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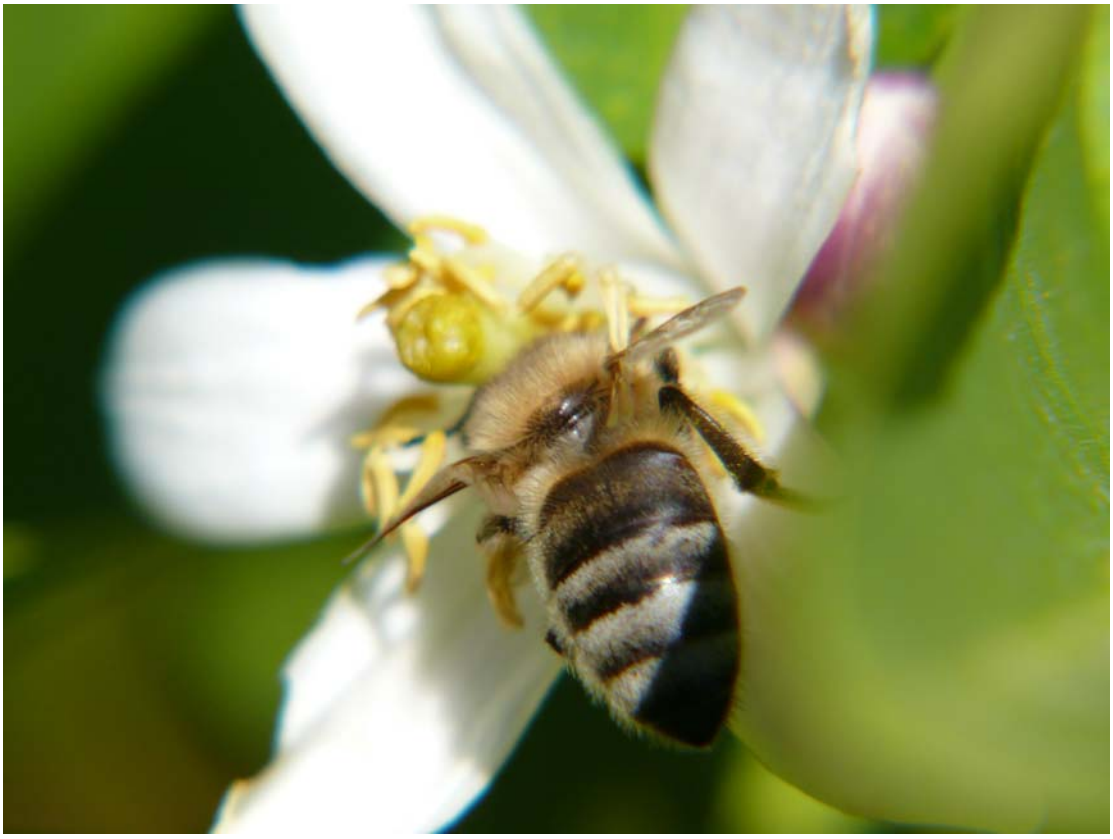
# Report of the Study Tour to Investigate the Occurrence of Colony Collapse Disorder in the Honey Bee Industry within the United States



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# Executive Summary

It is clear that during the fall and winter of 2006, the United States honey bee industry experienced a major mortality event with approximately 25% of bees being lost. Losses for individual beekeepers varied from 30 – 90%. This mortality event has been named Colony Collapse Disorder (CCD). What is less clear is the cause of the mortality event. In a normal year, the industry expects to lose only 15 - 20% of its bees over the winter period.

During the course of the study tour, the group spoke with a number of experts who expressed a diverse range of opinions for the cause of the mortality event. Members of the Colony Collapse Disorder consortium (core members - USDA Beltsville laboratory, Penn State University and several industry members) expressed strong opinions that CCD is a new phenomenon and, probably an infectious disease.

Conflicting opinions where presented by other scientists, notably Dr Robert Danka of the USDA Honey Bee Breeding, Genetics and Physiology Laboratory at Baton Rouge. Dr Danka believes that the exact same phenomenon has been observed previously throughout the past 50 years and that all colonies with high mortalities that he has personally inspected have a reasonable alternative explanation for the mortalities. Dissenting opinions were also expressed by Dr Marla Spivak of the University of Minnesota and Dr Gene Robinson of the University of Illinois. Alternative explanations for the mortality event have included the impact of Varroa mite, pesticides and bad management practices compounded by the development of resistance to coumaphos in Varroa mites. Resolution of the actual cause of the 2006 mortality event will require the results of further studies currently being undertaken in the USA.

