USDA

United States Department of Agriculture

Agricultural Research Service

Colony Collapse Disorder Progress Report

CCD Steering Committee
June 2010

Cover: Adult honey bee, *Apis mellifera Linnaeus*, photo courtesy of Susan Ellis, Bugwood.org.

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This report has been cleared by all agencies involved.

Executive Summary

Mandated by the 2008 Farm Bill [Section 7204 (h) (4)], this second annual report on Honey Bee Colony Collapse Disorder (CCD) research progress represents the work of a large number of scientists from 8 Federal agencies, 2 state departments of agriculture, 22 universities, and several private research efforts.

In response to the unexplained losses of U.S. honey bee colonies, now known as colony collapse disorder (CCD), the U.S. Department of Agriculture's (USDA) Agricultural Research Service (ARS) and National Institute of Food and Agriculture (NIFA) led a collaborative effort to define an approach to CCD, resulting in the CCD Action Plan in July 2007. Many universities and organizations—Federal, State, and private—were involved in developing this plan and are carrying out the work that addresses the CCD problem. The CCD Action Plan is organized under four topic areas:

1. Survey and (sample) data collection;
2. Analysis of existing samples;
3. Research to identify factors affecting honey bee health, including attempts to recreate CCD symptomology; and
4. Mitigation and preventive measures.

Highlights of progress in these topic areas are summarized below.

Topic I: Survey and (Sample) Data Collection

Several survey and data collection efforts have been underway to provide baseline information on CCD, resulting in better defined CCD symptoms, and documented evidence of increasing honey bee losses. Colonies with poor health, including colonies with CCD symptoms, exhibited increased pathogen levels and evidence of pesticide residues. General colony loss levels in 2009 were 29 percent and increased to 34 percent in 2010. Losses are a major problem for the beekeeping industry and potentially to commodities that depend on bees for pollination services.

Topic II: Analysis of Existing Samples

Based on an initial analysis of collected bee samples (CCD- and non-CCD affected), reports have noted the high number of viruses and other pathogens, pesticides, and parasites present in CCD colonies, and lower levels in non-CCD colonies. This work suggests that a combination of environmental stressors may set off a cascade of events and contribute to a colony where weakened worker bees are more susceptible to pests and pathogens. Testing this complex hypothesis is the focus of joint efforts between Federal and university scientists funded by ARS and NIFA.

Topic III: Research to Identify Factors Affecting Honey Bee Health, Including Attempts to Recreate CCD Symptomology

Survey and analysis results indicate that many pesticides, parasites, and pathogens are associated with CCD. Therefore, efforts to explore the role of these factors continue. Findings currently suggest an association of sub-lethal effects of pesticides with CCD. Two common miticides in particular, coumaphos and fluvalinate, which are pesticides registered for use in bee colonies to control varroa mites, are suspect, either acting individually or in combination (e.g., synergistically, where the combination of the two compounds is more toxic than either compound alone). The emerging evidence of pesticide exposure to pollinators and potential interactive effects indicates the need to further study pesticides for their potential interactions with CCD. Studies have also confirmed suspected links between CCD and poor colony health, inadequate diet, and long-distance transportation. Research results indicate that both supplemental protein diets and natural pollen feedings can increase colony strength and offset the negative impacts of stress caused by pests, pesticides, and long-distance transportation of bees by beekeeping operations.

Topic IV: Mitigation and Management Measures

Efforts to mitigate honey bee losses are highlighted by two national multi-year projects, the ARS Areawide Project on Honey Bee Health and a NIFA-funded Coordinated Agricultural Project (CAP), which are allowing researchers to test various hypotheses related to poor bee health and provide further insights into the problem, and strategies for better bee management. Key accomplishments to date include the development of new, varroa mite-resistant bee stocks, improved diets and timing of feeding, a strategy (comb irradiation and ozone treatment) to reduce pathogen levels, and identification of several alternative pollinator species. Progress has also been made toward developing new detection capabilities for pests and pathogens, integrated control strategies for varroa mites, and a set of comprehensive best management practices that will provide beekeepers with specific guidelines to improve the health of their bees.

As a method for potentially mitigating the occurrence of CCD, and in an effort to encourage the provision of improved and sustainable habitat for both managed honey bees and all pollinators, the Natural Resources Conservation Service (NRCS) is implementing its NRCS Pollinator Initiative, which is a multifaceted initiative involving the following: (1) the revision of conservation practice standards to better address pollinator habitat needs; (2) the identification and provision of recommended ecoregion-specific, pollinator-friendly vegetation as part of the implementation of the NRCS Plant Materials Centers' Pollinator Conservation Action Plan; (3) the provision of support and encouragement for enhancing pollinator habitat under a variety of conservation programs as identified in the NRCS Programs Pollinator Action Plan; and (4) the provision of awareness-building materials, training, and outreach about the importance of pollinators and helpful actions individuals may pursue.

During the past 3 years, numerous causes for CCD have been proposed and investigated. Although the cause of CCD is still unknown, research has provided support to the hypothesis that CCD may be a syndrome caused by many different factors that work individually or in combination. Looking ahead, studies will focus increasingly on the interaction of multiple factors in causing CCD.

More detailed findings follow in the body of the report.

Colony Collapse Disorder
Annual Progress Report

This report is the second annual report prepared in response to Section section 1672 (h) (4) of the Food, Agriculture, Conservation, and Trade Act of 1990 (7 U.S.C. 5925 (h) (4)), as added by section 7204 (a) (3) of the Food, Conservation, and Energy Act of 2008 ("2008 Farm Bill") (Pub. L. 110-246), which directed the Secretary of Agriculture to:

> ''submit to the Committee on Agriculture of the House of Representatives and the Committee on Agriculture, Nutrition, and Forestry of the Senate an annual report describing the progress made by the Department of Agriculture in—
> (A) investigating the cause or causes of honey bee colony collapse; and
> (B) finding appropriate strategies to reduce colony loss."

Background

After the large-scale, unexplained losses of managed U.S. honey bee (*Apis mellifera* L.) colonies during the winter of 2006-2007, investigators identified a set of symptoms that were termed colony collapse disorder (CCD). In response to this problem, Federal and State government, university, and private researchers, led by the U.S. Department of Agriculture's (USDA) Agricultural Research Service (ARS) and Cooperative State Research, Education, and Extension Service (now the National Institute of Food and Agriculture (NIFA)), mobilized to define an approach to CCD, an effort resulting in formation of the CCD Steering Committee, and publication of the CCD Action Plan in July 2007. Many organizations, public and private, in addition to those represented on the Steering Committee, are involved in the work to address the CCD problem.

During the past 3 years, numerous causes for CCD have been proposed and examined. There have been many associations identified throughout the course of research; however, it is becoming increasingly clear that no single factor alone is responsible for the malady. Researchers continue to document elevated pathogen levels in CCD-affected bees, with no specific pathogen linked definitively to CCD. In addition, tests to examine hives for known honey bee parasites (varroa mites, honey bee tracheal mites, Nosema species), which pose significant problems for beekeepers, and once were highly suspected to play a major role in CCD, have not revealed these parasites, by themselves, at sufficient levels to explain the problem. Over the past year, several independent studies have shown that bees are exposed to a wide range of pesticides and that some pesticides have interactive effects (on bee mortality) with other pesticides, with bee pests, or with viruses. Taken together, these studies support the hypothesis that CCD is a syndrome of stress, caused by many different factors working individually, but more likely in combination. Insufficient data are available to confirm this, but studies continue, based on the groundwork that has been laid.

