the presence of living colonies in an area. Most dead out colonies showed no, or minimal, evidence of wax moth or small hive beetle damage. In colonies that still have bees, small clusters were reported with evidence of a laying queen. The surviving workers tended to look young in age.

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.

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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 brood were removed (split) from the surviving colony. Some beekeepers then introduced a mated queen or queen cell into the queenless unit while others 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 brood 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 others fed to hold off starvation in the summer during particularly severe drought.
 - a. Carbohydrates: some did not feed, some feed HFC, others sucrose. They used frame feeders, top hive feeders, and barrel feeders. Some added mineral salts to the feed, some added antibiotics, none used Fumaigillan.
 - b. Protein: most did not feed, some used pre-made protein supplement.
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 large losses.
 - b. Beekeepers have been asked to “interview” fellow beekeepers based on questions derived from initial interviews. (Appendix 1)
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.

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°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, nosema 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

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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 in 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 posterior end of the abdomen and pull the gastrointestinal 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 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. Both yeast and bacteria have been identified as causal agents for this condition (called H melanosis and B melanosis depending on casual agent). Fyg (1969) reported this condition in queens. In addition to their sting glands, evidence of melanosis was found in the ovaries of infected queens. These queens were superseded by colonies. Subsequent research has documented damage to hypopharyngeal glands of worker bees.

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

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mycelium were 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. These fungi are being characterized as to exact species/strain by David Geiser.

Samples from PA and GA were examined for known viral, bacterial, and fungal pathogens of honey bees using methods to detect the genetic material belonging to these organisms. Given that few adult bees or brood were available from the "dead out" colonies, the honey and bee bread were examined. 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 when infected bees were the ones making those food stores (Cox-Foster and Ostiguy). In the PA colonies (19 to date) and GA colonies (5 to date), the examination of the honey and bee-bread did not reveal significant infections in the dead-out colonies. The samples collected came from 15 dead-out colonies and the four living nucleus colonies. In the honey and bee bread samples from the 19 PA samples, only one had detectable deformed wing virus (DWV), four had sacbrood virus (SBV), and one had a virus similar to Kashmir bee virus (KBV). 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 (BQCV), or chronic bee paralysis virus were detected. In addition, no chalkbrood spores, AFB, or EFB were detected in the honey/bee bread samples from the PA operation. Likewise, the GA honey and bee bread samples had little viral contamination, except for two samples with DWV and one with BQCV.

Additional analyses were done on five queens and five workers from the living colonies that were declining from the PA operation. These samples revealed extensive viral and fungal co-infection, with all bees being infected by DWV, nearly all infected by BQCV, a significant number having KBV, some SBV, and almost all having chalkbrood infection. One sample tested positive for AFB. Given the extent of infection in these bees and the lack of corresponding detection in honey/bee bread in the same colonies, there is a chance that the reserves remaining in the colonies are remainder reserves present in the equipment used to make the new splits. These results suggest that it is best to gain data from any living bees present in colonies undergoing the dwindle disease. The extent of the infection levels in these bees is also unusual and not comparable to data reported by others in the literature to date or by the extensive studies done by Cox-Foster and Ostiguy on over 200 colonies. The characterization of pathogens needs to be performed more fully before any conclusions can be reached on underlying diseases causing the fall dwindle disease.

Ongoing activities:

1. Additional observation and a more careful review of the literature regarding gut contents will be initiated.
2. 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.
3. 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.

Chemical Analysis for Pesticide Contamination

Goals:

- 1) To analyze pollen, honey and bees for the presence of neonicotinoid pesticides (and possibly certain fungicides)
- 2) To analyze beeswax (brood nest combs) for possible accumulation of Fluvalinate, Coumaphos and/or Amitraz

The neonicotinoids, for example imidacloprid, are a rather new class of pesticides. There have been new chemicals of this sort introduced over the past few years (clothianiden and thiamethoxam). There is conflicting information about their effect on honey bees, however the EPA identifies these chemicals as highly toxic to honey bees. "Clothianiden is highly toxic to honey bees on an acute basis (LD50>0.0439 mg/bee). It has the potential for toxic chronic exposure to honey bees, as well as other non-target pollinators through the translocation of clothianidine residues in nectar and pollen. In honey bees, the effects of this toxic chronic exposure may include lethal and/or sub-lethal effect in the larvae and reproductive effects on the queen". [EPA Fact Sheet on Clothianiden]. Some researchers have not found this effect but most were looking for mortality and not chronic

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or behavioral effect. In addition, a study in NC found that some of these neonicotinoids in combination with certain fungicides, synergized to increase the toxicity of the neonicotinoid over 1,000 fold in lab studies. Both the neonicotinoids and the fungicides (Terraguard and Procure) are used widely.