The recommendations of the study group are:

1. Continuation of a watching brief on the latest developments in determining the cause of the 2006 mortality event.

2. Cooperation with the CCD consortium members in the exchange of samples and conduct of collaborative studies.

# Introduction

In November 2006, members of the United States honey bee industry reported unusually high mortalities amongst their bees and described a syndrome characterised by the rapid loss of bees from the hive without the accumulation of dead bees within or surrounding the hive. The mortality was further characterised by the unusual finding that the hives with this syndrome did not suffer from predators such as the small hive beetle (*Aethina tumida*) or wax moths (*Galleria mellonella*). Approximately 23% of beekeepers were affected and they lost an average of 45% of their bees. The mortality event was subsequently named colony collapse disorder (CCD). The occurrence of CCD has received substantial coverage in the US and Australian media.

In the Senate Estimates hearings of May 2007, senior members of the Australian Government Department of Agriculture, Fisheries and Forestry were strongly encouraged to send a study group to the USA to explore the phenomenon of CCD, hold discussions with a range of experts in the field and identify potential threats to the Australian honey bee industry. A team of three visited the USA from 21 June to 2 July 2007 to conduct the study.

# Group Membership

The study group comprised three members:

1. Dr Iain East, Epidemiologist, Office of the Chief Veterinary Officer, DAFF.
2. Dr Denis Anderson, Principal Research Scientist, CSIRO Division of Entomology.
3. Ms Paula Dewar, beekeeper and queen bee breeder, Dewar Apiaries.

For meetings with the USDA and APHIS held on 25 June, Drs East and Anderson were joined by Dr Rob Williams, Agricultural Counsellor, Embassy of Australia, Washington DC.

## Itinerary

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| 21 June | Depart Australia – Travel to Champagn-Urbana. |
| 22 June | Meetings with:<br>i. Prof. Gene Robinson, University of Illinois<br>ii. Prof. Hugh Robertson, University of Illinois |
| 23 June | Travel to Washington DC |
| 25 June | Meeting with:<br>i. Dr Jeff Pettis, Bee Research Laboratory, USDA<br>ii. Karen Ackerman, Trade Director, APHIS, USDA<br>iii. Prof. Diana Cox-Foster, Penn State University |
| 26 June | Travel to Minneapolis<br>Meeting with Prof. Marla Spivak, University of Minnesota<br>Travel to Ames, Iowa |
| 27 June | Attendance at the 9th International Pollination Symposium on Plant-Pollinator Relationships – Colony Collapse Disorder Workshop.<br>Meeting with Dr Robert Danka, Honey Bee Breeding, Genetics and Physiology Laboratory, USA<br>Travel to Minneapolis |
| 28 June | Travel to Penn State University |
| 29 June | Meeting with:<br>i. Dr Jeff Pettis<br>ii. Prof. Diana Cox-Foster<br>iii. Dr Ian Lipkin, Columbia University<br>Field trip to Hackenberg Apiaries |
| 30 June | Travel to Australia |

# Discussions with Gene Robinson, University of Illinois

On the 22nd June, Drs East and Anderson met with Prof. Gene Robinson, head of the Bee Research Laboratory at the University of Illinois. Prof. Robinson has been cooperating with the CCD consortium through collaborating on studies examining the effect of CCD on gene expression in the bee using microarray technology. Three populations of bees are available, these are:

i. Bees with no known history of disease
ii. Remnant bees from hives affected by CCD
iii. Bees from hives believed to be in the early stages of CCD

Although, Gene's primary interest is in neural development, the study will not be done on gene expression in brain tissue. The changing dynamics of the population as bees are lost due to CCD will result in changes in gene expression in the brain as bees change their role in the hive eg. older foraging bees will need to work as nurse bees. Existing studies have shown that a change of role in bees is accompanied by biochemical changes including changes to lipid levels in the abdomen and changes in the levels of some hormones.

The gene expression studies to be undertaken will utilise a 'chip' populated with genes expressed in gut tissue. This may be opportunistic as being the only other set of genes available other than the brain 'chip'. The microarray analysis had not been completed at the time of the visit and results were not available.

A further point of interest in discussions with Gene was the fact that a resurgence is occurring in feral bee populations in the USA after 10 years of decline since the introduction of Varroa. It was not known whether feral colonies had been impacted by CCD.

Gene believed that CCD was a new phenomenon and not ‘spring dwindle’ because the bees disappeared more rapidly than previously observed during ‘spring dwindle’ events.

Gene was hosting a visiting professor from the University of Tennessee who indicated that he was not convinced that CCD was a new phenomenon and was not aware of any cases of CCD in Tennessee.