Although the causes of CCD have not been fully illuminated, the research response is coordinated and dedicated to resolving this issue, while simultaneously improving pollinator health. NIFA's Coordinated Agricultural Project and ARS' Areawide Project on Honey Bee Health are of particular importance for their ongoing efforts to examine bee effects from a broad, regional perspective, which will be a key component to solving the crisis. Funding from ARS and NIFA, with additional contributions by a number of other sources, including the National Honey Board, the Almond Board of California, Burt's Bees, Häagen-Dazs, the North American Pollinator Protection Campaign, Project "Apis m." (PAm), the Foundation for the Preservation of Honey Bees and others, is resulting in a variety of new studies and new expertise on bee health issues. The fruits of this increased research effort are being published, and a new "eXtension" website (http://www.extension.org/bee%20health) has been assembled to provide reliable research-based information to beekeepers and the general public.

The CCD Action Plan is organized under four topic areas:

1. Survey and (sample) data collection;
2. Analysis of existing samples;
3. Research to identify factors affecting honey bee health, including attempts to recreate CCD symptomology; and
4. Mitigation and preventive measures.

Following up on the 2007-2008 Report, below is a summary of progress in meeting Action Plan objectives for each of these topic areas over the past year.

Topic I: Survey and (Sample) Data Collection

In response to an immediate need for a baseline of both bee production and health, several survey and data collection efforts, supported both by ARS and NIFA, have been underway. Together, the different survey efforts have better defined CCD symptoms. Previous studies showed that symptoms included a rapid loss of adult honey bees, excess immature bees present in the combs, and the queen still present. Additional findings indicate an absence of damaging levels of the gut parasite Nosema or parasitic varroa mites at the time of collapse. Data on overall honey bee losses for 2010 indicate an estimated 34 percent loss, which is statistically similar to losses reported in 2007, 2008, and 2009. Survey work will continue to assess the status of honey bee health and further refine CCD symptomology.

The Animal and Plant Health Inspection Service (APHIS) has completed a pilot national survey, which will be expanded this coming year to include sampling in 13 states to look for exotic pests and diseases in honey bee colonies.

The National Agricultural Statistics Service (NASS) has discussed methods and procedures to incorporate colony loss questions into the current NASS national honey production survey. The Environmental Protection Agency (EPA) recently hosted a USDA-led workshop to discuss the development of new toxicity testing protocols to determine the potential effects of pesticides on bees. EPA and USDA officials are members of a steering committee composed of international representatives from government, industry, academia, and non-governmental

organizations who are organizing a more comprehensive workshop to be convened at the global Society of Environmental Toxicology and Chemistry (SETAC) Pellston[1] workshop in the coming year. The global Pellston is intended to develop an improved approach for characterizing risks of pesticides to bees and to identify the data needed to inform the risk assessment process. The SETAC conference will also examine the adequacy of tests performed with *Apis mellifera* to serve as a model to predict effects to other "non-Apis" pollinators.

Topic II: Analysis of Existing Samples

Following up on efforts from previous years, researchers supported both by NIFA and ARS continue to analyze bee samples for pesticide residues and pathogen loads to determine possible linkages to bee declines. Studies continue to demonstrate very high levels of pathogens in CCD-affected samples and lower pathogen levels in non-affected samples, consistent with the empirical observation that healthy honey bee colonies normally fend off pathogens. These observations have led to the hypothesis that bee declines are resulting from immune suppression. A large survey of healthy and CCD-affected colonies also revealed elevated levels of pesticides in wax and pollen, but the amounts of pesticides were similar in both failing and healthy colonies.

Supporting the observation that CCD is caused by a combination of factors is a series of studies within NIFA's Coordinated Agricultural Project (CAP). CAP scientists reported several important findings in the past year. In addition to the demonstration that pesticide application rates are highly variable regionally—again pointing away from one specific cause of bee decline—CAP scientists have identified interactive effects among pesticides, the parasite Nosema, and viruses—including the Israeli Acute Paralysis Virus (IAPV) and deformed wing virus, all known stressors to honey bees. Another multi-year, complex study within the CAP project is taking place across multiple states to examine additional interactions among pesticides, pests, and parasites. These studies are just beginning and are yielding working hypotheses based on preliminary, mostly unpublished data. For example, one study indicates potential interactive effects between varroa mites and small hive beetles on colony losses.

CAP-funded studies also identified sub-lethal effects of neonicotinoids[2] and fungicides on bees. It is hypothesized that these pesticides impair the bee's immune system, which leaves the bee more susceptible to three important bee viruses. Future experiments are needed to test these hypotheses and conclusively identify mechanisms of immune response, as well as how these interactions might affect mortality and colony health.

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[1] The first Pellston Conference was held in 1977 to address the needs and means for assessing the hazards of chemicals to aquatic life. Since then, many conferences have been held to evaluate current and prospective environmental issues. Each has focused on a relevant environmental topic, and the proceedings of each have been published as a peer-reviewed or informal report. These documents have been widely distributed and are valued by environmental scientists, engineers, regulators, and managers because of their technical basis and their comprehensive, state-of-the-science reviews. The first four Pellston conferences were initiated before the Society of Environmental Toxicology and Chemistry (SETAC) was effectively functioning. Beginning with the 1982 conference, however, SETAC has been the primary organizer, and SETAC members (on a volunteer basis) have been instrumental in planning, conducting, and disseminating conference results. Taken from http://www.setac.org/node/104.

[2] Neonicotinoids are a class of pesticide compounds registered on a wide variety of commercial agricultural crops.

Separate research has independently demonstrated synergistic effects between various pesticides and pathogens. A study by Pennsylvania State University scientists, funded by the National Honey Board, identified potential interactions among numerous pests, pathogens, and pesticides. Scientists also found that one fungicide (chlorothalonil) was linked with "entombing" behavior in bees (a defensive behavior associated with poor health) and had particularly detrimental effects. Studies are ongoing, and as more light is shed on the latent effects of these sub-lethal and synergistic factors, strategies to mitigate these effects may evolve along with the development of management strategies for beekeepers to protect their bees.

CAP-funded scientists from Pennsylvania State University recently conducted preliminary studies that indicate IAPV has an East Coast and a West Coast variant. IAPV was believed to be linked with CCD in 2007. The west coast IAPV variant appears to be more closely related to the predominant strain in Australia. Currently, studies are underway to compare the virulence of the East Coast and the West Coast strains of IAPV, and to better understand any association with other factors linked to CCD.

Continuing to capitalize on the honey bee genome, and with funding from NIFA, University of Illinois scientists have identified potential molecular markers for genes that play a role in CCD (Proceedings of the National Academy of Sciences, 2009). Although application of these results is a long way off, these markers will be highly useful for breeding bees with enhanced tolerance to pesticides and/or pathogens associated with CCD and declining bee health in general.

Improved diagnostic assays are ongoing with funding from both ARS and the NIFA-funded CAP to analyze CCD samples within the context of sample analysis and in Topic III, Hypothesis-Driven Research, described below.

Topic III: Research to Identify Factors affecting Honey Bee Health, Including Attempts to Recreate CCD Symptomology

Research efforts jointly supported by ARS and NIFA continue to investigate factors that may play a role in causing CCD, either alone and/or in combination. Factors include diseases (parasites and pathogens), pesticides, poor nutrition, beekeeping practices, and to a lesser extent, other pests such as the small hive beetle.

Certain pesticides are suspected as a contributing factor to CCD. Inclusion of pesticides as a contributing factor to CCD is predicated on the relative paucity of detoxification enzymes (which help insects fend off toxins in the environment) found in CCD samples. It is believed that bees have a relatively weak immune system as a result, and that this may be an evolutionary consequence of feeding on nectar and pollen, which are plant materials lower in toxin levels than foliage. However, it has been noted in the literature that nectar and pollen in certain plant species harbor secondary plant compounds that are toxic to bees. And recently, scientists[3] reported the occurrence of pesticides in pollen. Further studies are needed to determine whether these residues are affecting honey bees either sub-lethally or acutely, and whether any sub-lethal effects may impact the immune response of bees.