Recent research tested crops where seed was treated with imidacloprid. The chemical was present, by systemic uptake, in corn, sunflowers and rape pollen in levels high enough to pose a threat to honey bees. Additional research has found that imidacloprid impairs the memory and brain metabolism of bees, particularly the area of the brain that is used for making new memories.

Implication: If bees are eating fresh or stored pollen contaminated with these chemicals at low levels, they may not cause mortality but may impact the bee's ability to learn or make memories. If this is the case, young bees leaving the hive to make orientation flights may not be able to learn the location of the hive and may not be returning causing the colonies to dwindle and eventually die. It is also possible that this is not the sole cause of the dwindling but one of several contributing factors.

Action: Dr. Chris Mullen is a chemist in the Penn State department of Entomology who has worked on the toxic and behavioral effects of pollen feeding beetles, feeding on neonicotinoids in pollen. He is willing to work on this problem and will attempt to analyze pollen, honey, and bees from colonies involved in the die off, for the presence of neonicotinoides. He is capable of detecting ppm but to detect ppb we would have to do a more complex analysis (that would cost more) but this would still require the preliminary work that Dr. Mullen will need to do for the ppm analysis.

Ed Bogas worked for many years as the technical specialist in the Penn State Pesticide Research Lab and was responsible for most of pesticide residue work conducted by this lab. He now works for Dr. Consuelo DeMoraes, who is not involved in pesticide residue work but she has agreed to allow him to do this work, using her lab's equipment but we will need to cover the cost of the analysis. Ed will work on the analysis of wax and other hive materials for the presence of chemicals typically used in colonies for the control of varroa mites. We are hopeful that he will be able to detect pesticide residues at the ppb level. Pesticides residues at these levels might not have lethal effects but could have chronic or behavior effects

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Table 1: Findings in submitted samples

Sample	Varroa abundance	Sticky board	Malpighian tubule damage	Sting gland damage	Pylorus 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

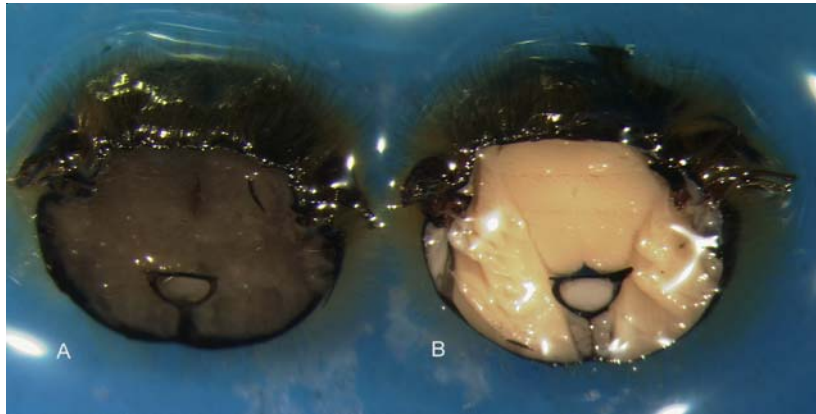


Figure 1: When thoracic discs were cut from sample GA-2 the musculature of bees was notably soft and discolored (A) when compared to healthy thoracic cuts (B). This discoloration suggests that the bees were dead upon collection. When questioned the beekeeper confirmed that the bees were alive at the 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).

