Colony Collapse Disorder Action Plan

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Colony Collapse Disorder
Action Plan
CCD Steering Committee
June 20, 2007

EXECUTIVE SUMMARY

Colony Collapse Disorder (CCD), a significant disappearance of honey bee colonies that may be affecting bees in more than 22 states, threatens the production of crops dependent on bees for pollination as well as honey production. Pollination is responsible for $15 billion in added crop value, particularly for specialty crops such as nuts, berries, fruits, and vegetables. Of the 2.4 million colonies of bees in the United States, the almond crop in California alone requires 1.3 million colonies, and this need is projected to increase significantly over the next few years. The bee industry is facing difficulty meeting the demand for pollination in almonds because of bee production shortages in California. Consequently, growers depend increasingly on beekeepers from other states to transport honey bee colonies across the country to meet the pollination demand (a phenomenon known as migratory beekeeping). If researchers cannot find a solution to CCD, beekeepers will be unable to meet demand for this and other crops.

Current theories about the cause(s) of CCD include increased losses due to the invasive varroa mite; new or emerging diseases, especially mortality by a new *Nosema* species (related to the microporidian giardia); and pesticide poisoning (through exposure to pesticides applied for crop pest control or for in-hive insect or mite control). In addition to these suspects, perhaps the most highly-suspected cause of CCD is a potential immune-suppressing stress on bees, caused by one or a combination of several factors. Stresses may include poor nutrition (due to apiary overcrowding, pollination of crops with low nutritional value, or pollen or nectar dearth), drought, and migratory stress brought about by the increased need to move bees long distances to provide pollination services (which, by confining bees during transport, or increasing contact among colonies in different hives, increases the transmission of pathogens). Researchers suspect that stress could be compromising the immune system of bees, making colonies more susceptible to disease.

Following the ad hoc formation of a CCD Working Team (a rapid response group comprised of academic, private, and Federal scientists), the Department of Agriculture (USDA) took the lead in the effort to determine causes contributing to CCD. Specifically, USDA organized a two-day CCD Workshop in Beltsville, Maryland, for various apiculture experts to identify research gaps and priorities as well as measures required to address these needs. Based on information gathered at the Workshop, a newly formed CD Steering Committee, composed of Federal program leaders and Land Grant university scientists/administrators, identified critical research and response needs and developed an Action Plan.

Direction of research to bee decline and protecting bee health has been accompanied by considerable direction of Federal resources. In fiscal year (FY) 2007, ARS had a honey bee research budget of $7.4 million, the focus of research being on controlling the varroa mite pest and microbial pathogens and on improving honey bee nutrition. Between FY 2000 and FY 2006, the Cooperative State Research, Education, and Extension Service (CSREES) spent an average of $1.7 million per year on honey bee and pollinator research; roughly one third to one half of this funding was spent on research on honey bee health. Additional funds are now being directed by ARS' Areawide Integrated Pest Management program to conduct a full-scale areawide project on honey bee health in the amount of $1 million per year for the next 5 years. Meanwhile, CSREES has tapped $117,000 in unexpended funds from the Critical and Emerging Issues Program to provide seed grants for CCD. In addition, CSREES is tentatively planning to direct additional funds in FY 2008. Land Grant University Experiment Stations have committed to the support of a new Multi-state Rapid Response Research project administered by the North Central region through the Hatch Multi-state Research allocation. This project will begin in FY 2008 and will include scientists throughout the United States. Furthermore, extension specialists are active in every State and many have specific responsibilities to apiculture. Many of their activities are supported by Federal Smith-Lever appropriations to States for the Cooperative Extension System.

The current strategy for addressing the CCD crisis involves four main components: 1) survey and data collection; 2) analysis of samples; 3) hypothesis-driven research; and, 4) mitigation and preventative action. Within each component topic area in this Plan, we have outlined the current status of ongoing research and future plans needed to address the problem of poor honey bee health, as well as the organizations(s) that will be involved in the effort. Furthermore, this plan identifies many areas of research where specific expertise is lacking, and it recommends long-term capacity building in these areas, accomplished through the hiring of new scientists. Finally, as noted in a 2006 National Academy of Sciences study on the status of bee populations in North America, honey bee health has been in decline for several years – long before the appearance of CCD – and a concerted, well-funded research and extension effort is urgently needed to ensure the viability of these essential pollinators in U. S. agriculture.

Survey and Data Collection

Despite the existence of several surveys for both honey production and bee health, these surveys are either limited in scope, fundamentally flawed, or otherwise unable to provide an accurate picture of bee numbers or products (honey and pollination services). New surveys are needed to determine the extent of CCD in the United States and the current status of honey bee colony production and health. At a minimum, this process will require participation from the National Agricultural Statistics Service (NASS), and likely the Animal and Plant Health Inspection Service (APHIS).

Analysis of Samples

Researchers must collect and then analyze the bee samples collected. Presently, analysis is being conducted to determine the prevalence of various pests and pathogens, bee immunity and stress, and exposure to pesticides. These and other analyses will help researchers determine the exposure of worker bees to various toxins and pests and pathogens and potentially to identify any new pathogens. Various Federal agencies, universities, and private institutions will continue to expand on this work, with the goal of identifying and characterizing pathogens, pests, and pesticides or environmental contaminants that may be associated with CCD.

Hypothesis-Driven Research

The largest component of this Plan is research. Scientists have identified four categories of candidate factors based on the most reliable information available concerning what impacts bee health and on recent analysis of affected bees: 1) new or re-emerging pathogens; 2) bee pests; 3) environmental and nutritional stresses; and 4) pesticides. Research will focus on determining whether these candidate factors, or specific stressors within these categories, are contributing causes of CCD, either individually, in combination, or synergistically.

Mitigative and Preventative Measures

Since little is known about the cause(s) of CCD, mitigation, at present, must be based on improving bee health and habitat and countering known mortality factors. Goals identified under this topic include: developing general best management practices for honey bees and non-*Apis* bees; developing strategies to maintain bees with resistance to parasites and pathogens; improving the regulatory framework for better protection against pathogens, pests, and parasites; developing an Areawide Program to improve honey bee colony health; and developing Web-based sites for the dissemination of science-based information on bee health and CCD.