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[3] van Englesdorp et al, 2010

Over the past year, given the knowledge of high pathogen loads in diseased bee colonies, ARS- and NIFA-supported researchers have increasingly focused on possible interactive effects between pesticides and pathogens, including Nosema and bee viruses, as noted in the analysis studies under Topic II. Two recent studies have demonstrated increased levels of the gut parasite Nosema following exposure to sub-lethal levels of imidacloprid. As noted previously, a survey of healthy and CCD-affected colonies revealed an elevated level of pesticides in wax and pollen, but the amounts were similar between failing and healthy colonies.

In addition, noteworthy bioassay studies, conducted by NIFA-funded scientists at the University of Illinois at Urbana-Champaign and University of Nebraska at Lincoln, revealed synergistic effects of different chemicals on honey bee health. The studies revealed that these compounds (such as the pyrethroid pesticide fluvalinate, the organophosphate pesticide coumaphos and the fungicide prochloraz, if used in combination) produced an increase in toxicity to bees over what should be expected from use of the products individually. These studies show that the pesticides interfere with the bees' detoxification processes, indicating that these pesticides can be safely used individually, but point to specific pesticide and fungicide combinations that may harm bees and should be avoided. If confirmed in follow-up studies, this information will be highly useful for informing bee health management plans that permit optimal crop pest and disease control with minimal impact on pollinators.

ARS researchers in the Areawide Project on Honey Bee Health have initiated a study to investigate possible interactive effects among pesticide and pathogen load, pests, and diet quality on honey bee health. Results, to become available in spring 2011, will help identify these possible interactive effects.

Researchers have completed sequencing and annotating the genomes of several major bee pests and parasites, including *Nosema ceranae* and *Nosema apis* as well as partial sequencing of the varroa mite genome. With industry support, ARS completed the *Nosema* spp. sequencing effort last year, and work continues with varroa, which is being supported by ARS and NIFA. Analysis is ongoing to pinpoint important genes and capitalize on specific vulnerabilities that will allow development of better control strategies for these pests and parasites.

Topic IV: Mitigation and Management Measures

Significant progress has been made, and work continues, in the area of mitigating the honey bee crisis. This work is highlighted by two national multi-year projects, the ARS Areawide Project on Honey Bee Health and a NIFA-funded CAP, through which researchers continue to test possible causes of CCD and develop strategies to improve bee management.

Efforts to enhance mite-resistant stocks and breed-resistant bees maintain an important component were conducted in the ARS Areawide Project and NIFA supported research efforts. In particular, research continues to focus on developing germplasm (i.e. bee lines) with the varroa sensitive hygiene (VSH) trait, shown to protect bees against the most significant bee pest, the varroa mite. As these efforts continue, varroa-resistant bee stocks will become increasingly available to beekeepers and will reduce the overall impact of this major known cause of bee

decline. Also along these lines, university scientists have several training efforts underway to help bee producers improve breeding and maintenance of healthy bee stocks.

The Areawide Project has also supported several studies informing honey bee diet recommendations for beekeepers. Studies have supported prior findings that supplemental protein feedings can strengthen honey bee colonies, while high-fructose corn syrup may cause colony stress.

Meanwhile, other researchers continue to produce tools and recommendations that help beekeepers limit the impact of common bee pests and pathogens. For instance, ARS researchers have developed chemical attractants to help control the small hive beetle. Other ARS research has shown that ozone—a common means of controlling contamination and rotting of agricultural products—can also be used to kill bee pests and pathogens.

Research is ongoing regarding the development of non-Apis pollinators intended to provide alternatives to honey bee pollination. It is important to note that ARS- and NIFA-funded scientists have made significant progress toward developing the blue orchard bee (*Osmia lignaria*) for almond pollination ("How to Manage the Blue Orchard Bee," Bosch and Kemp, USDA-NIFA Sustainable Agriculture Research and Education (SARE) and ARS, 2001). Development of the blue orchard bee may potentially reduce pressure on honey bee colonies which are the primary pollinators for almond. With funding from SARE, the Minnesota Department of Entomology, and the Xerces Society for Invertebrate Conservation, bee extension specialists and conservationists published a book in 2010 entitled "Managing Alternative Pollinators." This book provides the latest information on the importance of native bees to pollination of agricultural crops.

Pollinator studies have also focused on exploring bumble bees (*Bombus* spp.), seeking to determine the relationship between bumble bee and honey bee pests. Studies have revealed that bumble bees are affected by IAPV and other viruses and parasites that affect the honey bee. These findings may have potential implications for the origin of these factors in bee decline and may assist in understanding and ultimately controlling bee parasites.

To meet the need for an improved regulatory framework for honey bee imports/exports, APHIS and ARS have developed guidelines to coordinate regulation and importation of bees between Mexico, Canada, and the United States, seeking to avoid introducing new bee pathogens and parasites during export.

Also supporting this objective, APHIS has carried out a pilot national pest survey in three states (Hawaii, Florida, and California) and will expand it this coming year to 13 states. This survey will provide increased knowledge of pest and pathogen impacts on bee health and will determine if exotic new pests are present in the United States. Coupled with international collaboration (OIE (World Organization for Animal Health) and COLOSS (Colony Loss Prevention Working Group of the EU)) to identify similar patterns in bee health worldwide, this survey will both provide information that could inform actions to improve pollinator survival and allow the United States to better meet World Trade Organization (WTO) standards with regard to international trade.

To transfer these and other technologies to the field, a number of websites have been created, including the Bee Health Community of Practice eXtension website, which will contain Best Management Practices for beekeepers, along with several scientific publications to relay findings quickly and easily to beekeepers and other users (http://www.extension.org/bee%20health). These resources will facilitate dialogue and enhance information sharing as new discoveries are made regarding bee health and CCD.

Increasingly, CCD results have found a presence on a variety of social networking and public sites, from Facebook to Twitter and YouTube. These newer venues are an innovative mechanism to educate the public and raise awareness of the plight of pollinators.

The Appendix that follows provides specific results and findings from the past year of research, listed within the framework of the Action Plan.

APPENDIX: Specific Accomplishments by Action Plan Component

Topic I: Survey and Data Collection

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

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

ACCOMPLISHMENT

Definition of CCD. A recent publication (vanEngelsdorp et al. 2009, PLoS ONE, vol. 4, issue 8) described in detail the field symptoms used to define colonies as suffering from CCD. Symptoms include (1) the apparent rapid loss of adult worker bees from affected colonies as evidenced by weak or dead colonies with excess brood populations relative to adult bee populations; (2) the noticeable lack of dead worker bees both within and surrounding the hive; and (3) the delayed invasion of hive pests (e.g., small hive beetles and wax moths) and kleptoparasitism (stealing food) from neighboring honey bee colonies. A Descriptive Study gives a detailed account of how to diagnose CCD in the field and the use of laboratory tests for varroa mites and nosema that should also be excluded as causative if these parasites are present at low levels. This paper lays the groundwork for comparative studies as it clearly defines the parameters of CCD and thus helps refine the symptoms associated with this disorder and helps to guide hypothesis-driven research designed to determine causation.

FUNDING

National Honey Board
ARS Areawide Program
Pennsylvania Department of Agriculture
Pennsylvania State Hatch Funds
North Carolina Agriculture Foundation
NIFA

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2. Develop and conduct an expanded, systematic, nationwide, epidemiological survey, based on existing models.

ACCOMPLISHMENT

Colony Losses Remain at High Levels, Similar to Previous Two Beekeeping Seasons. ARS scientists and the Apiary Inspectors of America surveyed U.S. honey bee colonies and estimated total losses, from a variety of causes, to be more than 29 percent from the fall of 2008 through winter of 2009, representing absolute colony losses ranging from 580,000 to 770,000 hives. The 2009-2010 data indicate losses of approximately 34 percent. These data indicate a statistically similar loss rate to the 31 percent and 36 percent losses recorded during the 2006-2007 and 2007-2008 seasons, respectively. The 2008-2009 colony loss survey appeared in a special issue of the Journal of Apicultural Research (January 2010), and a preliminary report of the 2009-2010 study was released in May 2010.