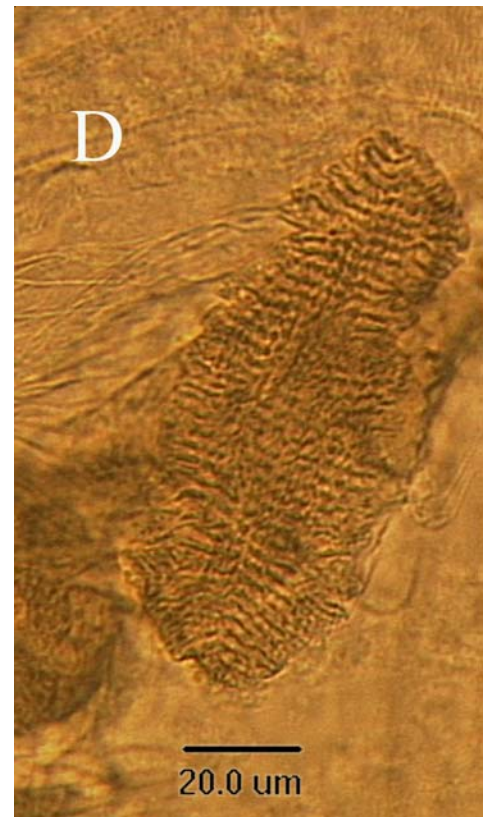
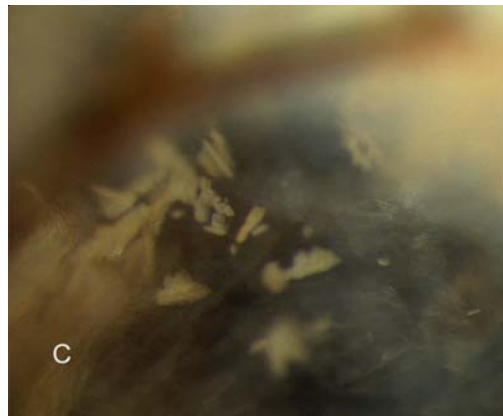
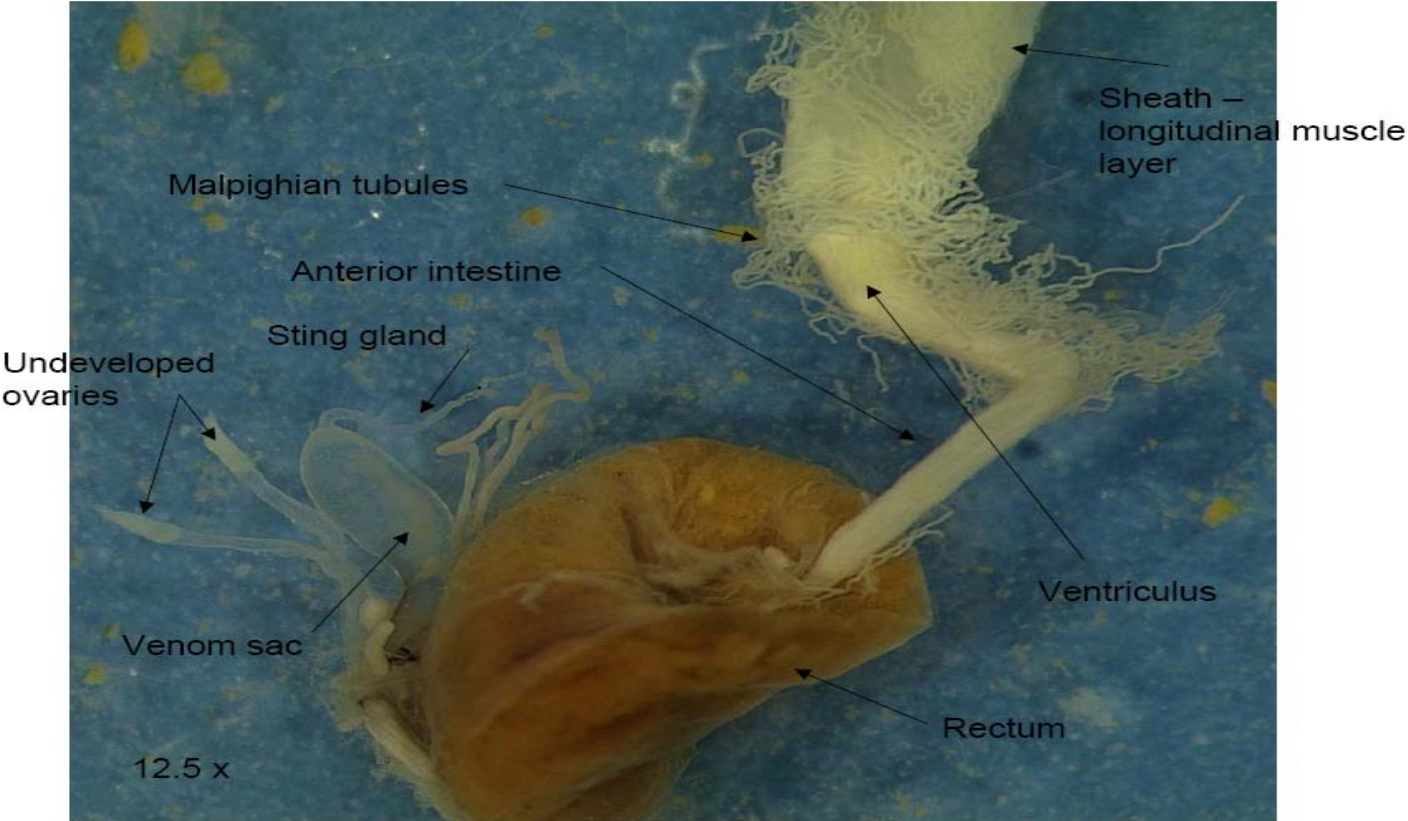