Acronyms and Definitions

AAPA	American Association of Professional Apiculturists
AIA	Apiary Inspectors of America
AMS	Agricultural Marketing Service (USDA)
APHIS	Animal and Plant Health Inspection Service (USDA)
ARS	Agricultural Research Service (USDA)
CCD	colony collapse disorder (an unexplained rapid die-off of honey bee colonies)
CRP	Conservation Reserve Program (NRCS)
CSREES	Cooperative State Research, Education and Extension Service (USDA)
DoD	U.S. Department of Defense
EPA	U.S. Environmental Protection Agency
EQIP	Environmental Quality Incentives Program (NRCS)
GMO	genetically modified organism
Hatch Fund	provides funding for Land Grant Universities (CSREES, States)
in vitro	testing done in a laboratory setting under artificial conditions outside the living host
in vivo	testing done in living organisms
IR-4	program that funds research on minor use pesticides (USDA, States)
MAAREC	Mid-Atlantic Apicultural Research Extension Consortium
NAS	U.S. National Academy of Sciences
NASS	National Agricultural Statistics Service (USDA)
Nosema	a microsporidian pathogen related to the fungi
NHB	National Honey Board (USDA)
NRCS	Natural Resources Conservation Service (USDA)
NRI	National Research Initiative (CSREES)
OIE	Office of International Epizooties (defines tests for pests and pathogens of animals)
PIPE	Pest Information Platform for Extension and Education (CSREES)
PSU	Pennsylvania State University
qRT-PCR	quantitative real time polymerase chain reaction (quantifies PCR product)
RMA	Risk Management Agency (USDA)
SARE	Sustainable Agriculture Research and Education (CSREES)
Smith-Lever	provides funding for Extension (CSREES)
USDA	U.S. Department of Agriculture
WRP	Wetlands Reserve Program (NRCS)
WTO	World Trade Organization

Key to Priority and Duration Designations for Each Objective:
Priorities are ranked: Urgent, Very High, High, and Medium in order of importance
Duration is ranked: Short (less than 1 year), Medium (1-3 years), Long (more than 3 years)

INTRODUCTION

Value of Bee Industry to U.S. Agriculture

Beekeeping is an essential component of modern agriculture, providing pollination services for over 90 commercial crops grown in the United States. The honey bee adds $15 billion in value to agricultural crops each year, and the demand for honey bees is growing. The California almond crop alone uses 1.3 million colonies of bees for pollination, approximately one half of all honey bees in the United States. Furthermore, this demand is projected to grow to 2.1 million colonies by 2012, a number nearly equal to all the colonies currently in the United States (2.4 million). Honey bees also provide a fundamental level of pollination that enables home gardeners to produce many of these same crops without having to be concerned about adequate pollinators, and, together with wild bees, honey bees play a critical role in many food webs that support wildlife. The importance of the beekeeper and managed bees is greater today than ever, because parasitic mites have destroyed most of the feral honey bees across the United States.

Bee Industry in Crisis

In early 2007, the National Academy of Sciences National Research Council's (NRC) "Status of Pollinators Committee" issued the findings of a two-year study detailing the serious problems facing the beekeeping industry, which was described as being in crisis mode. In brief, beekeeping has suffered several major setbacks during the last two decades:

- Invasive parasitic mites have decimated honey bee populations throughout the country, creating instability in the supply of bees rented for pollination and greatly increasing the costs of managing bees and of renting hives for pollination. Mite-related losses reached catastrophic proportions during the winters of 1995 to 1996 and 2000 to 2001, when colony deaths in the northern United States ranged between 50 and 100 percent in many beekeeping operations. Despite considerable efforts at both State and Federal levels, effective and sustainable controls have not been found for these mites. Pesticide (*i.e.*, miticide) resistance is a major problem that may be contributing significantly to losses.

- The Africanized honey bee has begun to move into regions of the country critical to the sustainability of the U.S. beekeeping industry. These areas, in the southern United States, are the major wintering grounds for migratory bees and the major source of queen and package bees purchased by northern beekeepers to replace high winter losses, which are high. Africanized bees out-compete traditional European bees in these areas and make it difficult to maintain pure lines of European ancestry. If germplasm from this highly-defensive race of bees becomes common in the commercial population, colonies will become less manageable, and liability issues for both beekeepers and growers may become significant.

- American foulbrood, the major bacterial disease affecting honey bees, has developed resistance to the antibiotic used to prevent it. Although an alternative compound is now available, it is only a matter of time until resistance to this compound develops.

- The small hive beetle is stressing bees in the southern U.S, and additional pathogens (viruses, bacteria, and fungi, including microsporidia) are causing extensive bee mortality.

- Insecticides used in crop protection have been associated with bee mortality.

- Cheap, imported honey has maintained strong downward pressure on prices paid to U.S. honey producers. Combined with increased production costs attributable to mites and disease, this has contributed to a reduction in the number of beekeepers and colonies, despite increases in rental income.

Colony Collapse Disorder: Perfect Storm for Beekeepers

During the winter of 2006 to 2007, beekeepers in the United States became alarmed that honey bee colonies were dying in large numbers, with reported losses of 30 to 90 percent in some beekeeping operations. While many of the colonies lost during this time period exhibited symptoms consistent with those typically observed when under attack by parasitic mites, as many as 50 percent of all colonies were reportedly lost, demonstrating symptoms inconsistent with mite damage, or any other known causes of death. This suggested that increased stress or a new, unidentified agent could potentially be responsible. This unexplained cause of death has been given the name “Colony Collapse Disorder,” or CCD. Subsequent investigations suggested that these outbreaks of unexplained colony collapse have been occurring for at least two years.

Symptoms of CCD include: (i) sudden loss of the colony’s adult bee population with very few bees found near the dead colonies; (ii) several frames with healthy, capped brood with low levels of parasitic mites, indicating that colonies were relatively strong shortly before the loss of adult bees and that the losses cannot be attributed to a recent infestation of mites; (iii) food reserves that have not been robbed, despite active colonies in the same area, suggesting avoidance of the dead colony by other bees; (iv) minimal evidence of wax moth or small hive beetle damage; and (v) a laying queen often present with a small cluster of newly emerged attendants.