# Discussions with Jeff Pettis – United States Department of Agriculture

Dr Jeff Pettis is Research Leader at the Bee Research Laboratory in Beltsville, MD. Drs East and Anderson were accompanied by Dr Rob Williams, Veterinary Councillor, to discussions with Dr Pettis and his staff at their Beltsville laboratory on the 25th June. Dr Pettis' team included Dr Yanping (Judy) Chen and Dr Jay Evans. Mr Danny Weaver, a major apiarist was also present during the discussions.

The group was particularly interested in collaborating with Denis Anderson due to his extensive experience investigating bee diseases and his extensive knowledge of bee parasites particularly Varroa. Denis indicated that there was potential to collaborate but that his laboratory had no funding for this work and that collaboration would be difficult without a source of funding. Dr Pettis indicated that the possibility existed to include Denis as a collaborator on some of his grant applications and thus provide funding to Denis if the applications were successful.

The discussion then became more wide ranging and covered several aspects of the suite of work that has been done within the CCD portfolio. Pesticide levels in hives had been measured and several hives had levels of sufficient concern that the Food and Drug Administration had been informed of the results. There had been no consistent differences in pesticide levels between hives affected with CCD and unaffected hives. An opinion was also expressed that if the die off of bees was due to pesticides then, dead bees would be observed in the vicinity of the hives.

Dr Pettis also spoke about the finding of *Nosema ceranae* in imported Australian bees. Samples obtained from the Australian bees were predominantly *N. apis* but *N. ceranae* had also been detected. Denis reported that all Australian samples

characterised by himself and Michael Hornitsky of NSW Agriculture were *N. apis* only. Dr Pettis offered Denis the sequence of Judy Chen's primers for Nosema – both generic and species specific – to assist Denis in his work.

Danny Weaver offered some insight from a beekeeper's point of view and indicated that bees were leaving the almond orchards after three weeks of pollination work in poorer condition than when they commenced at the almond orchards. This was previously unheard of. Whether this was a symptom of the CCD syndrome was uncertain however, Danny raised the issue of the impact of imidacloprid, an insecticide that has been the subject of considerable controversy in France over whether it has a deleterious impact upon bees foraging on crops where it has been used. Despite a lack of evidence, political pressure has resulted in bans on the use of imidacloprin based insecticides in France. No evidence was provided to suggest that Imidacloprid had been implicated in CCD in the USA.

In mid-afternoon, the group was joined by Prof. Diana Cox-Foster from Penn State University and several staff members of APHIS including Trade Director, Karen Ackerman and Senior Entomologist, Dr Wayne Wehling.

Prof. Cox-Foster provided a briefing on aspects of the studies conducted to look at pathogens present in bee samples. Whilst fungi of the genus *Mucor* had been found associated with CCD affected hives, the association with fungi of the genus *Pandora* was no longer thought to be an issue. *Pandora* had not been found in the samples of Royal Jelly from China although a range of other fungi had been isolated from the Chinese samples.

Denis then raised the issue that they had not looked at the full range of potential viral pathogens and that one particular virus, cloudy wing virus (CWV), was known to be a primary pathogen. There was acknowledgment from the Americans that they had not

considered CWV because they had no sequence data for it. Denis agreed to supply some material to Ian Lipkin at Columbia to allow for determination of the sequence of CWV. Denis also agreed to supply antisera against CWV and bee virus X and bee virus Y to aid the Americans in their studies. Denis also suggested that the American studies would be aided by studies in basic bee pathology. The Americans agreed but appeared to indicate that they lacked the necessary expertise.

Prof. Cox-Foster foreshadowed the visit to Dave Hackenburg's apiary undertaken on 29 June and indicated that in this instance, there were hives affected by CCD in close proximity to hives unaffected by CCD suggesting that the causative agent is not highly contagious or easily spread.

# Discussions with Marla Spivak, University of Minnesota

Drs East and Anderson and Ms Dewar met with Marla Spivak and two of her staff. Marla's strong belief is that CCD is not a new phenomenon but that the most likely cause is the Varroa mite and incorrect management of the Varroa mite. Marla also believes that current management practices are not optimal and that the bees often suffer from a low quality and low diversity of pollen. Corn and soybean pollen are useless for honey bees and without sufficient pollen of good quality, the bees cannot make brood food for feeding larvae.

Varroa presents several problems because as well as being a direct parasite, it acts as the vector for spreading deformed wing virus and acute paralysis virus.

The only available miticide treatment for bee hives involves a narrow treatment window between the end of the honey flow and the winter shutdown of the colony. This treatment also weakens the bees and they are thus less likely to be able to over winter successfully.