Figure 2: Digestive tract of healthy bee.



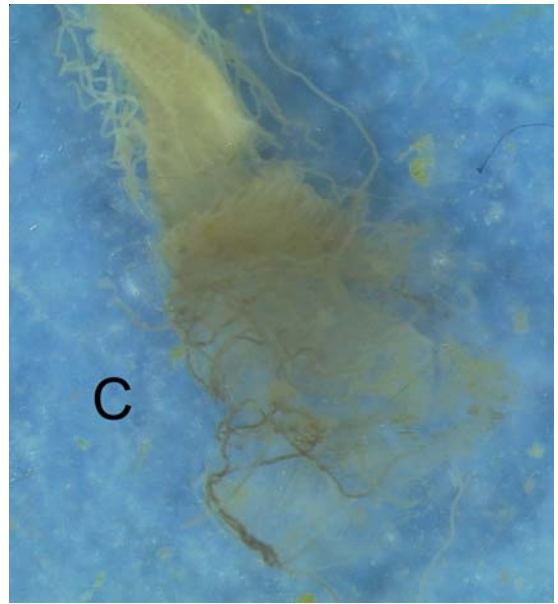
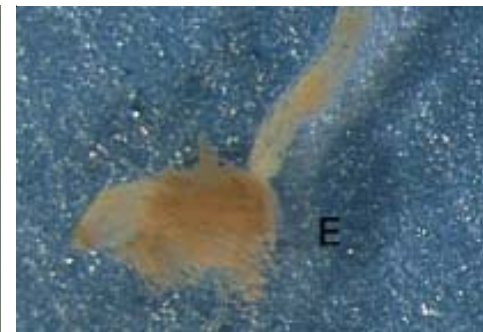
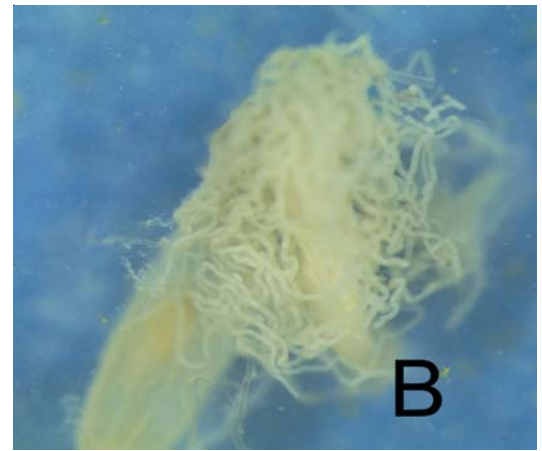


Figure 3: The Malpighian tubules were examined for the presence of Amoeba disease. Only the occasional amoeba cyst was found in tubules, but never at levels that would seem pathogenic (A). When compared to apparently healthy tubules (B), many samples had Malpighian tubules that were obviously discolored (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).



