



*Colony Collapse Disorder*  
Progress Report

CCD Steering Committee  
June 2009

## CCD Steering Committee Members

### *Federal:*

Kevin Hackett	USDA Agricultural Research Service (co-chair)
Rick Meyer and Mary Purcell-Miramontes	USDA Cooperative State Research, Education, and Extension Service (co-chair)
Robyn Rose	USDA Animal and Plant Health Inspection Service
Doug Holy	USDA Natural Resources Conservation Service
Evan Skowronski	Department of Defense
Tom Steeger	Environmental Protection Agency

### *Land Grant University:*

Bruce McPheron	Pennsylvania State University
Sonny Ramaswamy	Purdue University

*This report has been cleared by all USDA agencies involved, and EPA. DoD considers this a USDA publication, to which DoD has contributed technical input.*

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## Executive Summary

*Mandated by the 2008 Farm Bill [Section 7204 (h) (4)], this first annual report on Honey Bee Colony Collapse Disorder (CCD) 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), USDA's Agricultural Research Service (ARS) and Cooperative State Research, Education, and Extension Service (CSREES) 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) mitigative and preventive measures.**

***Topic I: Survey and (Sample) Data Collection:*** Several survey and data collection efforts are underway to provide baseline data for CCD, resulting in better defined CCD symptoms, documented evidence of increasing honey bee losses, and evidence of increased pathogen and pesticide levels in colonies with poor health.

***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 the lower levels in non-CCD colonies. This work has provided evidence that a combination of stress factors is likely to set off a cascade of events in the colony where weakened worker bees are more susceptible to pests and pathogens.

***Topic III: Research to Identify Factors Affecting Honey Bee Health, Including Attempts to Recreate CCD Symptomology:*** Based on survey and analysis results indicating that many pesticides, parasites, and pathogens may be involved in CCD, efforts continue to explore the role of these factors. Findings to date indicate the sub-lethal effects of two common miticides on honey bees, as well as a synergistic effect of two pesticides (where the combination of the two compounds was shown to be more toxic than either compound alone), indicating the reality of these threats to bees and need for further research. Studies have also confirmed suspected links between poor colony health and inadequate diet and long distance transportation, indicating that both supplemental protein diets and natural pollen feedings can increase colony strength and offset the negative impacts of stresses such as pests and pesticides.

***Topic IV: Mitigative and Preventive Measures:*** Efforts to mitigate the honey bee crisis are highlighted by two national multi-year projects, the ARS Areawide Project on Honey Bee Health and a CSREES funded Cooperative Agricultural Project (CAP), which are allowing researchers to test various hypotheses related to poor bee health and provide

further insight into the problem and strategies for better bee management. Key accomplishments to date include the development of new, varroa-mite-resistant bee stocks, a new strategy (comb irradiation) to reduce pathogen levels, and several alternative pollinators to honey bees. 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.

During the past two years, numerous causes for CCD have been proposed and investigated. Although the cause of CCD is still unknown, research has lent credence to the hypothesis that CCD may be a syndrome caused by many different factors, working in combination or synergistically. Looking ahead, studies will focus increasingly on combinations and synergistic effects of factors in causing CCD.

More detailed findings follow in the body of the report.

## Colony Collapse Disorder Progress Report

This first annual report has been prepared in response to the 2008 Farm Bill, Section 7204 (h) (4), which directed the Secretary 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.”

### Introduction

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, or CCD. In response to this problem, Federal and State government, university, and private researchers, led by USDA’s Agricultural Research Service (ARS) and Cooperative State Research, Education, and Extension Service (CSREES), mobilized to define an approach to CCD, an effort resulting in formation of the CCD Steering Committee, and its 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 that addresses the CCD problem.