# The Colony Collapse Disorder Workshop

The Colony Collapse Disorder Workshop was held as part of the 9th International Pollination Symposium on Plant –Pollinator Relationships at Iowa State University, Ames Iowa on 27 June 2007.

The four speakers in the symposium were:

i. Dr Robert Danka, United States Department of Agriculture, Baton Rouge, LA.

ii. Prof. Marla Spivak, University of Minnesota, Minneapolis.

iii. Dr Jeff Pettis, Unites States Department of Agriculture, Beltsville, MD.

iv. Prof. Diana Cox – Foster, Penn State University.

The abstract for each presentation is included as Appendix 2. In addition to their presentations at the workshop, the study group spoke to each presenter individually. In general, the four presenters provided more up-to-date, more detailed information in the private conversations than they did in their workshop presentations.

## Bob Danka

Dr Danka stated that there had been no CCD in Louisiana to date. He indicated that the industry lost a lot of commercially managed bees each year and that this had been happening for many years and that the situation was getting worse each year. One grower known to Dr Danka lost 30% of his bees each year and these losses were related to the intensive pollination industry.

Dr Danka believes that CCD may exist but that the major losses in the industry are due to Varroa. Dr Danks believes that the solution to these losses will be through the development of parasite resistant stocks. The rest of his talk was a review of the three known lines of Varroa resistant bees:

i. Minnesota hygienic

ii. The Varroa sensitive hygiene line

iii. The Russian strain.

## Marla Spivak

Prof. Spivak presented a historical review of the bee industry and the major changes that had taken place since 1945. The arrival in the USA of a series of diseases (chalk brood, Nosema etc) hampered the industry as did increasing use of pesticides. Tracheal mite arrived in 1984 and Varroa in 1987 and both have had major impacts on the industry. Varroa also transmits a number of viruses including deformed wing virus and acute paralysis virus. Varroa has now developed resistance to apistan and coumaphos and thus treatment for Varroa is more complex and can only be completed in the window between the end of the honey flow and winter shutdown of the hives. The industry is under stress from a number of sources. Artificial feeding of bees on brewers yeast, soy flour and corn syrup to sustain them during their time in holding yards prior to and after transport does not provide high quality nutrition.

## Jeff Pettis

Dr Pettis reviewed the industry and the decline that has occurred in the last 60 years due to disease. The major losses have been since the 1980's when tracheal mite and Varroa were introduced into the USA.

Apiary inspectors expect winter to result in the loss of 17-20% of the bees in the industry. In 2006-07, the industry lost an estimated 25% overall with some beekeepers losing up to 90%. These losses are occurring against a background of many diseases and parasites.

Dr Pettis believes that the 2006-07 mortalities had a unique set of new symptoms not previously seen. He stated that he believes a range of primary stressors that may

include Varroa, transport, nutrition or pesticides make the hive susceptible to a secondary infection with an uncharacterised pathogen.

Some historical die-offs that have occurred in the industry have had similar symptoms. Dr Pettis admitted that people investigating CCD suffered from a lack of good data on incidence of CCD and identification of hives experiencing CCD.

## Diana Cox – Foster

Prof Cox-Foster's studies had included examining hives with and without CCD for the presence of a range of pathogens. No results were presented although Prof. Cox-Foster did indicate that multiple viruses are normally found in bees as a latent or persistent infection with no overt symptoms or pathology. Wide ranging studies examining the potential causes of CCD were foreshadowed. In some cases, high levels of pesticides were measured in the bees and hives. There was however, no consistent association between and class of pesticides and the occurrence of CCD.

## Panel Discussion

During the panel discussion, a representative of the onion seed industry stated that none of the beekeepers servicing his industry has reported CCD.

# Discussions with Robert Danka

Dr Danka does not believe that CCD is a new syndrome and quoted some examples of reports of major bee losses from the 1960's where the symptoms were virtually identical to those reported for CCD. One of his industry contacts kept some hives in Louisiana year-round and these hives did much better than the hives used in pollination contracts that were moved eight times per year and only wintered in Louisiana.

In addition to the nutritional stress, Dr Danka believes that the movement of hives around the country due to significant changes in climate. Bees that finish the season in Maine or New York start to shut down because of the winter. They are then moved to Louisiana where the weather is much warmer and the bees become active again and start laying brood. When the weather gets cold in Louisiana, the bees are in very poor condition and do not survive winter well.

# Presentation by Ian Lipkin

During a visit to Penn State University at State College, we received a formal presentation from Dr Ian Lipkin, Director of the Jerome L. and Dawn Greene Infectious Disease Laboratory at Columbia University. Dr Lipkin is part of the CCD Consortium.