Many affected beekeepers indicated that their colonies were under some form of stress at least two 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, exposure to pesticides, or high levels of varroa mites. Case studies of beekeeping operations suggested the possible involvement of a pathogen or toxin in CCD. Some beekeepers losing colonies to CCD placed the abandoned “dead out” hive boxes on top of boxes containing strong colonies. These strong colonies also then suffered CCD.

Fortuitously, new information on honey bees and new technical approaches are available to help determine the underlying causes of CCD in honey bees. At the end of 2006, the honey bee genome was fully sequenced, permitting the creation of new molecular approaches in honey bee genomics and molecular physiology. Using these tools, scientists can identify which genes are being turned on and off in bees, in effect allowing the bees themselves to show how they are being impacted, and helping scientists identify the most likely causal factors underlying CCD. These analyses have the potential to reveal how the bees are responding to potential pathogens, environmental toxins, or other stressors. Likewise, new approaches (*e.g.*, a new generation of sensors) for the detection of new or re-emerging pathogens or for the sensitive detection of environmental chemicals may help in unraveling the underlying causes of CCD and other problems in the health of honey bees and other pollinators.

The following document contains four major topic areas identified by the NRC committee report and by participants at a two day workshop on CCD held in Beltsville, Maryland, in April 2007.

These topic areas, reflecting the response team's plan for focusing research efforts on CCD, include: 1) survey and data collection; 2) analysis of samples; 3) hypothesis-driven experimentation; and 4) mitigative and preventative measures. Within each topic area are outlined the current status of research and future plans needed to address the problem of CCD and inadequate honey bee populations.

Topic 1: Survey and (Sample) Data Collection

Current Status of Survey and Data Collection: Apicultural industry groups, researchers (Federal, State and private), and apicultural Extension specialists all agree that there is an immediate need to establish uniform and consistent data collection methodologies to provide a baseline for both bee production and health (epidemiology) measures. While several surveys have been or are currently being conducted, none meet the criteria needed to enable researchers to evaluate increases or decreases in these measures across the U.S. or North America. The National Agricultural Statistics Service (NASS) currently conducts an annual survey of the beekeeping community that focuses on honey production. Since pollinating colonies are not monitored unless they also produce honey, there are limits on the extent to which those data can be extrapolated to estimate pollination services provided by the honey bee. NASS methods also result in undercounting, because the annual survey does not include beekeepers with fewer than five hives; *i.e.*, there is no mechanism to count hobby beekeepers who contribute to the supply of honey-producing or pollinating colonies. An additional complication of significance is that migratory beekeepers' colonies, leased in different regions of the country for different seasons, may be counted more than once. Several other one-time surveys have been conducted on either a National or regional level within the last two years, including surveys by Bee Alert Technology, Inc., the Mid-Atlantic Apiculture Research and Extension Consortium (MAAREC), the Pennsylvania Department of Agriculture (PSU), and the Apiary Inspectors of America (AIA).

As the NRC has concluded: "Improved information gathering for the beekeeping industry is critical, and NASS should modify its data collection methodologies." The committee has specifically recommended that NASS:

- Refine its assessment of honey bee abundance, specifically by collecting data annually, eliminating double-counting, recording pollination services, and monitoring winter losses.
- Collect commercial honey bee pollination data, including crops pollinated, and leasing fees from beekeepers and crop growers.
- Coordinate and reconcile data collection on honey bee colonies throughout North America. NASS should make its annual survey definitions compatible with its five-year census of agriculture.

In addition, the beekeeping industry has called for a National Honey Bee Pest Survey program to be developed and conducted under the auspices of APHIS with the participation of AIA. APHIS has the necessary expertise and experience to conduct an Office International des Epizooties (OIE)-compliant program and has begun to develop feasibility studies to determine requirements, components, and costs of such a program. Currently, this program is being planned, with a goal of identifying potentially invasive pests such as the mite *Tropilaelaps* spp., the large hive beetle, and problematic *Apis* species such as the Cape bee. This could be expanded into an epidemiological survey that would meet the goal of developing a long-term overall health survey.

Goals established for this topic area of the Action Plan are predicated on an urgent need to establish uniform standardized survey instruments; these instruments are needed as a basis to assess fluctuations in bee populations that may be attributable to disease or pest outbreaks such as CCD or to the economics of both honey production and pollination services. Comprehensive surveys should address both production/management and bee health issues.

Goal 1: Determine the extent of CCD in the United States.

Objective 1: Refine CCD symptomology to determine what CCD is and what it is not.

Priority: Urgent; Duration: Short

Plan: The American Association of Professional Apiculturists (AAPA) will refine CCD symptomology based on field observations of affected hives.

Objective 2: Develop and conduct an expanded, systematic, Nationwide, epidemiological survey, based on existing models.

Priority: Urgent; Duration: Short

Plan: Federal and State participation is being sought to compare management techniques and environmental conditions (stressors) between CCD-affected operations and non-CCD-affected operations.

Goal 2: Determine current status of honey bee colony production and health.

Objective 1: Develop an annual NASS survey that includes information on pollination services, colony loss, and honey production.

Priority: Very High; Duration: Long

Plan: APHIS, CSREES, and ARS will collaborate with NASS to modify current survey questions as recommended by the 2006 NAS/NRC status of pollinators report.

Objective 2: Develop a long-term annual APHIS survey on the overall health status of U.S. honey bees.

Priority: High; Duration: Long

Plan: APHIS, CSREES, and ARS will collaborate to coordinate a Bee Diagnostic Network (based on the Plant Diagnostic Network jointly maintained by CSREES and APHIS). This would expand a yearly survey *(in planning stages by APHIS) that is currently narrowly defined, but, as expanded, could collect data on current levels of invasive and re-emerging pests and pathogens in U.S. bee populations. This would help provide long-term data needed to determine the causes of bee mortality and ultimately the cause(s) of CCD or other potential invasive pests and diseases.*

Note: There is also the potential to develop and use sentinel colonies scattered across the U.S. to monitor bee health and environmental chemical contamination, as is done for soybean rust. This was suggested for the Pest Information Platform for Extension and Education (PIPE) program. An additional idea is to have a set of mobile diagnostic laboratories for honey bees and to couple these with sentinel colonies dispersed around the country as an early warning system for pathogen or pest introductions.