During the past two years, numerous causes for CCD have been proposed, but it now seems clear that no single factor alone is responsible for the malady. To date, researchers have documented elevated pathogen levels and a wide array of pesticides present in wax and pollen in both CCD-affected and non-affected apiaries, with none linked definitively to CCD. Additionally, tests for known honey bee parasites (varroa mites, honey bee tracheal mites, *Nosema* spp.), which have posed significant problems for beekeepers and were once highly suspected to play a major role in CCD, have not found these parasites, by themselves, at sufficient levels to explain the problem. Thus, the cause of CCD is still unknown, but research is beginning to lend credence to the hypothesis that CCD may be a syndrome caused by many different factors, working in combination or synergistically; a prime example is the finding that varroa mites can vector viruses to honey bee colonies, of which elevated levels are highly associated with CCD. Some studies are focusing on combinations and synergistic effects of factors, such as the synergistic effects of *Nosema* and pesticides, and of pesticides and other pathogens, and will continue based on the groundwork being laid.

While the causes of CCD have not been fully illuminated, the research response is coordinated and dedicated to solving this issue, while improving pollinator health. Funding from ARS and CSREES, with additional contributions by a number of other sources, including the National Honey Board, the Almond Board of California, Burt’s Bees, Haagen-Daz, the North American Pollinator Protection Campaign, and others, is

resulting in a variety of new studies and new expertise working on bee health issues. The fruits of this increased research effort are being published and a new e-Xtension website is being assembled to provide results 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) mitigative and preventive measures.** A summary of progress in meeting Action Plan objectives over the past two years is provided below for each of these topic areas.

***Topic I: Survey and (Sample) Data Collection***

In response to an immediate need for a baseline for both bee production and health, several survey and data collection efforts are underway. These collection efforts have better defined CCD symptoms, as documented in two forthcoming publications, and have produced evidence of increasing honey bee losses [31% (2007); 35% (2008)] beyond the already-high losses experienced in 2006 (20-25%); no data for 2009 is yet available. Surveys have also documented increased pathogen and pesticide levels in colonies with poor health.

Work will continue to assess the status of honey bee health and refine CCD symptomology. The Environmental Protection Agency (EPA) is considering a workshop on assessing risks of systemic pesticides to bees, and the Animal and Plant Health Inspection Service (APHIS) and the National Agricultural Statistics Service (NASS) are laying the groundwork for proposed national and local surveys on colony health.

***Topic II: Analysis of Existing Samples***

With funding from Federal and numerous nongovernmental sources listed above, ARS has led completion of an initial analysis of collected bee samples (CCD- and non-CCD-affected). From these samples, a better picture of the factors involved in CCD is emerging. Published and submitted reports have noted the high number of pathogens, pesticides (over 73 different types), and parasites present in CCD colonies, and the lower levels in non-CCD colonies. The role each of these factors plays in CCD is the subject of on-going studies under Topic III; however, it is becoming apparent that no one factor alone is responsible for CCD and that a combination of stress factors is likely to set off a cascade of events in the colony where weakened worker bees are more susceptible to pests and pathogens.

Since publication of the CCD Action Plan, several new diagnostic services have been made available to beekeepers, some free of charge and others on a fee-for-service basis. This capability is allowing beekeepers several options to monitor bee health and make more informed pest management decisions. Increased sample analysis has demonstrated that levels of *Nosema* (a common parasite of honey bees) are elevated during certain times of the year but cannot be directly tied to CCD-related losses, and has also made the first documented find in the United States of a European virus (*Varroa destructor* virus, or VDV-1). Several surveys have linked elevated levels of various viruses, e.g., Israeli acute paralysis virus (IAPV), Kashmir bee virus, etc., to poor bee health, but to date have not identified a single virus or other pathogen that can account for CCD.

In addition to viruses, analysis has also been focused on identifying pesticides and environmental contaminants, which can be found in honey bee colonies and are likely affecting bee health. Foraging bees are great samplers of the environment and bring back most things they encounter, even when present at extremely low levels. The Agricultural Marketing Service's (AMS) excellent analytical capabilities, which can detect contaminants at very low levels, have found a wide range of contaminants, including miticides used by beekeepers, and agricultural insecticides, fungicides, and herbicides. The three most common compounds identified are two miticides, fluvalinate and coumaphos, followed by the compound chlorpyrifos. Although no definitive conclusions will be available for several months, preliminary results are becoming available. For instance, results may indicate that different pesticides have different effects: At least one may be associated with increased prevalence of CCD, and another has been linked with decreased prevalence; the latter association may be due to more vigilant pest control by beekeepers. This research paves the way for further studies to assess these associations more definitively and to develop effective recommendations for pesticide use that balance its risks and benefits in maintaining colony health. Numerous studies are already examining the effects of these pesticides on bee health in greater detail, with more definitive conclusions expected in the coming months (Topic III).