Dr Lipkin commenced his presentation with a review of molecular techniques available for disease surveillance including Mass Tag PCR, Greene Chips and pyrosequencing. The technology behind the 454 Pyrosequencer combined with novel bioinformatics (sequence matching and identification) software has been used to identify the pathogens present in bees taken from CCD affected colonies. This approach identified the presence of a range of pathogens in the samples. Standard PCR and sequencing was used to examine for the presence of the pathogens in a panel of CCD affected and normal hives.

# Discussions with Dennis Van Engelsdorp

During travel to Dave Hackenberg's apiary, Dr East had the opportunity to talk with Dennis Van Engelsdorp, Chief Apiary Inspector for the state of Pennsylvania. After recognition of the problem with CCD, Dennis collected eight hives affected by CCD from Dave Hackenberg's apiary and is maintaining them at his own home. At the present time, two of the hives have collapsed completely, two have recovered and are thriving and the remaining four are just holding their own.

Dennis indicated that they are now starting to conduct the necessary epidemiological studies including a longitudinal study of hives to provide a detailed hive history.

Further discussions suggested that APHIS would be considering the results obtained thus far by the CCD consortium and may review import policies based on their findings.

# Field trip to Hackenberg Apiaries

The study group visited Hackenberg Apiaries in Lewisburg, PA. The owner, Dave Hackenberg, was the first person to report the occurrence of unexpectedly high mortalities in his bee hives during the fall and winter of 2006-07. We were informed that the majority of the Hackenberg hives were out on site at a pollination contract but that the 80 hives present at the apiary were the poorest hives and were hives that had been identified as being affected by CCD. Upon inspection of the hives, a number of them had CCD chalked on the outside of the hive confirming that the members of the CCD consortium believed that these hives were affected by CCD. Upon inspection of the hives, it was clear that they were not strong hives and did not contain a large number of bees.

Upon inspection of these CCD affected hives, Denis Anderson and Paula Dewar identified a number of problems impacting on the hives. These included:

i. Significant chalk brood infestations

ii. Colonies lacking queens

iii. Colonies that had substandard or under-performing queens

In summary, Denis and Paula believed that the poor condition of the inspected hives could be explained by factors other than CCD.

## Conclusions and Recommendations

During the study tour, it became apparent that all people interviewed were in agreement that during the fall-winter of 2006-07 the US honey bee industry lost significantly more bees than would be expected in a normal winter. The cause of the abnormally large number of mortalities has not been identified however, the mortality event has been named colony collapse disorder (CCD). There was also general acknowledgement that a proportion of the hive losses attributed to CCD may well have been caused by other problems and that bee keepers were demonstrating a "band wagon" effect in their search for reasons for loss of hives.

A large and capable group of scientists from a range of institutions together with honey bee industry members have come together to form the CCD consortium. This group has completed a substantial amount of work investigating CCD. There are gaps apparent in the CCD consortium's studies including a complete lack of any pathology results from hives affected by CCD. Whilst this was attributed to the fact that bees from hives affected by CCD disappeared, it seems apparent that the consortium does not have access to appropriate pathology skills.

The problem with the case definition was encountered throughout the study tour. Upon questioning, doubt was placed over the seasonal timing of CCD and the lack of robbing by small hive beetle and wax moths was attributed by at least one scientist to CCD occurring in winter when these insects were not normally active. Thus, the case definition reduces to "hives that lose a lot of bees quickly", patently an inadequate description to be of any use.

Dr Anderson was also concerned that a range of simple experiments that would examine the performance of the queen in infected hives had not been conducted and this was reinforced during the field trip to Hackenberg Apiaries where the "CCD

affected hives" examined by Dr Anderson were suffering from a range of identifiable problems including the fungal disease chalk brood, absence of queen bees or the presence of underperforming queen bees.

Dr Anderson was also concerned that, due to a lack of available sequence data, the CCD consortium members had not considered the possible involvement of the cloudy wing virus (CWV) in causing CCD. CWV is one of the few bee viruses that can act as a primary pathogen causing clinical disease in the absence of precipitating or predisposing factors. Dr Anderson has agreed to provide Ian Lipkin with a sample of CWV so that the sequence can be determined.

It is the conclusion of the study group that, based on the evidence presented, it is not possible to reach a meaningful conclusion as to the cause of CCD and whether it is a new phenomenon or not. The study group recommends that:

1. Continuation of a watching brief on the latest developments in determining the cause of the 2006 mortality event.
2. Cooperation with the CCD consortium members in the exchange of samples and conduct of collaborative studies.