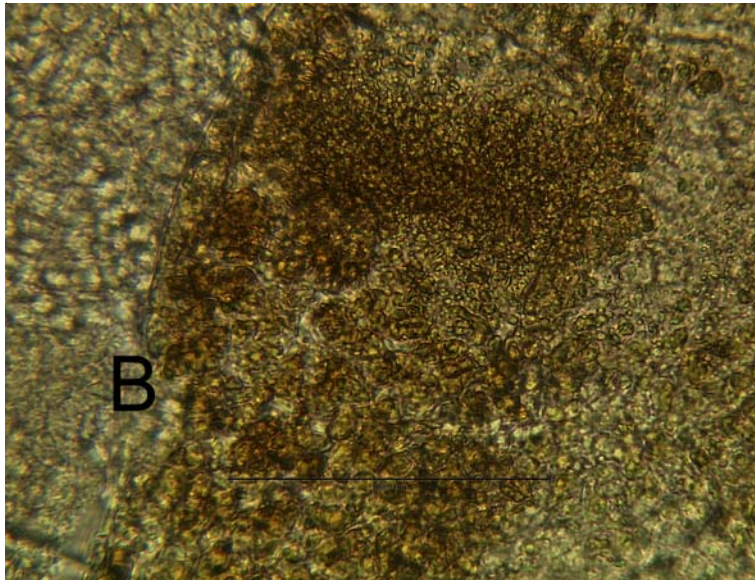
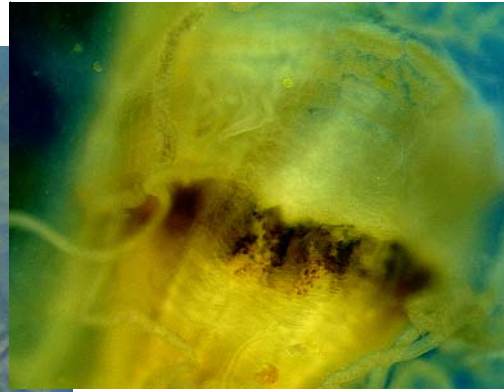
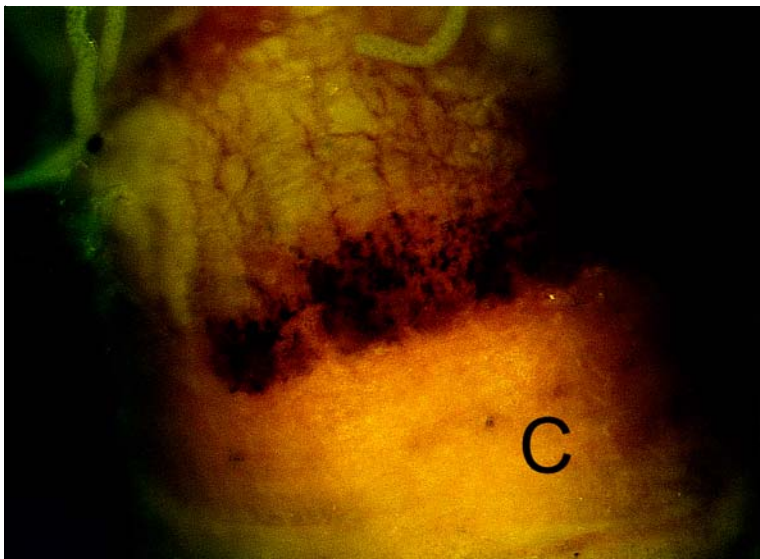


Figure 4: Pylorus scarring was evident in some samples (A). Wet mounts of the scar area showed extensive melanization (B) that may be the result of Morison's cell inclusion. This immune response has been previously reported in association with chronic bee paralysis, accumulation of flagellates, or possibly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