Topic 2: Analysis of existing samples

Current Status of Analysis: Samples were collected in the fall of 2006 and early 2007 from over 200 colonies, representing beekeeping operations in 10 states (California, Florida, Georgia, Idaho, Minnesota, Montana, Pennsylvania, South Dakota, Texas, Washington). These samples included colonies exhibiting CCD and others that were apparently healthy and owned by the same beekeeper or by neighboring beekeepers not experiencing CCD. Additionally, symptomless control colonies were sampled in Georgia, Pennsylvania, Hawaii, and Australia.

Adult bee samples are currently being analyzed using high-throughput 454 sequencing at Columbia University, with follow-up pathogen detection at PSU, whole genome array analysis at the University of Illinois, and a bee immunity/stress/pathogen panel [qRT-PCR assay (see list of acronyms)] developed by ARS in Beltsville, Maryland. This "Bee Path Chip" is used to determine how bee genes respond to pathogens, and therefore can be used to fingerprint pathogens by their effects on bees. Comb samples from each colony were also taken for chemical analysis of bee bread (pollen stores), wax, and brood. These analyses together should provide clues as to possible exposure of worker bees to pesticides and pathogens and may help to identify novel pathogens if they exist. Concurrent with the aforementioned analyses, adult bees are undergoing autopsies to catalogue abnormal scarring of the digestive tract, presence of fungal growth, and other physical abnormalities. Additionally, sample analysis is nearing completion for determining the prevalence of tracheal and varroa mites and *Nosema* levels in adult bees. There is also ongoing work to identify fungi (including microsporidia), bacteria, and viruses from adult bees. Outstanding needs include the examination of comb samples to detect if a fungal toxin or a repellant is present and to determine its identity and origin.

Goal 1: Identify and characterize pathogens associated with CCD.

Objective 1: Analyze samples using:

- **High-throughput sequencing for pathogen detection in individual colonies.**
- **Microarray analysis and quantitative gene expression studies to determine stressor or pathogen effects on bee gene expression.**
- **Integrated Virus Detection System (IVDS) for identifying pathogens by particle size.**

Priority: Urgent; Duration: Short

Plan: Research teams that include university researchers, the Federal Government (ARS, DoD), and private industry will use new strategies for detecting bee gene expression and for detecting and identifying pathogens of bees to determine the cause(s) of CCD.

Objective 2: Isolate, purify, and quantify microbes associated with CCD.

Priority: Very High; Duration: Short

Plan: Research teams will develop methods to try to isolate, purify, and detect pathogens associated with CCD. Several unique organisms have already been identified in bees through high-throughput sequencing efforts. Some of these organisms may be commensals or potential pathogens that had been described previously, and a few appear to be relatively new introductions. It is important to differentiate between the organisms and known bee pathogens associated with CCD-affected colonies and those associated with unaffected colonies or colonies not exhibiting CCD.

Goal 2: Identify pests associated with CCD and quantify pest levels associated with the disorder.

Objective 1: Use standard sampling methods to analyze samples for tracheal and varroa mites and *Nosema* spp.

Priority: Very High; Duration: Medium

Plan: Analysis of current samples is near completion by CCD Working Group Teams (WT Teams).

Goal 3: Identify pesticides or environmental contaminants associated with CCD.

Objective 1: Examine wax, pollen, honey, and adult bee samples for pesticides and environmental contaminants.

Priority: Urgent; Duration: Medium

Plan: A laboratory associated with USDA's Agricultural Marketing Service (AMS) is analyzing control pollen samples from historical collections as well as incoming pollen samples from bees foraging on trees sprayed with pesticides. In addition, pollen stores from CCD and non-CCD colonies will be sent for analysis of over 100 different compounds, representing common chemicals applied for pest control in apples and other bee pollinated crops and for in-hive control of varroa and tracheal mites. Metabolites of some of these compounds are being analyzed as well, since some breakdown components can be more toxic to bees than the original parent compound. In the future, wax, nectar, and brood samples will be analyzed, with honey being examined last. These analyses will help determine if colonies experiencing CCD have been exposed to significantly higher levels of environmental chemicals than non-CCD colonies. In addition, volatile chemicals in colonies are being measured using new technologies.

In the future, additional studies are needed to determine how sub-lethal levels of any detected pesticides or other environmental compounds affect the physiology of bees. Potential effects could include a suppressed ability to learn or increased physiological stress that may impair immune responses.

Objective 2: Determine whether interactions between pesticides applied inside bee hives and pesticides applied to crops contribute to CCD.

Priority: Urgent; Duration: Medium

Plan: WG Teams will conduct studies on potential interactions between chemicals used by beekeepers and those applied to crops visited by bees. Research will focus on candidate chemicals that have not yet been thoroughly examined, and will establish dose-response curves for chemicals if curves do not yet exist. Researchers will then conduct bioassays to test for synergistic chemical effects and quantify any that are found.

Goal 4: Develop analytical tools to assess bee health.

Objective 1: Develop the use of molecular markers to determine the physiological status of bees as indicators of bee health.

Priority: Very High; Duration: Medium

Plan: Specific molecular markers may be indicative of colony heath, but work is needed to validate these markers by exposing bees to stress, pesticides, and pathogens and documenting the level of response. Researchers at ARS and universities will contribute to this effort.