FUNDING

ARS
AIA
Pennsylvania Department of Agriculture

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

1. Adapt the annual NASS honey production survey to include information on pollination services and colony losses.

   There are currently no resources for this activity at NASS.

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

ACCOMPLISHMENT

Workshop Held to Examine the Potential Effects of Pesticides on Honey Bees. APHIS hosted a USDA-funded workshop at EPA with ARS, California Department of Pesticide Regulation, Canadian Pesticide Management Regulatory Agency, and the French Agency for Food Safety, and university representatives to discuss laboratory, semi-field, and field toxicity testing protocols for honey bees and other non-target invertebrate pollinators. With the knowledge that pesticides could potentially have significant effects on bees, EPA recognizes the need for more comprehensive testing of some pesticides and possible revision of past standards. The proceedings of this workshop will be made available in the coming year.

FUNDING

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Topic II: Analysis of Existing Samples

Goal 1: Identify and characterize pathogens associated with CCD.

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; and
   - Integrated Virus Detection System (IVDS) for identifying pathogens by particle size.

ACCOMPLISHMENTS

High Virus Prevalence in Local and Migratory Honey Bees Demonstrated. University of Massachusetts researchers analyzed local and migratory honey bee hives from eastern Massachusetts for seven viruses and identified three with a significant presence in the samples. Over 98 percent of the bees were infected with at least one virus; of the seven viruses examined, black queen cell virus (BQCV), deformed wing virus (DWV), and sacbrood virus (SBV), in that order, had a high presence. These results will help researchers pinpoint the factors most likely to play a role in bee decline and will help target development of pathogen control strategies.

Methods Developed to Detect Virus Replication in Honey Bees. The assessment of virus effects on bee colony health requires not only the detection of viruses in bees and bee colonies, but also the indication of virus replication in the host. Scientists at the University of Massachusetts have developed a simple RT-PCR-based assay to detect the most active stage of virus replication—production of a material known as "negative strand, replicative intermediate"—and determined that 50-80 percent of the virus-positive bees had these intermediates. These results indicate that actual virus infection rates were much lower than previously understood.

FUNDING

NIFA

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Interactions of Israeli Acute Paralysis Virus (IAPV) and Deformed Wing Virus (DWV) on Honey Bee Health Examined. In a Cooperative Agricultural Project between ARS and the University of Illinois, scientists examined the interactions among IAPV, DWV, pesticides, and Nosema. Results showed that IAPV-infected bees exhibited 90-100 percent early (3-5 day post-infection) mortality rates and paralytic-like seizures before death. Behavioral changes included increased brood feedings and release of Nasanov pheromones, which draw bees back to the hive and are associated with worker recruitment to the hive or food and water sources. These results implicate the virus as being associated with both mortality and behavioral changes, which are possible links to CCD. The behavioral changes, in particular, deserve further investigation since CCD might be a consequence of such changes.

Studies also showed that bees exposed to neonicitinoid insecticides and fungicides at sub-lethal levels had impaired bee immune systems and increased levels of the viruses noted above. Since

CCD might be a result of such interactions and damaged bee immunity, further studies are planned to examine immune responses and to determine the mechanisms underlying these interactions.

Research is also characterizing levels of *Nosema ceranae* and *Nosema apis* to determine possible interactions and synergies with the viruses above.

FUNDING

NIFA
ARS

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High Pathogen Loads Identified in Diseased Bee Populations. Researchers compared 61 variables (e.g., adult bee physiology, pathogen loads, and pesticide levels) between healthy and diseased bee populations. They found no single CCD causal agent, but rather that CCD colonies had higher total pathogen loads and were infected with a greater number of pathogens than non-CCD colonies, suggesting that bee immune systems had been compromised (vanEngelsdorp et al., PLoS One, 2009). This work will pave the way for future studies to identify specific mechanisms leading to collapse and to reconstitute CCD in laboratory experiments, as well as strategies to mitigate pesticide and pathogen buildup and otherwise protect honey bee health.

FUNDING

National Honey Board
ARS Areawide Program
Pennsylvania Department of Agriculture
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NIFA-AFRI

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Diagnostic Tools Developed for High-Throughput Detection and Monitoring of Honey Bee Diseases. ARS and Pennsylvania State University scientists developed a new diagnostic tool for detection of Nosema infection in field honey bee samples. This new highly specific, sensitive test, based on an antigen capture assay that detects Nosema spore wall protein, is capable of detecting Nosema antibodies. Researchers are pursuing leads to transfer this technology to private industry.

FUNDING
ARS
NIFA

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Differential Susceptibility of Bees to IAPV. Honey bee workers of different ages were found to be differentially susceptible to IAPV infection. Newly emerged adult bees were highly susceptible to infection by IAPV with median mortality occurring at 46 h post emergence. The susceptibility declines as bees age, with 5- to 6-day-old bees exhibiting 40 percent mortality at 80 h. Bees parasitized as pupae (with normal wings) by varroa are approximately twice as susceptible to IAPV infections as non-parasitized bees.

FUNDING
USDA-NIFA

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

ONGOING RESEARCH
In Vitro Production of Bee Viruses. Research into the molecular biology, pathology, replication, and transmission of viruses in honey bees has been greatly hindered by lack of an in vitro, cell culture system for the propagation of these viruses. University of Massachusetts researchers are currently developing methods using insect cell lines to produce RNA viruses outside of the bee. At present, scientists have been able to produce double-stranded DNA molecules of deformed wing virus RNA genome by making cDNAs and are developing methods to express viral gene products. This will be useful for determining the role of viruses in CCD.

FUNDING
NIFA

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ARS-Ft. Pierce is producing IAPV and other viruses in bee larvae and pupae through microinjection of purified viruses. Progress has also been made in producing bee hemolymph cell cultures, with cells remaining viable for several months. Efforts to culture bee viruses in the cells have begun. If successful, this will provide a fast and readily available system for bee virus production.

FUNDING
ARS

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

Use standard sampling methods to analyze samples for tracheal and varroa mites and Nosema species.

ONGOING RESEARCH

Interactions between Pests, Pathogens, and Pesticides Being Investigated. With funding from the NIFA Coordinated Agricultural Project, scientists are examining the potential interactive effects of pests, pathogens, and pesticides in seven states across the United States. Researchers have been correlating infestation of varroa mite and small hive beetle with colony losses, seeking to determine patterns. To date, research confirms the detrimental effect of pest infestation on colony health. This long and complex study is critically necessary to evaluate the effects of interactions and synergies and bee health, which are little understood but suspected to play a role in CCD.

FUNDING

NIFA

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

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

AND

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

ACCOMPLISHMENTS

Link Between Queen Status, Brood Pattern, Varroa/Nosema, Pesticides, and Colony Health Studied. ARS and Pennsylvania State University (PSU) researchers are completing an epidemiological analysis of the relative risk of various conditions in colonies in three migratory operations. In a study of pesticide residues in bee colonies across 23 states, PSU researchers found numerous pesticides and metabolites in the hive samples studied, increasing the

opportunity for toxic interactions between the chemicals. Initial analysis discovered a relatively new condition in bee colonies—termed "entombed pollen"—a defensive behavior correlated with poor health and increased mortality rates (Journal of Invertebrate Pathology, 2009). This condition is thought to be the result of the fungicide chlorothalonil in colony pollen stores. Data analysis continues, with queen failure and diseased brood being critical predictors of colony mortality.

FUNDING

National Honey Board
Project Apis mellifera
Foundation for the Preservation of Bees
Pennsylvania Department of Agriculture
NIFA

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Pesticide Variability Analyzed. Scientists supported by NIFA's Coordinated Agricultural Project analyzed pesticide residues in bee hives and identified a variety of different chemicals and their metabolites in hives. Results indicate that exposure is highly variable depending on sampling location and that further research is necessary to understand the spatial and temporal variability.