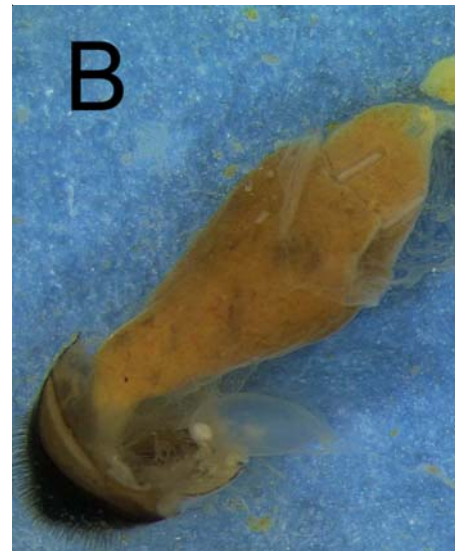
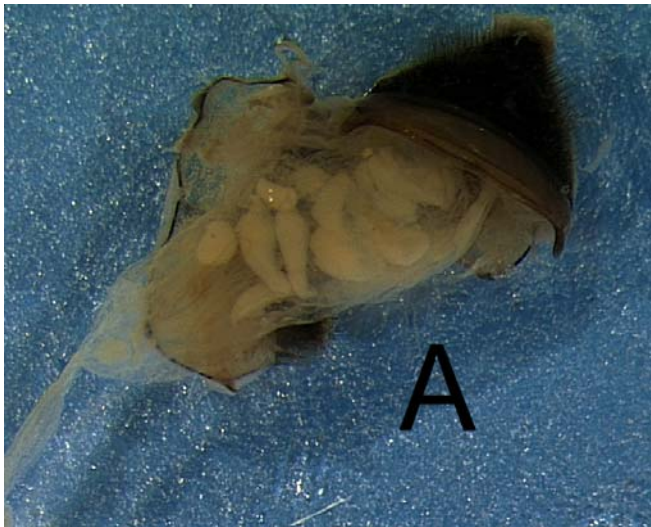
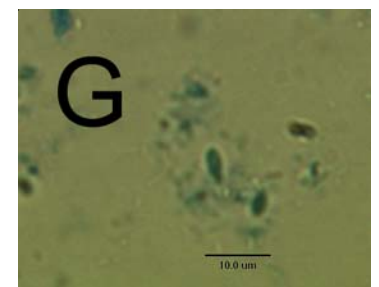
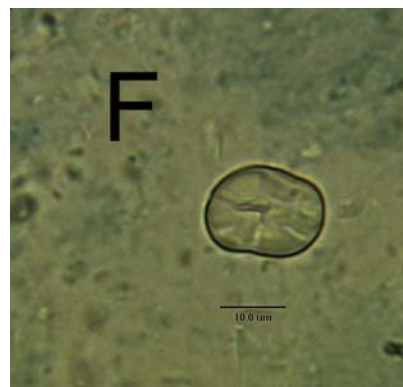
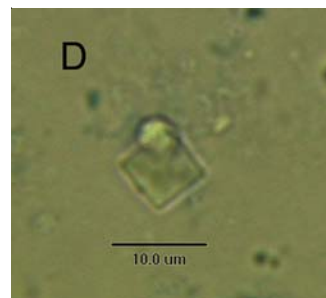
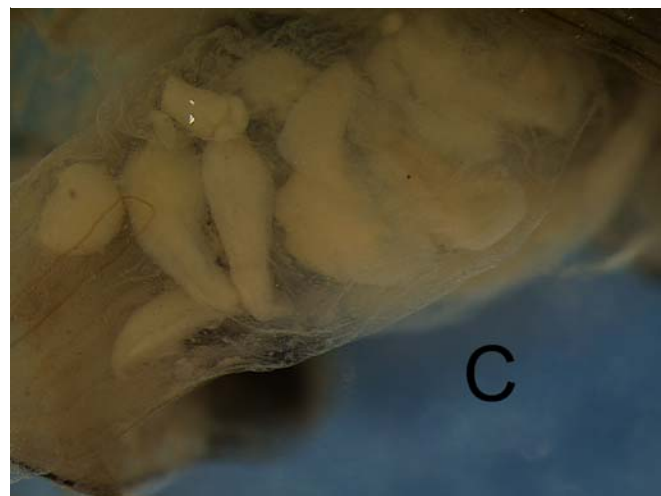


Figure 5: The rectal contents of GA bees (A) were distinctly different than the contents of PA bees (B). The rectal wall of GA bees were notably transparent revealing contents that looked like small stone packets (C). While (Fyg 1964) describes similar stone like contents in poorly laying queens, the 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.