To further the assessment of bee health and CCD, new analytical tools are being made available and developed. For example, scientists have identified molecular markers associated with stress, a possible genetic marker for CCD-affected colonies, and unique sonic signals that may be emitted by colonies in poor health. This work continues.

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

Research efforts have not identified any single cause as linked to CCD. Efforts continue to identify factors suspected to play a role in causing CCD, alone and in combination, particularly pesticides, parasites, beekeeping practices, pathogens, and to a lesser extent, other pests.

Pesticides have been a prime suspect in CCD research due to the known weakness of honey bees in detoxification (a likely evolutionary consequence of their habit of feeding on nectar and pollen, plant materials that are low in toxin levels). Although there are no conclusive findings to date, research continues to follow up on the analysis studies (Topic II) examining the effects of individual compounds and combinations of pesticide use on bee health to identify linkages to CCD. To date, scientists have demonstrated a synergistic effect of two pesticides in at least two studies, where the combination of the two compounds was shown to be more toxic than either compound alone. In addition, sub-lethal effects of the two common miticides, fluvalinate and coumaphos, have been demonstrated, and scientists are testing a possible link to a depressed immune system. Additional research is exploring a possible link between sub-lethal pesticide exposure and increased pathogen levels in honey bee colonies, which will help determine whether certain pesticides are indirectly contributing to poor colony health and CCD. Results will



be used to develop improved recommendations for beekeepers on pesticide use and exposure.

Beekeeping practices such as inadequate diet and stressful transportation are other key suspected factors in CCD. Results to date indicate that both may indeed be linked to poor bee health and are being remedied by new options and recommendations to beekeepers to improve the health of their colonies. For example, supplemental protein feeding has been shown to increase colony strength and offset the negative impacts of varroa mites, allowing a colony to tolerate higher parasitism rates. Additionally, natural pollen, which contains the flavanol quercetin, was shown to increase tolerance to pesticide exposure and thus may be a useful feeding strategy for beekeepers. Meanwhile, studies identified a harmful substance (hydroxymethylfurfural, or HMF) in some high fructose corn syrup (HFCS) samples (particularly from syrup exposed to high temperatures in truck tanks). These results have provided a possible explanation for alleged linkages between HFCS feedings and poor bee health and led to recommendations for beekeepers regarding proper storage of HFCS. As for migratory beekeeping, transportation of colonies over long distances has been shown to increase brood mortality, and studies are now investigating links between transportation and pathogen abundance. If suspected linkages are confirmed in these studies, recommendations will be developed for beekeepers to reduce losses directly or indirectly related to colony transportation.

While the honey bee tracheal mite and the small hive beetle have not been demonstrated to play a role in CCD, it seems clear that many pathogens may be involved in CCD, and their respective roles are being explored. One such pathogen includes the virus IAPV, which was the best predictor of CCD in one initial survey. ARS scientists are working to sequence two microsporidian parasites (*Nosema ceranae* and *N. apis*) as well as the varroa mite. Scientists have finished sequencing and annotating *N. ceranae*, with results to be published in the spring of 2009, and are sequencing *N. apis*, with results expected sometime in 2009. Also, with industry support, ARS initiated a genome project on *Varroa destructor*, the primary honey bee parasite, as part of a worldwide consortium to identify mite genes implicated in virulence, viral disease, and miticide resistance. Results from these sequencing efforts will support analysis concerning these factors' respective roles in CCD and the identification of vulnerabilities to promote new strategies for pest and disease management.

***Topic IV: Mitigative and Preventive 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 CSREES funded Cooperative Agricultural Project (CAP), which will allow researchers to test various hypotheses related to poor bee health and causes of CCD and provide further insight into the problem and strategies for better bee management. Also under development is a set of Best Management Practices that will provide beekeepers with specific guidelines to improve the health of their bees.