FUNDING

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Insecticide Novaluron Found to Have Limited Effects on Bee Eggs. In studies on female alfalfa leaf-cutting bees, ARS scientists evaluated whether the insecticide novaluron could affect the health of bee eggs and larvae produced by exposed mother bees. Although bees fed high levels of the pesticide experienced significantly greater larval losses than unexposed bees, in follow-up studies using typical field treatment levels of the pesticide, mortality rates were similar for unexposed and exposed bees.

FUNDING

ARS
NIFA

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Rimon Affects Bee Egg Viability. Novaluron (Rimon) is a relatively new insecticide that is an insect growth regulator, labeled for lygus control in alfalfa fields during bloom. Some alfalfa leaf-cutting beekeepers have complained about poor bee returns in fields where novaluron was used, and laboratory trials have shown this pesticide to be toxic to larvae and eggs. Field experiments were conducted in 2009, in a collaboration between the University of Idaho and ARS, to evaluate the effect of novaluron (Rimon 0.83 EC) on alfalfa leaf-cutting bees. The mean number of bee cells in plots treated with novaluron and plots treated with another insecticide typically used by growers did not differ, but both the total number and the percent of pollen balls (an early stage mortality, or eggless cells) was higher in plots treated with novaluron, with about 20 percent more pollen balls in the novaluron treatment. The percentage of live larvae was also lower in novaluron-treated fields. Dissection of pollen balls revealed that plots treated with novaluron had a significantly lower percentage of live eggs. Novaluron did not appear to affect adult bee survival or nesting activity, but had a negative impact on egg viability and the number of live larvae at the end of the season.

FUNDING

ARS
NIFA
Chemtura Inc.

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Effects of Pesticides on Alfalfa Pollinators Studied. In large-scale field trials carried out by ARS scientists cooperating with Washington State University, scientists evaluated the effects of several insecticides on pollinators.

FUNDING

Washington State Alfalfa Seed Commission
Washington State Commission on Pesticide Registration
ARS

PROJECT CONTACTS

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ONGOING RESEARCH

Stationary Apiary Health Parameters Comparisons. In the Coordinated Agricultural Project (CAP), scientists assessed the presence and quantity of tracheal and varroa mites, viruses,

Nosema, and pesticides in wax, as well as quantity of pollen, queen health parameters, and brood and adult bee populations, evaluating possible linkages between pest/pathogen/pesticide presence and colony survivorship. Data show high variability among virus presence, and evaluations continue to identify possible linkages. There was also a high variability in the pesticide load in the pollen brought back to the hives.

Pesticide Levels in Honey Examined. CAP scientists are evaluating the type and level of pesticides in honey extracted from bee hives and in honey that overwinters with the bees. This overwintered honey is the source of carbohydrates for the bees during winter and pesticides present in the honey will be ingested by the bees. The miticides coumaphos and fluvalinate were observed more frequently and at higher concentrations than any other pesticide, and results indicate that coumaphos may have migrated from brood chamber use.

FUNDING

NIFA

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Goal 4: Develop analytical tools to assess bee health.

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

ACCOMPLISHMENT

Honey Bee Detoxification Genes Identified. Certain chemicals commonly found in plants may be toxic to bees, and University of Illinois researchers have found that genes in the honey bee genome may have a role in processing chemicals produced by the plant and encountered by honey bees when they feed on nectar. Recent studies revealed that several genes inside bees were activated when fed extracts of honey, pollen, and propolis. Findings indicate the possibility that genetic differences among honey bees could explain differences in their ability to detoxify compounds in nectars with high flavonoid content. This information could help beekeepers breed strains of honey bees that tolerate plants with toxic compounds in nectar and pollen.

Genes Associated With Pesticide Metabolism Identified. Using the honey bee genome sequence, University of Illinois researchers have identified genes that facilitate metabolism of pesticides. Genetic analysis showed that certain enzymes metabolized the varroa mite-killing product Apistan (fluvalinate). Work is ongoing to characterize the metabolites, and molecular modeling will help identify key enzymes involved in breaking down pesticides. This work may potentially explain why certain pesticides are relatively benign in the context of acute toxicity to bees. This research is consistent with prior findings when Apistan (fluvalinate) is not combined with other pesticides (see Goal 4: Elucidate the synergistic and sub-lethal effects of in-hive miticides on colony health), and could be used to assist in breeding efforts to develop more bees that may tolerate or resist pesticide exposure.

FUNDING

NIFA

PROJECT CONTACT

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Topic III: Research to Identify Factors Affecting Honey Bee Health, Including Attempts to Re-create CCD Symptomology.

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

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

ACCOMPLISHMENTS

Synergistic Effects of Pesticide Exposure on Pathogen Growth and Bee Health Discovered. There has been significant concern about possible interactive effects of various pesticides and pathogens on bees. When bees were exposed to the gut parasite Nosema and insecticide imidacloprid, researchers found evidence of an interactive effect on honey bee health. Worker bees exhibited up to a fourfold increase in Nosema levels when they originated from colonies that had been fed imidacloprid, indicating a subtle sub-lethal interaction between pesticides and pathogens. This research has been submitted for publication, and similar findings have recently been published from a study in France in Environmental Microbiology 2009.

FUNDING

ARS
NAPPC

PROJECT CONTACTS

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Interaction between Pesticides and Bee Viruses Found. Pennsylvania State University researchers found that consumption of sub-lethal levels of certain pesticides elevates viral titers in infected bees and appears to alter the immune responses of the bees. In cage studies, nurse or house bees were fed sugar solutions containing pesticides (myclobutanil, acetamiprid, fluvalinate or chlorothalonil) at dosages previously determined to be sub-lethal. These dosages approximated lower concentrations found in incoming pollen. The titers of several picorna-like viruses (deformed wing virus, black queen cell virus, and IAPV) were found to be elevated by one or several of the pesticides. The expression of several immune-related genes was altered by the pesticide exposure as compared to untreated bees that were also immune challenged. The identity of the viruses was confirmed via genome sequencing.

FUNDING

Pennsylvania Department of Agriculture.

PROJECT CONTACT:

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Movement of Imidacloprid in Trees Explored. APHIS has used soil and trunk injection of imidacloprid to treat hardwood trees in controlling the invasive Asian longhorned beetle. There is the potential for movement of the pesticide into pollen and nectar of treated trees. APHIS is collaborating with ARS to monitor the movement of imidacloprid into pollen and nectar and to evaluate the potential effects on honey bees and other non-target species over a 3-year period.

FUNDING
APHIS
ARS

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

ACCOMPLISHMENTS
Reduced Longevity of Adult Worker Bees Related to Coumaphos Exposure in Wax During Larval and Pupal Development. There was a significant reduction in adult bee longevity following exposure to 100 ppb of coumaphos in wax during the larval and pupal stages in worker honey bees. A 4-day reduction in summer bee lifespan was observed equaling 16 percent of the total lifespan of summer bees. Reduced adult longevity could impact honey production and or overwintering ability. This study has been accepted for publication in Apidologie.

FUNDING
ARS
Pennsylvania Department of Agriculture.

PROJECT CONTACTS
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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.

No accomplishments to report.

4. Test effects of supplemental protein and carbohydrate (e.g., high-fructose corn syrup (HFCS)) feedings on bee health.

See Topic IV, Goal 3, Objective 2.

ONGOING RESEARCH

Plant Resin Identified to Improve Bee Resistance to Fungal Toxins. Honey bees and their resource-rich nests are hosts to a wide range of fungi, including species that produce toxic substances called mycotoxins, which workers detoxify using gut enzymes. In bioassay experiments, bees that consumed extracts of propolis, a complex mixture of plant resins collected by bees and used as a general caulking material in the hive, had an enhanced capacity to detoxify mycotoxins. These results suggest that the enzymes involved in mycotoxin detoxification are enhanced by ingestion of propolis, which may serve a hitherto unrecognized role in honey bee health.

PROJECT CONTACT

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

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

These objectives are being done as part of the Areawide project. See Part IV-6.