Topic 3: Research to identify factors affecting honey bee health, including attempts to re-create CCD symptomology

Current Status of Experimentation: It is uncertain whether CCD is a new phenomenon. Past literature has documented similar die-offs (*e.g.,* in 1898, and sporadically every 30-40 years in the United States), known then as "Dwindling Disease" or "Disappearing Disease". The causal mechanisms for all die-offs are unclear. However, scientists investigating these die-offs have now identified four candidate factors based on existing knowledge of what adversely impacts bee health and analyses of affected bees from recent CCD samples. Suspected causes include the following four factors: 1) environmental and nutritional stresses (*e.g.,* spring foraging followed by a cold weather period of 3 to 9 days, exposure to long periods of drought, inadequate nutrition, and migratory management practices); 2) new and/or re-emerging pathogens; 3) pests that attack bees; and 4) pesticides.

To support the first hypothesis involving stress, recent unpublished studies indicated that environmental and nutritional stresses may play a role in CCD. Studies showed that bees enduring a shortage of essential nutritional supplements in the fall were more likely to suffer from CCD in the winter.

As for the second hypothesis, initial surveys of CCD-affected bees have uncovered a multitude of different suspect pathogens. Even if CCD is cyclic, it could be caused by a different pathogen in each case; for instance, a new pathogen could be causing significant bee loss (CCD) until the bees are able to develop resistance, at which point the problem disappears until the emergence of the next new pathogen. Transmission of pathogens may be on the increase due to migratory beekeeping practices that confine hives under a net during transport, thereby increasing hive-to-hive exchange of inocula. Other research has shown that viruses and spiroplasmas (cell wall-less bacteria) attack the bee brain directly, which could conceivably affect their navigational abilities.

Current pathogen suspects include the single-celled organism *Nosema ceranae* (a microsporidian parasite related to fungi), which was responsible for large bee die-offs in Spain. *Nosema ceranae* has been identified in some affected bee hives in the United States since as early as 1995. Infected bees attempt to rid themselves of the pathogen by flying from the hive and defecating (taking what are called "cleansing flights"), which can expose bees to lethal winter temperatures of $4^{o}C$ or below. Other pathogen suspects include *Nosema apis*, a related organism, which was associated with CCD in the 1970s in the United States. In addition, *Aspergillus* spp. fungal pathogens are infecting bees at high incidences in CCD-affected hives. Further research is needed to conclusively demonstrate whether pathogens are involved with CCD and whether immune suppression is associated with this disorder.

As for pests, the varroa mite, which parasitizes honey bees and transmits bee viruses that may also be associated with CCD, has caused devastating losses to honey bee populations throughout the United States. These mites have developed resistance to pesticides, with control failures well documented. To combat this problem, varroa mite-resistant strains of honey bees have been developed; however, resistant stocks have not yet been widely adopted because of other bee stock characteristics.

Regarding pesticides, a new class of insecticides known as neonicotinoids is broadly and commonly used in most cropping systems and on turf and forest pests. One of the compounds in this class, imidacloprid, was banned in France, because it is acutely toxic to bees and since sub-lethal doses have been shown to impair honey bee short-term memory; short-term memory is critical to bee navigational abilities necessary for foraging flights and for returning to the hive. USDA-funded research in North Carolina suggested that widely used fungicides synergize the effect of neonicotinoids 1,000-fold. Imidacloprid, applied as a systemic, has been found in corn, sunflower, and rape pollen at levels high enough to harm bees. [Although bees do not pollinate corn, they do collect corn pollen.]

Several other factors have been suggested as causal mechanisms of CCD, for example, the use of genetically modified (GMO) crops. However, large bee die-offs have also occurred in Europe, where GMO crops are not widely grown. Also, in the United States, the patterns of CCD-affected colonies do not appear to correlate with the distribution of GMO-crops such as Bt-corn. Furthermore, extensive laboratory and field testing has indicated a lack of acute and sub-lethal effects on bees exposed to GMO-pollen.

Other hypotheses are even less likely. For example, based on misleading news reports, the public has become concerned that cell phone use may be causing bee die-offs; however, scientists have largely dismissed this theory because exposure of bees to high levels of electromagnetic fields is unlikely. Similarly, shifts in the Earth's magnetic field, which could conceivably affect bee navigation, have not been correlated with bee die-off episodes, but cannot be completely ruled out at this time.

A key tool in the fight against CCD includes the recently sequenced honey bee genome. The genome has already shown that bees are weak in detoxifying enzymes (which would make them particularly vulnerable to pesticide poisoning) and have weak immune systems; they likely depend on sociality to protect their colony from diseases, *e.g.,* depending on hygienic behavior to remove infected brood from the hive.

CSREES has provided significant intramural and extramural funding for research that is making use of the honey bee genome, including an NRI-funded grant that resulted in the creation of a genome-wide map in late 2006, obtained by a custom designed microarray. The array is publicly available to researchers to identify and characterize the genes associated with CCD, and ARS researchers are now using the microarray to perform studies relevant to bee-associated microbes that may be causing CCD. In addition, CSREES' Critical and Emerging Pests and Diseases Program is providing emergency funding in 2007 to make use of the whole-genome microarray to identify detoxification enzymes that may be associated with CCD. As a result of these and other efforts, the honey bee genome is helping researchers better understand basic bee biology, breed better bees, and diagnose bee pests and pathogens and their impacts on bee health and colony collapse. The use of this genomic information will have great applications in improving honey bee breeding and management.

Goal 1: Confirm or eliminate potential environmental stressors as contributing causes of CCD.

Objective 1: Test effects (lethal and sub-lethal) of neonicotinoids and other pesticides used for crop protection.

Priority: Very High; Duration: Medium

Plan: WG Teams will conduct laboratory and field experiments to examine the level of pesticide exposure that bees may be receiving while working crops treated with insecticides. Incoming nectar and pollen loads from worker bees on specific crops will be analyzed for pesticides. Cage studies may be used to simulate "worst case" scenarios where bees are confined to a single diet.

Researchers will test the effects of lethal and sub-lethal doses of insecticides on the development of honey bee brood, in vivo and in vitro, and on the longevity of adult bees. Specifically, scientists will be testing the life span, learning ability, and orientation of reared bees, particularly as this relates to CCD.