Of great importance to beekeepers is the need to protect their bees from pests, particularly the varroa mite. Scientists' understanding of mite-resistant bee stocks is

improving, and some mite-resistant stocks are now available to the beekeeping industry; their value is being demonstrated as part of the ARS Areawide Project. Better management tools and strategies are also being developed to counter varroa mite resistance to current miticides. Further research has established new thresholds for both the mite and small hive beetle that should allow beekeepers to reduce chemical treatments for these pests. Research continues to look for alternative chemical and nonchemical mite controls, including biopesticides and more integrated control strategies.

To reduce the large numbers of pathogens commonly found in bee colonies, researchers studied and demonstrated the value of comb irradiation in used wax comb prior to reuse. Some beekeepers are already using this technology to reduce pathogen load. Impacting both pest and pathogen control, increased detection capabilities are under development for both agents and should improve our understanding of their role in bee health and CCD.

To transfer these and other technologies to the field, a number of Web sites are being created, such as the University of Tennessee's e-Xtension site, along with several scientific publications to relay findings quickly and easily to beekeepers and other users. These resources will facilitate dialogue among researchers and beekeepers as new discoveries are made regarding bee health and CCD.

The development of non-*Apis* pollinators continues to increase offering alternatives to honey bee pollination. Importantly, scientists have identified the best natural forage plants to increase plant diversity in areas of poor pollinator forage. Research has also made advances in understanding best practices for developing the alfalfa leaf-cutting bee as an alternative pollinator. Further, advances in rearing and maintenance of mason bees (e.g., *Osmia* spp.) will result in their increased use as pollinators in orchards. Other bees are being developed as important pollinators of greenhouse tomatoes (bumble bees), berries, and squash. In disease research, scientists have discovered that non-*Apis* bee disease transmission can be minimized by the use of fungicides, a finding that should contribute to increased survival of alfalfa leaf-cutting bees and perhaps other species as well.

Also, to meet the need for an improved regulatory framework for honey bee imports/exports, APHIS, in collaboration with ARS, is carrying out a pilot pest survey that will provide increased knowledge of pest and pathogen impacts on bee health. Coupled with international collaboration to identify similar patterns in bee health worldwide, this survey could improve pollinator survival.

The Appendix that follows provides specific results and findings, 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:**

**Annual symptoms of CCD detailed in print and electronic publications.** Investigators from Bee Alert, the Florida Department of Agriculture, and California published a detailed description of changes in the symptoms of CCD throughout a year. This description is intended to assist beekeepers and researchers in identifying the disorder in its early phases. The article appeared in the February 2009 issue of "Bee Culture" and on several blogs.

PROJECT CONTACT: Jerry Bromenshenk ([beeresearch@aol.com](mailto:beeresearch@aol.com))

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

##### **Accomplishment:**

**Colony losses shown to increase over previous beekeeping season.** ARS 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 35% from the fall of 2007 through winter of 2008, representing absolute colony losses of 0.75 – 1.0 million hives. These data indicate an increase over the 31% losses recorded during the 2006-2007 season.

##### PROJECT CONTACTS:

Dennis van Engelsdorp ([Dennis.Vanengelsdorp@gmail.com](mailto:Dennis.Vanengelsdorp@gmail.com))

Jeff Pettis ([Jeff.Pettis@ars.usda.gov](mailto:Jeff.Pettis@ars.usda.gov))

**Oregon colony health being surveyed.** Oregon State University researchers are distributing a survey to Oregon apiculturists to assess what diseases and pests are impacting their hives and what control methods are being used, chemical and cultural. A copy of the survey can be found on the Honey Bee Diagnostic Service Web page at [www.science.oregonstate.edu/bpp/insect\\_clinic/bees.htm](http://www.science.oregonstate.edu/bpp/insect_clinic/bees.htm).

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

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

There are currently no resources for this activity for NASS.

**Accomplishment:**

**Subcommittee appointed to examine pesticide incident reporting.** The Environmental Protection Agency (EPA) is working with stakeholders regarding protocol development for the reporting of beekill incidents, exploring ways for beekeepers to report incidents rapidly and easily. A beekill incident reporting form is currently being finalized, and both long- and short-term processes are being developed to allow beekeepers to facilitate reporting of bee incidents directly to the agency.