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

1. Test pathogenicity of the following CCD-associated microbes against honey bees and non-Apis bees:
    - Viruses
    - Fungi (chalkbrood; stonebrood)
    - Microsporidia (Nosema)
    - Bacteria
    - Trypanosomes and other microbes

ACCOMPLISHMENTS

Viral Diseases and Varroa Mites Linked to Colony Losses. Since 2008, ARS scientists in Weslaco have collaborated with university scientists to determine key factors (e.g., parasites, diseases, and pesticides) contributing to honey bee colony losses in apiaries across the United States. First-year data from this Coordinated Agricultural Project (CAP) indicate that varroa mite infestation and viral diseases contribute strongly to colony losses.

FUNDING

ARS
NIFA

PROJECT CONTACT

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

Varroa Mite and Nosema Species Being Sequenced. ARS scientists in Beltsville have finished sequencing, annotating, and publishing the *Nosema ceranae* genome and have sequenced *Nosema apis*, with annotation and publication expected sometime in 2010. Also, with industry support, ARS initiated a genome project on Varroa destructor, the primary honey bee parasite, as part of a worldwide consortium. Further analysis of these results will allow researchers to identify genes implicated in virulence, viral disease, and chemical resistance and to develop new strategies to control their impact on bees.

FUNDING

ARS
NIFA

PROJECT CONTACTS

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

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

   No results to report at this time.

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.

   No results to report at this time.

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.

ONGOING RESEARCH

Interactive Effects of Pollen Quality, Pesticides, Pests, and Pathogens Being Studied. As part of the ARS Areawide Project on Honey Bee Health, researchers from ARS-Beltsville, U.S. Geological Survey-North Dakota, and the University of Minnesota are investigating the interactive effects of pollen quality and availability, pesticide load, and relevant pests and pathogens on honey bee health. Researchers are comparing the performance of honey bee colonies in selected sites in North Dakota during the summer with their ability to overwinter and be available for almond pollination in the early spring. Results will help identify possible interactions between bee stressors and colony health.

FUNDING
ARS
USGS

PROJECT CONTACTS
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Interactive and Sub-lethal Effects of In-hive Miticides Evaluated. Scientists at the University of Nebraska-Lincoln have been studying the synergistic effects of various varroacides and fungicides on honey bee health, testing for lethal and sub-lethal effects. Research indicated that combinations of varroacides that are detoxified by the cytochrome P450 system (e.g., the miticides tau-fluvalinate, coumaphos, and fenproximate) tend to be significantly more toxic when applied in combination. A similar interaction occurred when bees were exposed to a cytochrome P450-inhibiting fungicide and a varroacide that was metabolized by P450s concurrently. Normally these varroacides are well tolerated by honey bees, but pre-treatment with a P450-inhibiting fungicide greatly increases mortality in bees by a synergistic ratio of nearly 2000X in the most extreme case. While this interaction was found using fungicide doses substantially higher than would likely occur through consumption of fungicide-contaminated pollen (Mullin et al. 2010, PLoS ONE 5: e9754) the potency of this interaction raises concerns about lethal and sub-lethal effects that may be occurring at field-relevant doses. The results suggest that honey bees should not be treated with more than one P450-detoxified varroacide concurrently or sequentially since varroacides are known to accumulate in beeswax. They also suggest that beekeepers should avoid applying P450-detoxified varroacides when honey bees are placed in orchards or other crop settings where exposure to P450-inhibiting fungicides is likely. These results may be useful for incorporation into a bee health management plan. Additional studies are needed to determine the range of bee exposure to fungicides and varroacides in the field and how these concentrations affect the bees.

FUNDING
NIFA

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Topic IV: Mitigation and Preventative Measures

Goal 1: Develop best management practices for honey bees.

1. Develop best management practices for migratory beekeeping.

ONGOING RESEARCH

Best Management Practices Being Established for Bee Conservation. In July 2009, NIFA's Coordinated Agricultural Project (CAP) and the USDA-ARS Areawide Project launched the Bee Health CAP website (www.beeccdcap.uga.edu), a university eXtension network designed to serve as a clearinghouse for objective, research-based information. The site provides a platform for communities of experts to share articles and resources for download. A Best Management Practices Guide for honey bee health is in progress. For more information on the eXtension site, see Goal 7 (page A-30).

FUNDING

NIFA
ARS

PROJECT CONTACTS

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Adoption of Sustainable Bee Management Practices Studied. Researchers are surveying beekeepers to determine how many implement best management practices in their operations. Using a web-based assessment, beekeepers are being evaluated in the winter of 2009-2010 and again in 2012. This will provide critical information about how well scientists and beekeepers are able to improve the needs of the bee industry.

FUNDING

NIFA

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

ACCOMPLISHMENTS

High Ozone Concentrations Found to Kill Insect Pests, Degrade Pesticides, and Kill Pathogen Spores. High concentrations of ozone have been used for many years to decontaminate water and to control rot in stored agricultural products. ARS carried out experiments to determine the concentrations needed to decontaminate honeycomb containing wax moths, pesticides, and pathogens. Ozone was very effective in killing insect pests of stored comb, primarily the wax moth, and was somewhat effective in removing pesticide residues, but very high concentrations, used in combination with heat and humidity, were needed to eliminate pathogens like foulbrood. These studies will assist in developing effective ozone treatment strategies to reduce possible bee stressors in used honeycomb.

FUNDING

ARS

PROJECT CONTACT

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Chemicals Associated With Mite Control Identified. ARS scientists in Gainesville, Florida, have identified two chemicals produced by larval bees that cause reproductively mature mites to move from adult nurse bees into larval cells. These chemicals may be useful as lures to trap varroa mites and disrupt their ability to find larvae. ARS is in the process of patenting the chemicals, which have significant potential to help manage these pests.

FUNDING

ARS

PROJECT CONTACT

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Trap Developed for Small Hive Beetle. ARS-Gainesville scientists have developed and demonstrated the effectiveness of a trap for the small hive beetle. The trap can be placed at the entrance to bee hives to capture larvae of the small hive beetle, thus breaking the cycle of beetle infestation of hives by removing larvae without the need for pesticides. The trap has been demonstrated at beekeeper industry meetings as an effective new pest management technology.

FUNDING

ARS

PROJECT CONTACT

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Expanded Small Hive Beetle Range Demonstrated. The small hive beetle was believed to be dependent primarily on bee pollen, honey, and brood for nutrition, and control efforts focused primarily on areas near honey bee hives. However, ARS scientists have found that the beetle occurs in woodlands far from managed honey bee colonies and that the bee pest can grow on a wider range of foods than previously thought. These findings indicate that the pest's expanded

range may make it much more difficult to control and will aid scientists in improving their control strategies.

FUNDING
ARS

PROJECT CONTACT
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Small Hive Beetle Monitoring and Management Techniques Enhanced. Monitoring the small hive beetle is necessary to manage this pest effectively and mitigate the damage it causes. ARS scientists in Gainesville have found that a trap used to monitor the small hive beetle is most effective when placed in shaded rather than open locations, even if located farther from bee hives. These measures will be used by beekeepers to increase the effectiveness of their monitoring and management efforts.

FUNDING
ARS

PROJECT CONTACT
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Chemical Attractants for Small Hive Beetle Identified. In cooperation with the University of Florida, ARS scientists identified volatile chemicals associated with bee samples that were attractive to the small hive beetle. These chemicals could be useful in developing better management strategies for the pests.

FUNDING
ARS

PROJECT CONTACT
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Non-Chemical Control for Small Hive Beetle in Development. ARS scientists in Weslaco signed a new agreement with Mexican collaborators to develop new non-chemical control methods for the small hive beetle. Researchers will test purified Bt toxins shipped from Mexico for effectiveness in controlling beetle populations in bee larvae.