# Comments on industry hygiene

During the course of discussions, the practice of restocking empty hives was raised. Apparently, standard practice is to place old empty hives on the top of existing hives as ‘supers’ and allow the bees to colonise and expand into the empty hive. No cleaning of the old hive is undertaken and the old hive includes frames with the honeycomb beeswax from the old hive and in some cases, stored honey and pollen from the old hive. This is done because the construction of honeycomb on new frames would require substantial energy expenditure by the bees and this would direct efforts away from storing honey.

The CCD consortium is undertaking studies on the effect of irradiating CCD affected hives prior to reuse. Results are not yet available because the studies are on-going.

In virtually every other sector of primary industry, the effective cleansing of housing prior to restocking is seen as standard practice. Numerous studies have shown the value of all-in, all-out stocking and cleansing of the housing between batches. It is strongly recommended that the bee industry investigate options for the cleansing and/or sterilising of empty hives prior to their re-use.

# Appendix 1 – Abstracts of Papers Presented at the Colony Collapse Disorder Workshop.

# Sustaining and Exploiting Genetic Variation in Honey Bee Pollinators

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Honey bees continue to play an indispensable role as pollinators in U.S. agricultural systems. Biological and economic challenges to the beekeeping industry, however, have significantly decreased the number of managed colonies available for pollination during the past few decades. This threat underscores the need for methods to help maintain colony numbers and the diversity of honey bee germplasm [1].
The principal challenge is to keep colonies alive and healthy when threatened by parasitism from the mite Varroa destructor Chemotherapy is a first line of defense but has recognized drawbacks such as mites developing resistance to acaricides. Long-term sustainability of honey bees is expected to come in the form of bees bred to have reliable, economically useful levels of genetic resistance to mites. Work in the United States recently has produced three types of honey bees with resistance to V destructor One stock of bees was developed primarily for enhanced general hygiene and has moderate resistance to V destructor [2]. These bees are based on an Italian stock of honey bees and so are expected to have desirable pollination-related characteristics, e.g., the tendency to form large colonies early in the year when much crop pollination occurs.
Two breeding efforts by the USDA yielded bees with significant resistance to V destructor One effort identified and enhanced a specific heritable trait of bees-"varroa sensitive hygiene"-which is available to bee breeders for incorporation into any desired stock of bees [3]. The intention is that the trait can be added without compromising the existing suites of desirable characteristics and genetic diversity in whatever base stocks are chosen. New commercial breeding products with varroa specific hygiene will need to be assessed for pollination performance as they are released to the beekeeping industry.
A USDA-developed mite-resistant stock of bees that originated in the Far East of Russia is the best studied with regard to pollination attributes [4]. Several tests have examined the comparative flight activity of these bees in commercial pollination settings. Flight characteristics of Russian colonies compared favorably to those of Italian colonies during the late spring and summer pollination period of lowbush blueberries and upland cotton [5, 6]. During late-winter (February) pollination of almonds in California, Russian bees had flight activity similar to Italian bees when bee populations and other significant environmental variables (temperature and time of day) were the same for the two bee types [7]. However, Russian colonies on average were less populous than Italian colonies during this early season pollination, and so Russian colonies had less total flight. The early season limitation due to relatively small colony size is recognized as a management issue that warrants attention so that Russian bees meet the very large demand for colonies to pollinate almonds. Rates of pollen foraging generally were similar for Russian and Italian bees in each of the pollination settings that were examined. Initial studies of foraging behavior of individual bees indicate that Russian and Italian bees deposit equal amounts of pollen during single visits to flowers of rabbiteye blueberries and upland cotton [unpub. obs.].

Although the number of managed honey bee colonies in the United States is decreasing, the diversity of available germplasm is not yet well quantified. Importantly, there have been recent discoveries worldwide of honey bee strains that express enhanced potential for pollination of specific crops. These include bees with preferences for foraging on apples [8], avocados [9], and sunflowers [10]. The ability to select honey bees for increased pollen collection and hoarding also is well documented [11]. Thus, if the general health of honey bee populations can be maintained, there appear to be opportunities to use existing genetic diversity in breeding to support agroecosystem pollination.