**Subcommittee to examine the potential effects of pesticides on honey bees.** EPA is working closely with ARS, universities, the beekeeping industry, and other stakeholders to develop a set of laboratory, semi-field, and field protocols to test the effects of pesticides on honey bees and other nontarget invertebrate pollinators. EPA has, in the past, used acute contact values as a starting point for nontarget testing of pesticides and recognizes the need for more comprehensive testing of some pesticides whose effects may be subtle or sub-lethal in nature. The two national beekeeping organizations are preparing to offer input, and a workshop on testing protocols for systemic pesticides is being considered.

**PROJECT CONTACTS:**

Jeff Pettis ([Jeff.Pettis@ars.usda.gov](mailto:Jeff.Pettis@ars.usda.gov))  
Tom Steeger ([Steeger.Thomas@epa.gov](mailto:Steeger.Thomas@epa.gov))  
Gene Brandi ([GBrandi@sbcglobal.net](mailto:GBrandi@sbcglobal.net))

## Appendix: Accomplishments by Action Plan Component

### Ongoing Research:

**New pathogen screening service established.** The Oregon State University Extension Service has established a Honey Bee Diagnostic Service pilot project in Oregon, in conjunction with the Oregon State University Insect Identification Clinic. Screening services are now available for American foulbrood, European foulbrood, chalkbrood, stonebrood, *Nosema*, varroa mites, tracheal mites, and insect pests (wax moth, hive beetle, bee louse, etc.). More Information on the service can be found at [http://web.science.oregonstate.edu/bpp/insect\\_clinic/bees.htm](http://web.science.oregonstate.edu/bpp/insect_clinic/bees.htm).

PROJECT CONTACT: OSU Extension Service

**Honey Bee Center for Disease Control and Prevention established.** Bee Alert Technology, the University of Montana, and Montana State University have established a Honey Bee Center for Disease Control and Prevention. Screening services are available for all bee diseases, bee pests, and pesticides with rapid turn-around times. Unique services offered include sample analysis via Proteomics MS, rapid sample analysis, and screening for viruses using IVDS, PCR for *Nosema* speciation, and pesticide analysis. Additional capabilities and services include sampling and analysis for volatile and semi-volatile chemicals in hive atmospheres, as well as in field sampling and investigation. More information on the service can be found at <http://beealert.info>.

PROJECT CONTACT:

Jerry Bromenshenk ([beereseach@aol.com](mailto:beereseach@aol.com))

David Wick ([mrwick@bvs-inc.us](mailto:mrwick@bvs-inc.us))

**Pilot bee survey developed.** With support from the Apiary Inspectors of America and the Pennsylvania State Department of Agriculture, ARS developed a pilot bee survey to screen bees from 20 states for the Israeli acute paralysis virus. Researchers have proved the concept of using bee inspectors for collecting and processing bee samples by molecular-genetic techniques.

PROJECT CONTACTS:

Dennis van Engelsdorp ([Dennis.Vanengelsdorp@gmail.com](mailto:Dennis.Vanengelsdorp@gmail.com))

Jeff Pettis ([Jeff.Pettis@ars.usda.gov](mailto:Jeff.Pettis@ars.usda.gov))

**Nationwide honey bee pest survey under development.** APHIS accomplished two important steps in the past year. First, the agency funded and completed a project to test collection methods and logistics for designing the proposed survey; in the pilot project, honey bee samples were collected from Florida and Montana and are now being analyzed at the ARS-Beltsville laboratory. Additionally, APHIS funded an ARS project to develop a rapid and efficient sampling method for identifying the parasitic mite *Tropilaelaps* sp., a Southeast-Asia-based mite that may spread with global trade and is a major concern for beekeeping worldwide; the new sampling method is also available for use in the proposed national survey.

PROJECT CONTACT: Robyn Rose ([Robyn.I.Rose@aphis.usda.gov](mailto:Robyn.I.Rose@aphis.usda.gov))

## **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.
  - Integrated Virus Detection System (IVDS) for identifying pathogens by particle size.

#### **Accomplishments:**

***New virus discovery made.*** U.S. Army scientists discovered the first *Varroa destructor* virus (VDV-1) in North American bee hives.