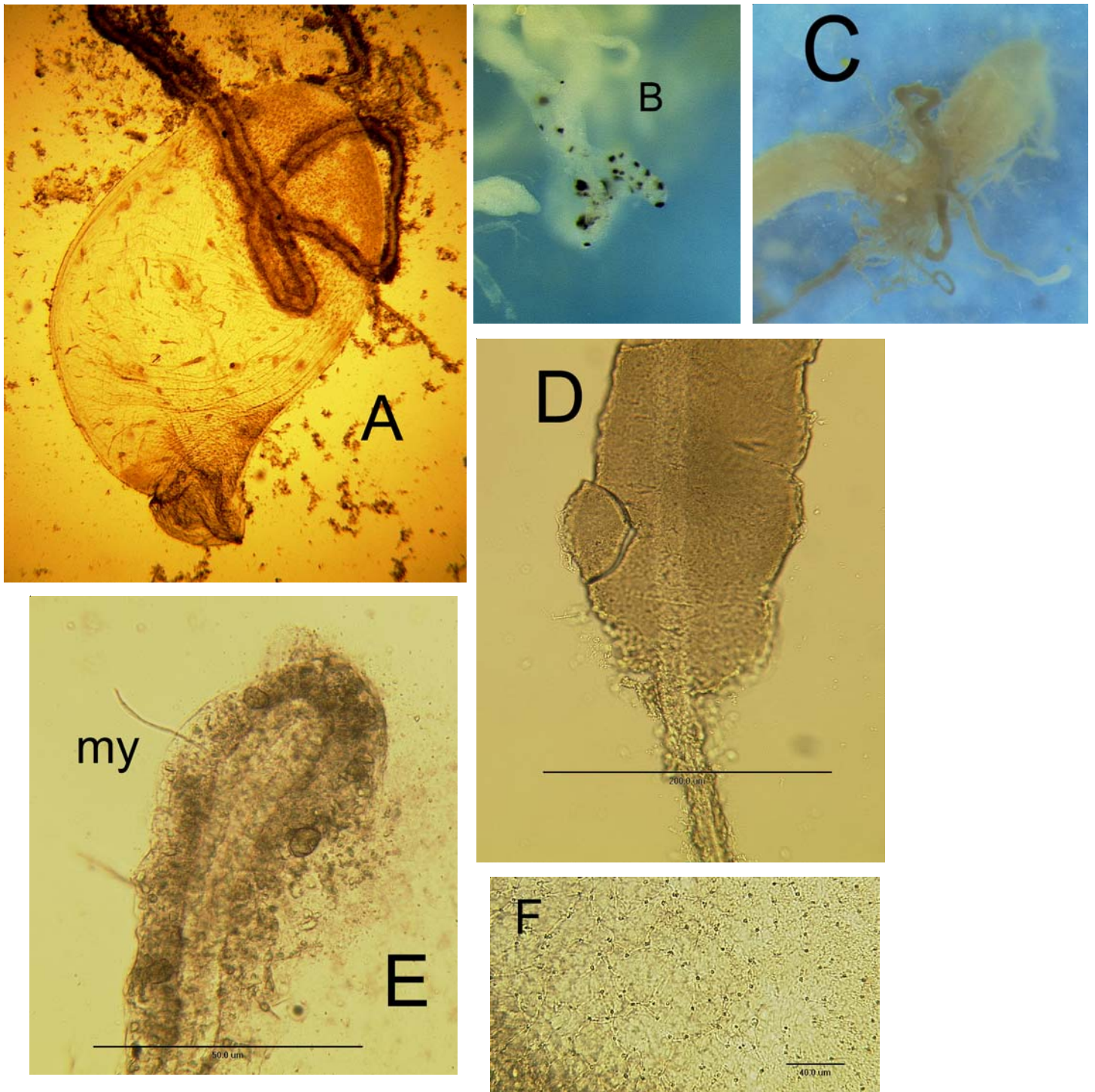


Figure 6: The venom sac and sting gland of bees were examined. In many examined bees (Table1) there were obvious black scaring. In some cases the marks were small specks (A), while in other cases damage was easily visible to the naked eye (B). The sting gland in some bees appeared “swollen” (C). What 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.

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Appendix 1

Honey Bee Management Questionnaire

The following questionnaire is intended to help us identify common and not in common practices in beekeeping operations. Beekeepers are encouraged to interview other beekeepers, even if those beekeepers are not experiencing losses. Differences between those with losses and those without could highlight some of the underlying causes of this disease. The identity of those who are interviewed **will remain confidential**.

