COLONY COLLAPSE DISORDER WORKING GROUP
PATHOGEN SUB-GROUP PROGRESS REPORT
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This document is intended to act as an "update" on a portion of research efforts of some of the members of the CCD working group. In large part it summarizes information that was provided to the participants invited to attend the Colony Collapse Disorder Workshop held April 23-24 2007 in Beltsville MD.

BACKGROUND INFORMATION

CHRONOLOGY OF AN EMERGING CRISIS:

During October-December 2006, beekeepers became alarmed that honey bee colonies were dying suddenly across the continental United States. Beekeepers reported losses of 30-90%. Subsequent investigations suggested that these outbreaks of unexplained colony collapse may have been occurring for 3 or more years. Responding to a report of bee loss in California in late 2005, ARS had sent a team of scientists from Beltsville to take samples of bees. And, even earlier, in 2002 and 2004, ARS had responded with site visits after claims of bee loss in Alabama and Minnesota, respectively. There was no discernable common cause of bee mortality, and the mortality was isolated, not extensive.

- 2002-2006:
    - Varroa Mite Crisis; Hints of a Problem Beyond Varroa
    - ARS Site Visits to Alabama, California, Florida and Elsewhere
    - National Academy of Science Study on Pollinator Decline (released 2006)
    - Mini-area wide Project for Almonds in California (Summer 2006)

- Fall 2006-Winter 2007: Crisis Emerges
    - Working Group Formed,
    - CCD Symptoms Defined:
        - Rapid loss of bee colony's population with very few bees found near colonies, as evidenced by a large amount of brood and insufficient bee coverage (see Fig 1 and 2).
        - Laying queen present is accompanied by few apparently young attendant bees.
        - Honey and pollen present and remains not consumed by secondary invaders
    - Questionnaires sent to Beekeepers
    - CCD affected colonies sampled across the U.S.
    - Sample analysis initiated

In early 2007, ARS teamed with university scientists and state departments of agriculture to form a Colony Collapse Disorder Working Group. The first task was to sample affected colonies. Initial sample analysis revealed a large number of disease-causing organisms, with most associated with "stress-related" diseases (*Nosema*, European foulbrood, and others), but no

specific cause was determined. The magnitude of detected infectious agents in adult bees suggested some type of bee immunosuppression. Sample analysis is on going.
A comprehensive history of colony collapse over the last century has been prepared and will be published in an upcoming issue of Bee Culture magazine.

FOCUS AREAS ON POSSIBLE CAUSES OF CCD:
Theories about the cause or causes of CCD include infection by bacteria, fungi, viruses, spiroplasmas or new pathogens such as a new Nosema (related to the microporidian giardia), the invasive varroa mite and pesticide poisoning (particularly by neonicotinoids such as imidacloprid). Stresses include poor nutrition (due to apiary overcrowding, pollination of crops with low nutritional value, or pollen or nectar dearth) and migratory stress brought about by the increased need to move bees long distances for pollination. Stress could compromise the immune system of bees making colonies more susceptible to disease. While CCD could be caused by a single factor it is also possible that multiple factors are working together to cause colony loss. Exploring the interactions between causative agents for CCD is not a simple task.

CURRENT STATUS:
The analysis of samples collected from across the country is ongoing. The ARS Beltsville Bee Laboratory in conjunction with Penn State is exploring the known pathogens and pests and trying to rule them in or out as possible causes of CCD. A series of samples is being analyzed by Penn State and Pennsylvania Department of Agriculture to document the prevalence of unusual fungi and other pathogens in adult bees. In collaboration with Penn State, Columbia University is determining if new pathogens are present by performing high through put sequencing of CCD bees versus healthy bee colonies, using novel methods. To examine affected colonies for exposure to stress, pathogens and pesticides a series of samples is being simultaneously tested using four different diagnostic tools; the Univ. of Illinois is using a whole bee genome array; the Beltsville Bee Lab is using a bee health array to screen for exposure to the known bee pathogens; and Penn State will be quantifying new organisms identified by Columbia, and the Pennsylvania Department of Agriculture is conducting autopsies on diseased bees to document gross pathology. All of the aforementioned screenings tools could provide evidence for exposure of CCD colonies to specific classes of pathogens, stress and pesticides (please see http://maarec.cas.psu.edu/ website for additional information). Pesticide analysis of samples from each colony is being initiated by Penn State, first with analysis of bee bread with analyses of wax and brood to follow. The combination of results from these tests will help to focus future research. Even in the absence of this information, studies are underway or in the planning stage to examine migratory stress, nutrition, and various combinations of factors. Future experiments will be refined from the discussions at the workshop and as sample results become available in the spring and summer of 2007.

SAMPLING AND RESULTS TO DATE:
Sampling of affected hives has been accomplished in beekeeping operations from at least 10 states (see Fig 3 and 4). Samples include a 300 adult bee sample taken in alcohol from the brood nest area, 100 adult bees frozen on dry ice and held at -80 and sections of comb containing brood, honey and pollen. Sampling of control colonies, in apparent good health, has occurred

within the same beekeeping operations, in beekeeping operations near affected apiaries, or apiaries far from any reported collapse (i.e. Hawaii).