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# Honey Bee Colony Collapse Disorder in the United States

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During the fall of 2006, beekeepers in the United States became alarmed that honey bee colonies were dying in large numbers, with reported losses of 30-90% in some beekeeping operations. Subsequent investigations suggested that these outbreaks of unexplained colony collapse have been occurring for at least the last 2-3 years. The current phenomenon, without a recognizable underlying cause, has been tentatively termed "Colony Collapse Disorder" (CCD), and threatens the pollination industry and production of commercial honey. The almond crop alone in California uses 1.3 million colonies of bees, approximately one-half of all honey bees in the United States, and this need is projected to grow to 1.5 million colonies by 2010. Symptoms of CCD include sudden loss of the colony's adult bee population with very few bees found near the dead colonies; several frames with capped brood (see photo) indicating that colonies were relatively strong shortly before the loss of adult bees; food reserves that have not been robbed, despite living colonies in the area, suggesting avoidance of the dead colony by other bees; minimal evidence of wax moth or small hive beetle damage; a laying queen often present with a small, 100-bee, cluster of young attendants. Many affected beekeepers indicated that their colonies were under some form of stress at least 2 months before the first incidence of CCD. Stresses could include poor nutrition (due to apiary overcrowding, pollination of crops with low nutritional value, or pollen or nectar dearth), limited or contaminated water supplies, possible exposure to pesticides or high levels of Varroa mites. Results from tests conducted on dead and dying colonies along with control hives in apparent good health will be discussed.

Honey bee (Apis mellifera) colony from Georgia, USA, spring 2007, showing signs of colony collapse disorder (CCD); note abundant sealed brood on several frames and a lack of adequate adult worker bee coverage.

# Research Foci Addressing Colony Collapse Disorder

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These hypotheses are being addressed simultaneously via extensive collaboration among members of the CCD Working Group. We are sharing specimens, have agreed to share data, and are actively working toward resolving the causes of CCD. Funding to date has been provided by several beekeeper organizations, the National Honey Board, USDA, PDA, Penn State, and the Department of Defense (through SBIR funding to Bee Alert, Inc.); we greatly appreciate this funding for allowing us to begin addressing CCD. A summary of our activities follows.

Are there new or reemerging pathogens responsible for CCD?
It has become clear in recent years that many pathogens have the ability to impair the immune defenses of their hosts. Among the known bee pathogens in CCD bees, none have been identified as having immunosuppressive abilities. We have identified several routes of entry into the United States that may have permitted the inadvertent introduction of new pathogens. In collaboration with Dr. Ian Lipkin and associates at Columbia University and the Northeast Biodefense Center, we at Penn State are identifying the microbes and viruses associated with CCD colonies. We predict that any pathogens that may be linked to CCD will be found in multiple operations having CCD and will not be present in colonies lacking CCD. In this analysis, we will probably isolate many new organisms not previously known to be associated with bees. Determining which microbes are important and linked to CCD will require extensive study We will also need to investigate new methods to control or disrupt infections by these pathogens.
These studies are being performed in collaboration with Drs. Jay Evans and Jeff Pettis at USDA-ARS and with Drs. May Berenbaum and Gene Robinson at the University of Illinois. These collaborations are utilizing the newly developed knowledge of honey bee genomics and molecular physiology, to let the bees themselves tell us how they are being impacted and what are the most likely causal factors underlying CCD by asking what genes are being turned on and off in the bees. We expect that these analyses will reveal how the bees are responding to potential pathogens, environmental toxins, or other stressors.
Are environmental chemicals causing the immunosuppression of bees and triggering CCD? It is recognized that environmental toxins or pesticides can impair the immune systems of animals. In insects, sub-lethal effects of insecticides are being increasingly recognized as stressors that may impair immune defenses. Our surveys to date have failed to identify common chemicals or pesticides being used in the various beekeeping operations experiencing CCD. Bee Alert, Inc. is asking whether any environmental chemicals are present in CCD colonies by analyzing volatile chemicals in hives. At Penn State, international experts in environmental chemistry and toxicology (Drs. Chris Mullin, Ralph Mumma and others) are helping to direct the chemical analyses of the hive products. Wax, honey, and pollen stores will be analyzed for pesticides and other toxic compounds. Of particular concern are pesticides being widely used to control insect pests in agriculture, urban environments, and animal systems. Among these are the neonicotinoids, a class of

pesticides that have been extensively adopted for pest management. This class of pesticides is recognized as having extremely low toxicity in humans and other vertebrates and as highly effective in controlling insect pests; however, these chemicals are known to be highly toxic to honey bees and other pollinators. Some research has suggested that these systemic pesticides can translocate or move through plants to become localized in pollen and nectar at concentrations that may affect bees. Research is warrant¬ed to address the effects on the bees and other pollinators of these compounds at the concentrations found in pollen and honey made from nectar collected by the bees. It is essential to determine whether these pesticides play a role as a causal factor in the CCD symptoms.