*Additionally, laboratory, greenhouse, field-cage, and open-field experiments will be conducted to examine the effects of pesticide exposure on honey bees and non-Apis bees that forage on single and/or various crops treated with pesticides (e.g., insecticides, miticides, and fungicides). Nectar and pollen from honey bee and bumble bee foragers and from pollen-nectar provisions from solitary bee nests will be analyzed for pesticide contamination. The lethal effects of direct pesticide exposure of adult bees and brood will be determined by bee mortality, while sub-lethal effects will be determined through evaluation of adult foraging and orientation behaviors as well as reproductive success. The study of non-*Apis *bees addresses whether other bees serving as pollinators are susceptible to the same lethal/sublethal factors as honey bees and whether solitary bees can be reliable, readily accessible, surrogate species for evaluating pesticidal impacts on bees.*

Objective 2: Test the effects of current miticides used in hives on worker bee longevity and colony health.

Priority: Urgent; Duration: Medium

Plan: Researchers will examine the effects of miticides on colony health. Previous research with coumaphos has demonstrated sub-lethal effects on workers and queens, but no data exists on effects from commercial beeswax combs. Therefore, in addition to studies of direct effects of mortality on workers and brood, studies will be conducted using miticide contaminated comb. Researchers will follow survival in the larval and pupal stage as well as study the longevity of adult bees reared in commercial comb to test the effects of lethal and sub-lethal doses of miticides. Specifically, scientists will be testing the life span, learning ability, and orientation of in vitro reared bees, as well as the association between the use of these chemicals and CCD. Analysis of wax will determine miticide levels, and remediation methods may be used to try to neutralize residues.

Objective 3: Test the effects of antibiotics (especially new ones such as Tylosin) on the increase in pathogens (*e.g., Nosema ceranae*) and the overall viability of bees over winter.

Priority: Very High; Duration: Medium

Plan: The use of antibiotics could lead to unwanted results if they alter the natural gut flora of adult bees, resulting in an increase in infectivity of other pathogens (e.g., Nosema). Cage studies will be conducted to test this hypothesis and may result in larger field trials with whole colonies. In related research, we will investigate whether antibiotics have chronic effects on bee survival over winter, effects that might lead to early season bee die-off.

Objective 4: Test effects of supplemental protein and carbohydrate [*e.g.*, high fructose corn syrup (HFCS)] feedings on bee health.

Priority: Very High; Duration: Medium

Plan: Reports in the 1970s indicated that HFCS contains low levels of poisonous hydroxy-methyl-furfural (HMF). Using both cage and field studies, the effects of sugar-substitute HFCS and protein-substitute supplements on bee health will be explored. Nutrient content will be analyzed and the metabolism of bees will be monitored. Additional testing could involve examination of GMO corn products (HFCS) to determine their potential impact on bee health when incorporated into HFCS.

Objective 5: Test effects of availability and quality of natural food sources on bee health as affected by climatic factors (*e.g.*, drought).

Priority: High; Duration: Long

Plan: Poor weather could result in reductions in the availability or quality of both pollen and nectar, and bees foraging in such areas may therefore be rearing brood on an inferior diet. Plants such as clover that bees use for pollen and nectar will be reared under varying environmental conditions, and the quality and quantity of pollen and nectar will be measured. Feeding studies using pollen from "stressed" and control plants should document any existing adverse effects.

Objective 6: Test effects of management practices (*e.g.*, nutrition, migratory stresses) on bee health.

Priority: Very High; Duration: Medium

Plan: Migratory beekeeping is likely impacting colony health, but few studies have attempted to document the impact that migration has on bee colonies. Studies will be conducted to compare colonies used for honey production with those moved repeatedly for pollination. An ARS Areawide Project on bee health will address many of the issues raised regarding the impact of commercial management and migratory beekeeping on bee health. Ongoing research in this area has and will continue to be conducted through collaboration with the beekeeping industry. Additionally, researchers at several universities have proposed to conduct studies comparing the pathogens in migratory beekeeper colonies with those in resident beekeeper colonies. These studies will seek to determine the impact of migration on bee colonies and whether that impact could be sufficient to cause CCD.

Goal 2: Confirm or eliminate potential pathogens as contributing causes of CCD.

Objective 1: Test pathogenicity of the following CCD-associated microbes against honey bees and non-*Apis* bees:

- **Viruses**
- **Fungi (chalkbrood; stonebrood)**
- **Microsporidia (*Nosema*)**
- **Bacteria (including spiroplasmas)**
- **Trypanosomes and other microbes**

Priority: Very High; Duration: Medium

*Plan: Sample analysis to date has revealed a large number of pathogens present in CCD colonies. Controlled exposure experiments are needed to determine the pathogenicity of many of these organisms. Experiments are needed to determine pathogenicity (i.e., to fulfill Koch's postulates) and virulence of microbes isolated from sick or dead bees. Bioassays should be conducted on non-*Apis *bees, as well as honey bees, to determine whether CCD is a threat to the Nation's other pollinators or is likely to be* *transmitted between species of bees. If it is determined in bioassays that a potential pathogen is not highly virulent, yet still infects adult bees, then greenhouse or caged studies will be conducted on the adults to identify if any of the pathogens have chronic effects such as shortened life spans over winter or cognitive effects on adult behavior that may render the bees unable to return to their hives or nests.*

Objective 2: Compare genes expressed in response to specific pathogens or pesticides with those expressed in bees from CCD colonies.

Priority: Very High; Duration: Medium

Plan: Gene expression holds great promise as an indicator of exposure to pathogens or pesticides. For example, changes in detoxification gene expression might indicate that the bee has been exposed to a pesticide, whereas changes in immune gene expression would suggest response to a pathogen. Changes in bee cognitive genes might indicate pathogen or pesticide interference with bee ability to learn and forage. Using this gene expression tool, worker bees will be exposee workers to pesticides and pathogens and their responses compared with bees from colonies exhibiting CCD symptoms.

Goal 3: Confirm or eliminate pests as contributing causes of CCD.

Objective 1: Test the effects of varroa mites on bee health and robustness, particularly overwintering effects and association with CCD in early spring.