FUNDING
ARS

PROJECT CONTACT
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Chalkbrood Resistance Genes Discovered. ARS scientists in Weslaco have discovered genes involved in honey bee resistance to chalkbrood, a major disease affecting honey bees. This finding may lead to the development of new gene-specific pharmaceuticals to fight this disease.

FUNDING
ARS

PROJECT CONTACT
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Major Bee Pathogen Sequenced. Recently, ARS-Weslaco scientists finished sequencing one of the major honey bee pathogens, *Ascosphaera apis*, the causative agent of chalkbrood disease. Research focuses on discovering genes involved in the invasion and pathogenesis of the bee host. This information will lead to the development of new disease control methods.

FUNDING
ARS

PROJECT CONTACT
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3. Establish guidelines for floral gardens to maintain stronger honey bees.

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

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

ACCOMPLISHMENTS
New Detection System Developed for Studying Disease Transmission. ARS developed a new detection system to assess the number of chalkbrood spores in bee samples. Using this system, scientists evaluated contamination levels in various bee management systems and studied the ecology of disease transmission in farmers' fields. Results will be used to develop improved strategies for disease management.

Immune Systems of the Alfalfa Leaf Cutting Bee Studied. Honey bee immune systems are known to be weaker and smaller than those in other insects, making them more susceptible to pests and diseases. After developing a library of expressed genes from healthy and chalkbrood-infected alfalfa leaf-cutting bees, ARS scientists identified 116 immune response genes for this solitary bee. This finding will allow scientists to greatly improve knowledge of disease resistance in bees—about which very little is known—in order to develop better disease control methods.

High Concentrations of Ozone Found to Kill Chalkbrood. High concentrations of ozone have been used for many years to decontaminate water, and to control rot in stored agricultural products. ARS conducted experiments to determine the concentrations needed to decontaminate leaf-cutting bee nesting boards. The primary pathogen of this bee—chalkbrood—was eliminated with ozone concentrations obtainable in on-farm fumigation chambers, making this a promising strategy for promoting bee health.

FUNDING
ARS

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Honey Bees and Israeli Acute Paralysis Virus (IAPV) and Deformed Wing Virus (DWV) Studied. Researchers found that IAPV infects bumble bees and reduces colony lifespan. Bumble bees dying from IAPV infections returned to the colony to die inside the hive, unlike honey bees, which died outside the hive. Bumble bees, however, also exhibited the same paralytic-like seizures as observed in honey bees.

Non-Apis Bee Pathogens Identified and Characterized. University scientists have been studying several parasites associated with bumble bees—*Crithidia bombi* (a protozoan parasite of bumble bees), *Nosema bombi*, and the tracheal mite—to determine their transmission patterns, occurrence and distribution, and origin. Although the protozoan was found in commercial bumble bees, it does not seem to be transmitted from wild bumble bees and managed honey bees in the area of Massachusetts investigated. For the protozoan, sequencing studies indicate great similarity between American bumble bees and European bees. Results from these studies will assist in understanding, and, ultimately, controlling bee parasites.

FUNDING
NIFA

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Lethal and Sub-lethal Effects of Insecticides on Non-Apis Bees Elucidated. Using totally enclosed field cage systems, University of Massachusetts scientists assessed the impact of imidacloprid in flowering low bush blueberry on commercial bumble bees (*Bombus impatiens*). After bloom ended, the bees were released to forage on surrounding plants for the remainder of the spring and summer. Results suggest that imidacloprid reduced brood (immature bees) at the end of bloom but did not seem to affect the survival of adult workers.

FUNDING
NIFA

PROJECT CONTACT
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2. Establish guidelines for maintaining stronger populations of non-Apis bees in agricultural systems, home gardens, and wild lands.

ACCOMPLISHMENTS
Blue Orchard Bees Developed to Supplement Honey Bees for Almond Pollination. ARS scientists developed guidance for the management of blue orchard bees for almond pollination and initiated a demonstrated project with university extension personnel. Guidance on managing blue orchard bees for almonds is now available to promote use of this bee and reduce pressure on honey bee colonies.

FUNDING
ARS
University of California-Davis.

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Alfalfa Leaf Cutting Bee Improved With Proper Stocking Density. The alfalfa leaf cutting bee is commonly used to pollinate alfalfa for seed production in the United States and Canada, but these bees frequently have problems with disease, parasites, predators, and unexplained mortality of eggs and small larvae. ARS researchers have shown that typical release rates for alfalfa leaf cutting bees in alfalfa pollination are too high, decreasing bee efficiency in pollinating flowers and causing them to produce fewer healthy offspring. When too many bees are placed in fields, they compete for food and nesting sites, which results in poor bee returns. Research provides information on optimal numbers of bees needed for effective pollination.

FUNDING
ARS

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Historic Range Maps for Native Bumble Bees Constructed. Currently, at least 5 North American bumble bee species are disappearing, with one species thought to have gone extinct within the last 5 years. Despite the rapid loss of these important pollinators, little is known about the cause of their demise. ARS scientists developed a method for determining the extent and cause of these losses using geographic information systems (GIS) and historic records of bumble bee collections, and created models that can predict the geographic range of the bees at different points of time in history. This new method could be used to help scientists understand the effects of climate change or the accidental introduction of pathogens on other pollinators as well.

FUNDING

ARS

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Fire Shown to Increase Wild Pollinator Diversity. Controlled burns constitute one method used to manage fuel loads in natural areas, but the effects on pollinators remains unknown. ARS scientists completed a 2-year study in Zion National Park, home to 474 different kinds of bees, comparing bee pollinators in adjacent plots of burned and unburned forest and shrub land. In most cases, bee abundance was significantly greater in the burned areas 2 to 5 years after the burn. Burns thus represent opportunities for increasing pollinator populations during forest regeneration.

FUNDING
ARS

PROJECT CONTACT
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Economic Impact of Alternative Pollinators Evaluated. ARS scientists have developed a supply and demand model to evaluate the economic impact of introducing a new pollinator (in addition to honey bees) into orchard systems for almond and cherry production. Due to the differences in these crops, they are differentially affected by increases in production acreage and bee supply, and these results will help determine how alternative pollinators can be used effectively to reduce demand for honey bees.

FUNDING
Utah State University
ARS

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

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

ACCOMPLISHMENT
USDA Varroa-Resistance Stocks Found Well Suited for Commercial Pollination. In a year-long examination of USDA's varroa-resistant stocks for commercial pollination of almonds, apples, blueberries, and cranberries, ARS scientists tested Russian honey bees and bees containing a varroa-resistant trait (varroa sensitive hygiene, VSH) for size and productivity. At every pollination set, researchers found USDA stocks to be as large and productive as control stocks, making them well suited for commercial pollination.

FUNDING
ARS

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ONGOING RESEARCH
Improved Management Strategies for Russian Honey Bees Being Developed. ARS scientists are evaluating management procedures for pollination of Russian honey bees. Scientists are studying how quality and quantity of feed, hive size, and winterizing impact colony health and development. When optimized, these procedures will be integrated into the breeding program for improved bee health.

FUNDING
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Mite-resistant Bees With Varroa Sensitive Hygiene (VSH) Trait Selected For Migratory Crop Pollination. ARS breeders at Baton Rouge are developing a population of bees with the VSH trait, which makes them less susceptible to the varroa mite. Research shows that these bees perform well when used in intensive migratory crop pollination. This selected germplasm is expected to be released for use by commercial beekeepers to improve the resistance of their bees to this troublesome pest.

FUNDING
ARS

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See Goal 4, Objective 1: Identifying genes that confer resistance to Varroa and pathogens, and genes that respond to biotic challenges.

FUNDING
NIFA

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

Breeding for Hygienic Behavior. University of Minnesota researchers and extension specialists are evaluating commercial honey bee stocks for the level of hygienic behavior, a trait which is associated with the bee's resistance to varroa mite. A pilot certification program will be initiated for the hygienic trait. A "Tech Transfer" team is forming in California to assist bee breeders with stock selection for hygienic behavior and to reduce chemical use in breeder colonies. It is envisioned that this team will serve as a model for the Nation in producing local, regionally adapted queens that are resistant to mites.