Is a combination of stresses working together to weaken bee colonies and allowing stress-pathogens to cause final collapse? Several working group members (USDA-ARS, PDA, North Carolina State University, and Penn State) are collaborating to ask what stresses are encountered by bee colonies that are part of migratory operations. Recently, we are beginning to learn from migratory bee keepers that multiple stressors impact their operations and cause significant losses of honey bee colonies. Gaining this baseline information is important in determining how bees are being impacted and how these stresses can be eliminated to ensure adequate pollination of crops.

Finally, the CCD working group recognizes the importance of trying to breed honey bees that are more resistant to diseases and the impacts of parasites such as varroa mites. In addition, we anticipate that different genetic strains will respond differently to various stresses. Researchers at North Carolina State, University of Illinois, and Texas A&M are beginning to ask how genetic diversity in bee populations correlates with CCD and resistance traits. Developing new genetic strains of bees for commercial production may be essential to the future of beekeeping.

# The Perfect Storm: Setting the Stage for this Year's Loss of Honey Bee Colonies

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The aim of this talk is to give a general overview of the numerous pressures that are burdening honey bees and beekeepers in the United States, setting the stage for an understanding of this year's large loss of colonies.

Honey bees are maintained in man-made beekeeping equipment as a hobby, as a sideline profession, or commercially as a livelihood. Some beekeepers maintain their colonies in one location year-round, and some transport their colonies long distances to follow the blooming plants for honey and to provide pollination service to crops. Some beekeepers propagate "nursery" stock (more bee colonies and queen bees) in southern states, California, and Hawaii for sale around the country.

The beekeepers that transport their colonies across the nation to pollinate crops for our nation's food supply face extreme difficulties in supplying bees that are strong and healthy enough for pollination. Often, a large number of bee colonies are required to pollinate a particular crop. For example, over one million bee colonies are required to pollinate almonds in California during late February and March. Moving such large numbers of colonies into a relatively small area places stress on bees. In these conditions, the bees may become nutritionally stressed, they
may be at increased risk of pesticide exposure, and they are at increased risk of disease transmission among colonies.

Honey bees are subject to diseases and parasitic mites. Two mites, the tracheal mite Acarapis woodi, and Varroa destructor were inadvertently introduced into the United States in the 1980s. These maladies, alone or in combination, weaken colonies and can lead to the collapse and death of colonies. Viruses inherent at low levels in bees may be transmitted by V destructor leading to a host of secondary disease symptoms. Beekeepers are careful to control these pathogens and pests in their colonies, but it has become increasingly difficult because the diseases and mites have developed resistance to some of the treatments. New treatments are currently available that reduce the risk of the pests developing resistance, but these treatments must be applied during narrow windows of time and are not always effective in lowering mite levels below an economic injury level. When colonies are moved en masse for pollination, there is extreme horizontal transmission of mites and associated viruses and diseases, often negating the effects of previous treatments. Fortunately, colonies that die from diseases and mite parasites can be replaced with nursery stock produced in the United States. Since the last large die-off of honey bee colonies in 2004-2005, thousands of colonies of bees (called "packages") have been introduced from Australia to ensure adequate numbers of colonies are available for almond pollination. Bees from Australia are healthy, but they have never been exposed to A. woodi or V destructor, so are extremely susceptible to these parasites.

In addition to the stress that diseases and mites place on the immune system of bees, urban sprawl and agricultural practices have limited the amount of bee "pasture" available to the bees for their food. The use of pesticides on crops to kill pest insects can have the negative side effect of killing honey bees and other important bee pollinators. Many pesticide applicators choose pesticides with low residual and low

toxicity to bees. But new classes of pesticides, such as those that are systemic, may contribute to the stress on bees' detoxification systems because the pesticide may be incorporated into the pollen and nectar. While we know that pesticides can adversely affect bee health, genetically modified (GM) crops have not been shown to directly affect honey bees.

It is no wonder bees are collapsing. But the biggest question is: is the collapse of honey bee colonies this year due to yet another factor? Is there a new disease afflicting bees? Are the effects of new classes of pesticides contributing to bee deaths? What is the so-called Colony Collapse Disorder? At the time of this writing, it is unclear why so many bee colonies are dying, and the name Colony Collapse Disorder is a placeholder until its nature can be determined for certain. It is likely that the bees are dying from a number of contributing factors that collectively place an enormous burden on the immune and detoxification systems of bees, eventually "putting them over the edge." Subsequent talks by Dr. Jeff Pettis and Dr. Diana Cox-Foster will give more details about what is known about the cause(s) of colony collapse disorder.

Although the loss of honey bees is disturbing, the amount of press and attention given to honey bees this year presents a great opportunity to educate the public about the importance of honey bees, native bees, and all pollinators to our agro- and natural ecosystems.