Priority: High; Duration: Medium

Plan: If a history of previous varroa infestation is associated with CCD in the spring, this might be due to the effects of parasitism on hive vigor or bee health, or, alternatively, to transmission of a viral pathogen that persists in the bees. Researchers will conduct hive tests that allow varroa mite levels to be elevated in the summer and fall and then controlled. These colonies will be monitored to determine if a history of heavy parasitism impacts bee survival over the winter or during spring buildup. Colonies that have high levels of mites in the spring and colonies with a history of low mite levels will be used as controls.

Objective 2: Determine the importance of varroa as a vector of viruses associated with CCD or as a general immuno-suppressive agent on the colony itself.

Priority: Very High; Duration: Medium

Plan: Since varroa has been shown to vector bee viruses, a new virus association might make a deadly combination. Studies are needed to *further explore the relationship between varroa and the transmission of several bee viruses. Such studies will include the effects of transmitted viruses on bee mortality, short- and long-term; acute, chronic, and cognitive effects will be ascertained.*

Goal 4: Determine what factors (or interactions between factors) are most important in their contribution to CCD. This includes environmental factors (*e.g.*, temperature, humidity, and chemical exposure), pathogens and parasites, and bee genetics and breeding.

Plan: CCD is likely caused by a combination of factors. Many of the previous objectives have examined single factors as contributing to CCD. This goal will begin to test multiple factors for interactions and could include any or all of the factors above in a series of stepwise experiments. This area of research, with multi-factor experiments, will require large studies and multi-institution efforts to be successful.

*Non-*Apis *bees could be used as surrogates to test some of the factors. It is often easier to use solitary bees as test subjects because environmental conditions can be controlled; by contrast, a honey bee colony regulates hive temperature and humidity. Solitary bees and bumble bees also will fly in enclosures such as greenhouses and screen houses much more readily than honey bees, without any direct impact on bee health. The immune response in all bees is likely to be more similar than between more distantly related insects.*

Topic 4: Mitigative and preventative measures

Current Status of Mitigative and Preventative Measures. Since little is known about the cause(s) of CCD, there is little confidence in current mitigative measures. Such remedies are urgently needed, particularly since beekeepers report that, when equipment from dead hives is combined with live hives, the live hives begin to decline. An experiment to study this is now underway, using irradiation and acetic acid fumigation to reclaim comb from CCD colonies; the experiment involves two hundred colonies with package bees from Australia (which does not have varroa mites). Since irradiation will kill pathogens, but will not likely alter the composition or availability of pesticides in the beeswax, this experiment could provide valuable data to address the pesticide question as well. Another new method that is being developed for all bees (honey bees and solitary bees) is the use of high concentrations of ozone to treat hives during winter storage. Ozone has been found to degrade hive pesticides, kill pathogens, and kill storage pests (*e.g.*, the wax moth).

In addition, a more complete understanding of shared pathologies between bumble bees (genus *Bombus*) and honey bees (genus *Apis*) would provide a comparative framework to assess treatment and management of diseases for both taxa. Bumble bees are close relatives of honey bees, and they are social. There are notable similarities between the taxa with regards to diet, pests, and pathogens. Several species of bumble bees (*Bombus affinis, B. franklini*, *B. occidentalis* and *B. terricola*) are experiencing population declines pre-dating and concurrent with CCD in honey bees. [Some suspect that the native bee *B. franklini* is now extinct due to a crossover pathogen from imported *Bombus* spp.]. While it is known that there are some shared pests (wax moths, tracheal mites) and diseases (Kashmir bee virus, deformed wing virus), the extent to which *Bombus* and *Apis* share diseases is not fully known.

The CCD crisis in honey bees highlights a critical need to conserve our native bees, which are all non-*Apis* bees. One challenge needing to be addressed is the loss of habitat from farming and urban developments, which may be causing a decline in native bees (see National Academy of Sciences report); inadequate nesting or foraging resources can limit bee population sizes and, for native unmanaged bees, species diversity. However, we can increase bee populations by providing extra pollen and nectar resources (*i.e.*, appropriate flowers), at least in gardens and in some farm crops. In addition, we propose to better utilize our natural wildlands to save our valuable native pollination resource. Millions of acres of private lands are annually enrolled in Federal Government land preservation and restoration programs such as the Environmental Quality Incentives Program (EQIP) [NRCS]; the Conservation Reserve Program (CRP) [Farm Service Administration and NRCS], and the Wetland Reserve Program (WRP) [NRCS] and millions more acres of Federal forests and rangelands (protected by the U.S. public land management agencies) require rehabilitation following wildfires or on long-term degradation. Rehabilitation typically consists of seeding with grasses and shrubs to restore plant communities; yet while wildlife or grazing values have been considered in plant species selection, pollinator value has not. If conservation and rehabilitation plantings were to include shrubs or seeds of native forb species known to produce generous pollen and nectar rewards attractive to diverse bees, these land management programs would help restore and preserve pollinator communities across vast areas of private and public lands at little added cost, with forage benefits to honey bees as well.

Goal 1: Develop best management practices for honey bees.

Objective 1: Develop best management practices for migratory beekeeping.

Priority: Very High; Duration: Medium

Plan: AAPA is writing a set of best management practices that will serve as a guideline. A draft copy of the booklet is scheduled for completion by October 2007, with a final copy to be prepared by January 2008. This booklet will be widely distributed to beekeepers at no cost. Answers to basic management questions are needed, including how to manage the ongoing problem of Africanized bees.

Objective 2: Develop best management practices for pest and pathogen control.

Priority: Very High; Duration: Medium

Plan: As results from pathogen identification become available, studies on disease mitigation will be initiated. Typical controls include the following: for viruses, bee resistance breeding and stock maintenance; for bacteria, antibiotics and bee resistance breeding; and for fungi, fungicides, including fumigants. Maintaining bee colony health through ensuring nutritious diets, water availability, and reduced colony stress is also key to protecting colonies against all pathogens. Additionally, there is a need for regulatory agency controls to prevent entry of new pathogens into the United States.

Objective 3: Establish guidelines for floral gardens to maintain stronger honey bees.

Priority: Medium; Duration: Long

Plan: Demonstration plots could be used to illustrate the value of natural food sources on bee health. Much is already known about the value of various plants for bees, but these plants are not in routine use for bee forage. Experiments are needed to test mixed plantings on colony growth and winter bee production.