FUNDING

National Honey Board
California Almond Board

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ONGOING WORK

Genetic Techniques Used to Improve Bee Breeding. The University of Tennessee has replaced half its bee stock with varieties resistant to varroa mites (either the Varroa Sensitive Hygiene (VSH) strain or with hybrids raised from VSH queens). Equipment and apiary expansion is underway to conduct bee breeding demonstration workshops in 2011 and 2012. Workshop materials will be obtained or developed for inclusion on www.eXtension.org for use in any bee breeding workshop. These efforts will increase the use of robust bee stocks.

FUNDING

NIFA

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Groundwork Established for a Sustainable Market for Genetically Improved Queens. Researchers are working to train beekeepers in stock selection (e.g., VSH bees) and breeding techniques, as well as management and testing practices to reduce impact from pests, pathogens, and chemicals. Specifically, scientists are teaching a 2-day Queen Rearing Short Course every summer at the University of Minnesota to assist bee breeders. This course combines lecture and hands-on field work.

In 2008 and 2009, researchers conducted on-site visits with 17 queen producers in northern California. These operations produce at least half of the commercially available queens in the Nation and thus have major impact on honey bee genetics and improvement nationally. Scientists demonstrated how to test their stock for hygienic behavior, how to test their colonies for Nosema, and provided best management practices, including reducing chemical inputs into their breeding stocks.

Washington State University and University of California at Davis researchers have imported selected stock for two Carniolan and Italian bees for breeding purposes. In the future, the team will bring in a third subspecies favored by beekeepers, the Caucasian.

Over the 2-year study, two things became very evident: 1) bee breeders are very competent and produce high-quality, well-mated queens to supply beekeepers throughout the Nation; 2) breeding for pathogenic resistance is highly complex, while maintaining productive characteristics and pollination efficiency requires professional assistance to help bee breeders improve stock selection, enhance genetic diversity, and perform disease diagnostics.

FUNDING

NIFA

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

No accomplishments to report at this time.

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

See Topic IV, Goal 3, Objective 2.

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

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

ACCOMPLISHMENT

Genetic Analysis Completed and Tools Developed. Scientists are studying candidate genes that influence grooming behavior and confer resistance to varroa mites, allowing us to eventually identify genes that influence resistance and potentially lead to marker-assisted selection to speed breeding for varroa mite-resistant bee colonies. Scientists also developed a new laboratory assay

to speed the genetic selection for the Varroa Sensitive Hygiene (VSH) trait, which confers resistance to honey bees.

FUNDING
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2. Develop new methods of managing parasites and pathogens.

ONGOING RESEARCH
Identifying Genes That Confer Resistance to Varroa and Pathogens, and Genes That Respond to Biotic Challenges

See Goal 4, Objective 1 above.

Deliverables for this objective are expected next year.

FUNDING
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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.

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.

2. Explore opportunities to change regulations based on new molecular detection systems. For example, if a virus is shown to be pathogenic and reliable new detection methods are

found, movement of virus-contaminated bee stocks may be regulated by bodies such as OIE.

ACCOMPLISHMENTS

New Molecular Assay for Resistant Bacterial Strains Developed. ARS identified the mechanism of resistance of the bacterial honey bee pathogen (for American foulbrood) to the antibiotics oxytetracycline (OTC) and developed a new molecular assay for detecting OTC-resistant bacterial strains in bee samples. This new identification method is now available for regulatory officials considering ways of preventing the spread of resistant bacterial strains in bee shipments.

FUNDING

ARS

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Guidelines for Bee Imports Developed. ARS began working with the North American Plant Protection Organization to coordinate regulation and importations of non-Apis bees from Mexico, Canada, and the United States. Initial guidelines were developed for the safe importation of bees to avoid the accidental release of bee pathogens and parasites and to avoid the introduction of bee species that may cause a loss of native bees.

FUNDING

ARS

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

ACCOMPLISHMENT

Pilot Survey for Bee Diseases, Parasites, and Pests. APHIS, in collaboration with ARS, conducted a pilot survey in 2009 in Florida, California, and Hawaii to validate sampling protocols used to determine which diseases /parasites/pests of honey bees are and are not present in the United States. A limited national survey is being conducted in 2010 in 13 states.

FUNDING

APHIS
ARS

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

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

ACCOMPLISHMENTS

Areawide Project Initiated. ARS is continuing an Areawide Project on Honey Bee Health across multiple ARS locations (Tucson, Arizona; Beltsville, Maryland; Weslaco, Texas; and Baton Rouge, Louisiana). The project has several aspects, including documenting the impact of migration on bee colonies, examining the effects of supplemental feeding on colony health, developing more resistant bee lines and improved methods for their management, and developing better control methods for honey bee pests. Ultimately, the Project aims to develop a set of best management practices for migratory beekeepers to reduce stress on their bee colonies, thereby enabling bees to ward off threats. Specific results of these individual research projects are described elsewhere in this report.

FUNDING

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Brood Pheromone Shown to Improve Colony Strength. ARS scientists in Weslaco, Texas, are testing a new brood pheromone device in honey bee colonies to improve the health of honey bees, as well as to improve crop pollination. In a cooperative agreement with Contech, Inc. (formerly PheroTech International Inc.), ARS carried out field trials showing that treated colonies experienced increases in pollen collection and population.

FUNDING

ARS
Contech, Inc.

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

ACCOMPLISHMENT

Effects of Supplemental Protein Feeding on Colony Growth Assessed. Cranberry pollination is extremely stressful to honey bees, providing minimal nutrition for the amount of work performed. ARS scientists studied whether supplemental feeding with the MegaBee® protein supplement during cranberry pollination could reduce colony losses and improve colony population growth. Results showed that colonies fed the protein supplement grew more than those that were not fed, indicating that even a relatively small addition of supplemental protein to colonies during cranberry pollination improves their growth and survival.

FUNDING

ARS
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Effects of Protein Supplements on Worker Physiology and Immune Response Examined. To evaluate the potential benefits of nutritional supplements on bee health, ARS scientists compared protein levels, endocrine development, and immune response in adult worker bees that were fed protein, pollen, and high-fructose corn syrup supplements. Results showed that protein and pollen supplements produced similarly positive effects, but bees fed high-fructose corn syrup had significantly reduced immune responses. This study will help beekeepers improve management strategies for their bees.

FUNDING

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

1. Develop, maintain, and preserve a secure Web-based site for scientific collaboration (SharePoint).
2. Develop, maintain, and update a Web-based public Internet site, e.g. eXtension or PIPE (Pest Information Platform for Extension).

ONGOING WORK

Best Management Practices Being Established for Bee Conservation. NIFA and ARS (Areawide Project researchers) have facilitated several extension and outreach efforts to inform the public of CCD research findings and updates. In July 2009, NIFA's Coordinated Agricultural Project (CAP) and ARS launched two important new sites: The Bee Health Community of Practice CoP: http://www.extension.org/bee%20health. This is a university eXtension network designed

to serve as a clearinghouse for objective, research-based information. The site provides a platform for communities of experts to share articles and resources for download. A Best Management Practices Guide for honey bee health is in progress. For more information on the eXtension site, see Goal 7. In addition, the University of Georgia developed a new site defining the Managed Pollinator CAP Project including the following: background about pollinator decline and goals, objectives and management plan for this team approach of 22 investigators to reverse pollinator decline. See the CAP project website at www.beeccdcap.uga.edu.

FUNDING

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ONGOING WORK

Social Media Outreach Initiated. Researchers are pursuing additional outreach opportunities using social networking sites and public Web pages. Honey bee health is featured on YouTube at http://www.youtube.com/beehealth. Researchers also are now implementing pages on Facebook, Twitter, and Second Life; on beekeeper Web forums such as beesource.com; and on Wikipedia.

FUNDING

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