Three surveys of beekeepers have been undertaken, each with a different focus. One is a detailed questionnaire of a beekeeper's management practices that attempts to explore in detail all aspects of affected and non-affected beekeeping operation (Penn Dept. of Ag.). The second has been collected online and with printed questionnaires and explores a wide range of questions with affected beekeepers (Bee Alert Technologies Inc., results available at: Beesurvey.com). The third was recently completed by the Apiary Inspectors of America and results are now available at (MARREC.org). Survey results indicate a higher than expected loss of colonies in the 2006-2007 season. One item of interest is that these surveys attempted to separate the normal "background" noise of winter loss due to parasitic mites and starvation from other losses that the beekeepers themselves felt could not be explained by causes known to them through experience in their respective climates.

PARASITIC MITE, NOSEMA AND PATHOGEN LEVELS:

The results from dissections of 16 adult bees per colony for tracheal mites revealed that only one of the beekeeping operations had any significant levels of tracheal mites. This same operation from the Pacific Northwest had high tracheal and Nosema levels when sampled in 2004 as well. Tracheal mites were either not detected or at low levels in the remaining beekeeping operations. Nosema levels were very variable with individual colonies having high levels while other colonies in the same operation no Nosema was detected. The variation between colonies was similar for colonies rated strong or weak. Thirty bees per colony were used as the sample size.

Varroa mite levels are given below in the figure below. Adult bee samples were weighed to estimate the number of adult bees per sample; varroa counts were adjusted to reflect number of mites per 100 bees. The Varroa levels between the weak and the strong colonies did not differ and only a very few colonies in each group had high Varroa levels. These Varroa levels are well below what is considered an economic injury level. While we have not ruled out Varroa as a contributing factor, the brood patterns did not present characteristic bee parasitic mite syndrome (BPMS) symptoms and coupled with the low mite levels indicate that Varroa was not the leading cause of the loss of colonies for these particular beekeeping operations. As stated before, Varroa mites continue to be a threat and surely some losses this year have been as a result of high mite levels. However, with the samples collected from colonies in CA (see figure 4) Varroa mites levels do not explain the sudden loss of adult bees in these colonies. Further, initial examination of capped brood for mites, found no evidence of high mite loads.

The pathogen levels in adult bees from CCD colonies appear unusual (see Table). The table shows infection prevalence in live adult bees taken from multiple colonies from three operations having CCD symptoms and demonstrates a high number of disease organisms found in each bee at high prevalence rate in the operations. In particular, the high prevalence of fungi in adult bees seems indicative of stress or a compromised immune system; these symptoms have never been previously reported.

OTHER PROJECTS INITIATED

Two other projects have been initiated:

1) Equipment re-use from dead out colonies.
2) Year round monitoring of migratory sentinel hives

Penn State, USDA-ARS, NCSU, Penn. Dept. Ag., and the FL Dept. Ag. are all collaborators in these studies. Funding has been provided by he National Honey Board, NEIPM, numerous individual beekeepers and beekeeping organizations, and in-kind donations from collaborating institutions and beekeepers.

EQUIMENT RE_USE FROM DEAD OUT COLLONIES Preliminary results

In February 2007, 200 Australian packages were installed on equipment originating from colonies collapsed, presumably dieing from CCD. Comb from dead out colonies was either fumigated with acetic acid, irradiated, or left untreated before packages were introduced. Packages were also introduces onto drawn comb that had only been used in honey supers. Early preliminary results appeared to document a weakened state in packages installed on untreated comb (Table 2) as compared to irradiated comb.

Figure 1 and 2: The rapid loss of adult honey bees is a defining feature of CCD. Here evidence of large sheets of brood are evidence that a large population of bees populated this colony in the last 14 days. The sparse remaining bee population appears to be mostly young adults. (Photo Credit: Nathan Rice, USDA-ARS, 2007)

Figure 3 and 4: Comprehensive samples from beekeepers in at least 10 states were collected. Samples include a 300 adult bee sample taken in alcohol from the brood nest area, 100 adult bees frozen on dry ice and held at -80 and sections of comb containing brood, honey and pollen. Sampling of control colonies, in apparent good health, has occurred within the same beekeeping operations, in beekeeping operations near affected apiaries, or apiaries far from any reported collapse (ie. Hawaii) (Photo Credit Dennis vanEngelsdorp, PSU/PDA 2007).