Goal 2: Develop best management practices for non-*Apis* bees to provide alternative pollinators for crops, gardens, and natural areas.

Objective 1: Develop best management practices for pest and pathogen control in non-*Apis* bees.

Priority: Very High; Duration: Long

Plan: Solitary bee pests and pathogens will be identified and their prevalence in North America determined. The focus will be on bumble bees and pathogens shared with the honey bee, with investigation also of managed solitary bees such as the introduced alfalfa leafcutting bee and the native blue orchard bee. A more complete understanding of the extent of shared pathogens and diseases would provide a comparative framework to assess treatment and management of diseases for both taxa, and could impact regulatory decisions of bee importation. Specifically, there is a need to identify viruses and bacteria, which are believed to be responsible for considerable "unknown" mortality.

Objective 2: Establish guidelines for maintaining stronger populations of non-*Apis* bees in agricultural systems, home gardens, and wildlands.

Priority: High; Duration: Long

*Plan: In many urban and agricultural settings, both food and nesting resources can be scarce for non-*Apis *bees, limiting their ability to grow or establish. Public land management agencies are in need of information to help them improve the health of non-*Apis *bees in all settings. Therefore, researchers will identify plantings or habitat modifications and develop artificial domiciles for bumble bee nesting. Specifically, they will evaluate native wildflowers for restoration, or conservation seed mixtures to boost recovery, of wild bee communities.*

Goal 3: Maintain bees with resistance to parasites and pathogens.

Objective 1: Identify traits associated with resistance to parasites and pathogens.

Priority: Very High; Duration: Long

Plan: Bees are known to have multiple mechanisms of resistance. Researchers will determine the mechanisms of resistance and then identify the genes that provide resistance.

Objective 2: Introduce resistance traits into bee stocks favored by the industry.

Priority: High; Duration: Long

Plan: Since bees carrying resistance genes will not likely have all other important characteristics, it will be important to introduce resistance genes into preferred lines. The bee industry will be consulted to determine preferred lines, and genetic engineering techniques will be further developed and used to transfer genes.

Objective 3: Use genomic technologies and germplasm preservation to maintain quantities of desirable honey bee germplasm.

Priority: High; Duration: Long

Plan: To support the goal of maintaining resistant bees, researchers will use genetic markers [expressed sequence tags (ESTs) and quantitative trait loci (QTL)] for desirable traits to augment traditional breeding processes. Researchers should make use of markers already identified for defensive behavior and hygienic behavior to facilitate the development of commercially viable selected stocks of honey bee.

Objective 4: Transition to mite and pathogen-resistant honey bee stocks.

Priority: Very High; Duration: Long

Plan: The U.S. bee population must be replaced by one that is resistant to parasites and pathogens. This transition will require improved methods for identifying superior stock, such as mass screening of honey bees for desirable traits (see above), developing new stocks viable in several regions of the country, developing third-party certification for selected stocks, and educating queen producers on best methods for stock improvement and maintenance.

Goal 4: Develop ways to manage mite resistance to miticides and create alternatives.

Objective 1: Develop resistance management programs that provide beekeepers with tools for mite management.

Priority: High; Duration: Long

Plan: Mites have demonstrated resistance to pesticides such as fluvalinate and coumaphos, leading to increases in mite populations. Researchers will need to develop programs to combat this trend, building on research on mechanisms of resistance, the identification of alternative pesticides, and improvements in pesticides.

Objective 2: Develop new methods of managing parasites and pathogens.

Priority: Very High; Duration: Long

Plan: Develop non-chemical control methods, including cultural or biological control methods, as alternatives to pesticide use. In particular, investigate fungal pathogens as potential biocontrol agents for varroa mites. Important obstacles include the need to increase delivery and persistence of insect-pathogenic fungi in the hive environment.

Goal 5: Improve the regulatory framework to better protect against the introduction of new pathogens, pests, and parasites of bees to meet World Trade Organization (WTO) and International Committee of the World Organization for Animal Health (OIE) requirements for the importation and exportation of honey bees.

Objective 1: Develop new molecular detection systems that can be used to detect pathogens, pests, and parasites in introduced bee stocks and bee products used in beekeeping.

Priority: Very High; Duration: Long

Plan: Researchers will use the Bee Diagnostic Network (Topic 1, Objective 2) to establish a long-term capacity to detect, identify, and respond to pests and pathogens. They will also develop testing standards that all imported bee stocks and bee products have to meet, regardless of defined uses.

Objective 2: Explore opportunities to change regulations based on new molecular detection systems.

Priority: Very High; Duration: Medium

Plan: APHIS will take the lead in this objective.

Objective 3: Establish processes for periodic monitoring of the U.S. honey bee population to determine whether specific pests are present.

Priority: Very High; Duration: Medium

Plan: APHIS will need to monitor the U.S. honey bee population for various pests, parasites, and pathogens. In collaboration with cognate agencies in Mexico and Canada, APHIS will collect and analyze samples of honey bees from countries interested in supplying bees to North America to ensure that new honey bee pests, parasites, and pathogens are not inadvertently introduced to North America.

Goal 6: Demonstrate improved colony health by integrating research-derived knowledge and tactics into an Areawide Project.

Objective 1: Test and verify management approaches for mite control, improved diet, improved bee stock, and changes in migratory practice.

Priority: High; Duration: Long

Plan: The ARS Areawide Program is described previously.

Objective 2: Transfer technology for early spring bee availability for pollination.

Priority: High; Duration: Long

Plan: The availability of early pollination is critical to crops such as almond; research is needed to examine pollination requirements in early spring

Goal 7: Transmit or disseminate science-based information to manage bees.

Objective 1: Develop, maintain, and preserve a secure Web-based site for scientific collaboration (Sharepoint).

Priority: High; Duration: Long

Plan: This could be done at university or Federal facilities.

Objective 2: Develop, maintain, and update a Web-based public Internet site, e.g., *e*Xtension or PIPE (Pest Information Platform for Extension).

Priority: Very High; Duration: Long

Plan: This effort will be led by CSREES.