If the interviewed beekeeper does not know the answer to a question please indicate that by writing "I don't know" next to the question. If the interviewed beekeeper does not want to answer a question please write "refuse to answer" next to the question.

To ensure unbiased reporting the person interviewing should not comment on the answers given by the interviewed beekeepers. A summary report will be made available to all those who perform interviews, who can in turn pass them on to those they interviewed.

A typical interview will last between 1 and 2 hours. Some of the questions may seem redundant, this is on purpose, as it acts as an internal check, perhaps jogging beekeepers memories.

Please answer the following questions on separate pieces of paper. Write the question number beside the answer. Send the results of the survey to:

Dennis vanEngelsdorp
PA Department of Agriculture
2301 North Cameron Street
Harrisburg PA, 17110

Or email them to: Dennis.vanengelsdorp@gmail.com

A. Questions for the interviewer:

Record:

1. Your name (the interviewers).
2. Record your phone number, address, and email address (the interviewer's).
3. Record the date and time of the start of the interview
4. Assign a number to the beekeeper (note to interviewer: the people processing these answers will never know who the interviewed beekeepers are, but we may have follow up questions. In that case we will call you (the interviewer) and refer to the assigned number to allow follow up questions.

B. Questions for the beekeeper being interviewed:

1. How many living colonies did you have at the beginning of January 2006 and in which state were they located at that time?
2. How many living colonies did you have at the end of December 2006 and in what state were they located?
3. At the beginning of the year, was it your intention to increase colony numbers, keep the same number of colonies, or decrease the number of colonies you managed?
4. Did you move colonies from one location to another over the course of the year? If so, for each month of the year, how many colonies did you move and where and why did you move them?
5. Did you find queenless colonies in your operation at any time in the year? If so, in what months did you find them? How many did you find? What did you do about the problem? What rate of queenlessness do you consider "normal"?

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6. Did you find dead out colonies in your operation at any time in the year? If so, in what months did you find them? How many did you find each month? And what did you do with the equipment? What rate of dead-outs would you consider normal?
7. Did you make splits over the course of the year? If so, in what months did you make these splits? How did you make them? Where did you get the queens to make them? Where did you get the equipment to make them?
8. Did you monitor for *Varroa* mites over the course of the year? If so in which months? How did you monitor levels? What levels would you have thought problematic? What levels did you get? Did you ever get levels higher than you think acceptable?
9. Did you treat for *Varroa* mites over the course of the year? If so when? With what? Was the treatment effective? How did you know?
10. Did you apply antibiotics to colonies over the course of the year? If so, what? How did you apply it? When did you apply it? Why did you apply it?
11. Did you see any brood diseases or pests in your colonies over the course of the year (Small hive beetle, sac brood, European foulbrood, American foulbrood, chalk brood, deformed wing virus, parasitic mite syndrome, wax moth)? When did you see it? How severe were these infections? Would you have considered the rates of infection you found normal?
12. Did you provide supplemental feed (sugar or protein) at any time over the past year? If so in what months did you feed? Why did you feed? What did you feed? How did you apply the feed? Did you add anything to the feed?
13. Did you collect a honey crop over the course of 2006? If so in what months? Did you consider the crop average, below average, or above average?
14. Did your colonies experience a dearth at anytime over the last year? If so how bad was the dearth? When was it? How long did it last?

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15. Did you rent colonies for pollination at anytime of the year? If so when? And for what crops?
16. Did you rear queens over the course of the year? If so, when did you rear them? How many did you use yourself? How many did you give away or sell?
17. Did you buy queens or queen cells over the course of the year? If so when did you buy them? How many did you buy (Please indicate if they were queen cells or caged queens)? How did you introduce them? What was their rate of acceptance? Did you consider their rate of acceptance normal?
18. Did you experience the phenomena tentatively referred to as Fall Dwindle Disease? If so when did it start? How many colonies have died in your operation as a result of this condition? Is the rate of death equal across all your apiaries? If not, can you think of any difference between the colonies in apiaries with high rates of loss and those that do not? What do you think is the cause of this condition? Are your bees still dying? If not when did they stop dying? To what do you attribute the stop in the death rate?

Ask if the beekeeper would be interested in interviewing a couple of other beekeepers.
The more responses we get the more information we have.

Thank you.