Figure 4:

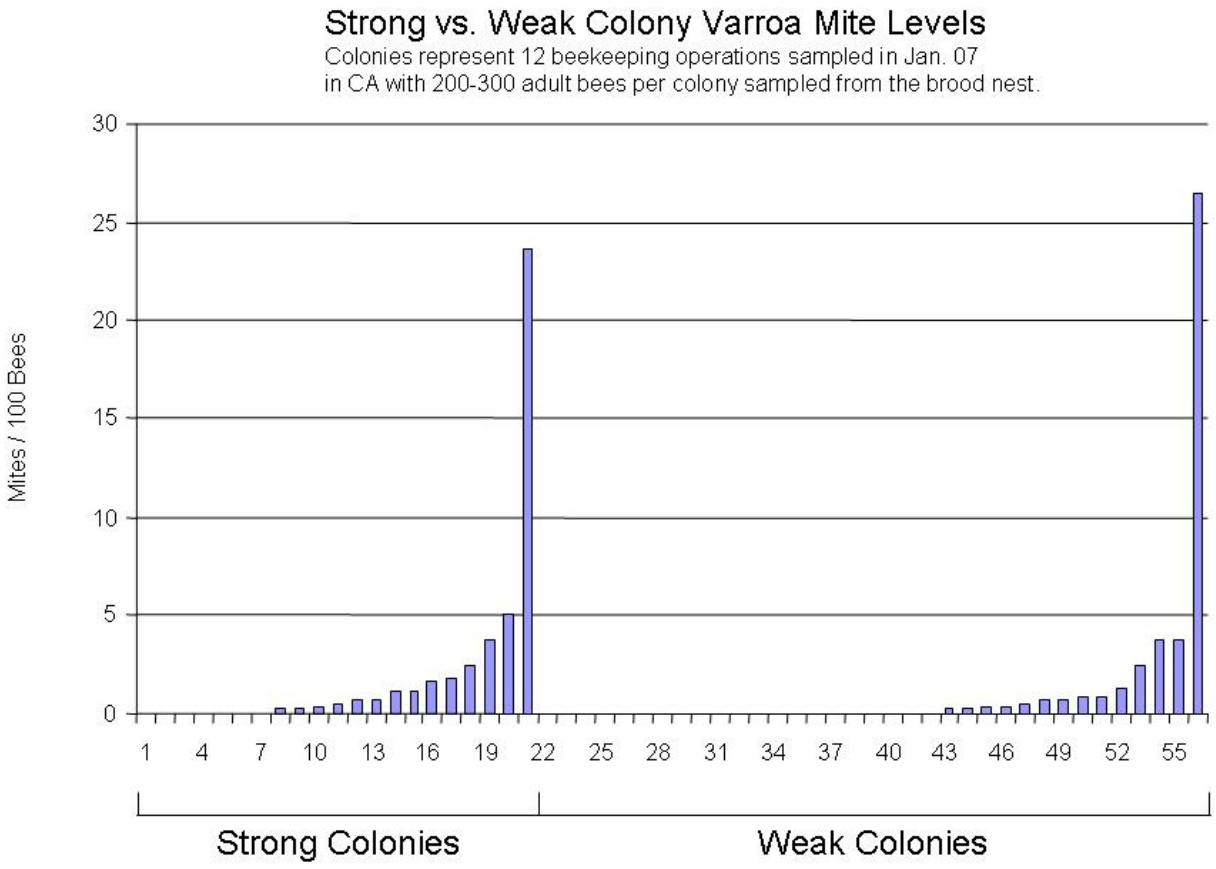
Strong vs. Weak Colony Varroa Mite Levels
Colonies represent 12 beekeeping operations sampled in Jan. 07
in CA with 200-300 adult bees per colony sampled from the brood nest.
Mites / 100 Bees
30
25
20
15
10
5
0
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55
Strong Colonies
Weak Colonies

Table 1. Infection prevalence in live adult bees taken from multiple colonies from three operations having CCD symptoms demonstrates a high number of disease organisms found in each bee at high prevalence rate in the operations. Several of the organisms are known to be "stress related diseases, in particular the bacterial infections and the fungal infections. The fungal infections in the live adult bees do not include chalkbrood. Diseases were detected using specific RT-PCR methods. Operations X, Y, and Z have no known connections and different localities.

		Viruses					Bacteria	
Operation	**# Bees**	**DWV**	**KBV**	**SBV**	**BQCV**	**Fungi**	**AFB**	**EFB**
X	11	11	6	10	10	8	0	0
		100%	55%	91%	91%	73%	0%	0%
Y	21	19	1	3	17	17	15	6
		90%	5%	14%	81%	81%	71%	29%
Z	10	10	7	5	9	7	1	0
		100%	70%	50%	90%	70%	10%	0%

Table 2: The mean number of frames of bees, frames with brood, and percent of cells missing in colonies established from packaged bees that were installed on comb from dead out colonies that was either left untreated, honey comb, fumigated with acetic acid, and irradiated with gamma radiation.

Comb Type	Frames of bees	Frames w/ brood	Missing Cells
Untreated	7.7 ± 3.0	6.1 ± 1.8	24.1 ±13.3
Honey Comb	8.3 ± 3.1	6.1 ± 1.7	17.4 ± 5.6
Acetic	9.5 ± 2.3	6.9 ± 1.8	19.7 ± 5.9
Irradiated	8.8 ± 3.3	6.5 ± 1.9	15.8 ± 4.5