#### PROJECT CONTACTS:

Charles Wick ([charles.h.wick@us.army.mil](mailto:charles.h.wick@us.army.mil))  
Michael Stanford ([mmichael.f.stanford@us.army.mil](mailto:mmichael.f.stanford@us.army.mil))  
Alan Zulich ([malan.zulich@us.army.mil](mailto:malan.zulich@us.army.mil))

***Honey bee viruses studied for links to CCD.*** Analyses of more than 800 samples indicate that there are at least 20 different viruses affecting honey bees, and efforts are ongoing to determine which have significant links to CCD. Using rapid methodology that gives results on the same day that testing is conducted, scientists were able to compile a history of hives that became affected by CCD. Efforts are ongoing to correlate virus names, data, and geographic area with the use of powerful proteomics and bioinformatics mass spectrometry methods.

#### PROJECT CONTACTS:

Charles Wick ([Charles.h.Wick@us.army.mil](mailto:Charles.h.Wick@us.army.mil))  
Michael Stanford ([Michael.f.Stanford@us.army.mil](mailto:Michael.f.Stanford@us.army.mil))  
Alan Zulich ([Alan.Zulich@us.army.mil](mailto:Alan.Zulich@us.army.mil))

***Virus concentration and distribution and management methods studied.*** Various management methods have been shown to directly minimize virus distribution and concentration in failing hives. The rapid turnaround data allows the effectiveness of different techniques, including isolation, in managing the health of the hive and controlling virus distribution and concentration to be rapidly assessed.

#### PROJECT CONTACT:

David Wick ([Mrwick@bvs-inc.us](mailto:Mrwick@bvs-inc.us))  
Jerry Bromenshenk ([BeeResearch@aol.com](mailto:BeeResearch@aol.com))  
Michael Stanford ([Michael.f.Stanford@us.army.mil](mailto:Michael.f.Stanford@us.army.mil))

*Appendix: Accomplishments by Action Plan Component*

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

**Ongoing Research:**

**Survey of microbes in honey bees underway.** University of Illinois scientists are working with researchers at Pennsylvania State University to identify and study microbes that may be associated with CCD. Researchers are sequencing microsporidian isolates to determine and confirm the presence of *Nosema apis* and/or *ceranae*, sacbrood, deformed wing virus, a paralysis virus, and any other viruses. Researchers have also been studying the progression of honey bee diseases and evaluating the specific effects on bees infected with multiple pathogens, including microsporidia and viruses.

**PROJECT CONTACTS:**

Leellen Solter ([lsolter@illinois.edu](mailto:lsolter@illinois.edu))

Diana Cox-Foster ([Dxc12@psu.edu](mailto:Dxc12@psu.edu))

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

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

**Accomplishments:**

**Elevated *Nosema* levels found in bee colony survey.** In a random sampling of honey bee colonies from across the Western U.S., ARS found unexpectedly high levels of the *Nosema ceranae* pathogen (many with over one million spores per bee). The study showed that colonies from the Pacific Northwest and Upper Midwest, particularly Minnesota and Washington, had significantly higher levels of the disease than those from other states. Findings suggest that one of the difficulties in meeting strength requirements for almond pollination may be due to the high levels of *Nosema*.

University of Minnesota researchers reported similar results, finding high levels of *Nosema* in 16 commercial California queen breeder operations.

In studies by University of Nebraska researchers, results suggest that *Nosema ceranae* was present at economically important levels in many colonies in 2008 and may contribute to colony decline and losses in the winter of 2008-2009.

**PROJECT CONTACTS:**

Frank Eischen ([Feischen@weslaco.ars.usda.gov](mailto:Feischen@weslaco.ars.usda.gov))  
Judy Chen ([Judy.Chen@ars.usda.gov](mailto:Judy.Chen@ars.usda.gov))  
Marla Spivak ([Spiva001@umn.edu](mailto:Spiva001@umn.edu))  
Marion Ellis ([mellis3@unl.edu](mailto:mellis3@unl.edu))

**Virus load and *Nosema* levels linked to CCD.** ARS researchers conducted a comprehensive genetic survey of honey bee colonies with CCD collected in California, Florida, and the Mid-Atlantic states from January and February 2007, examining them for pathogen levels and degree of immunity. Colonies from certain geographic locations showed greater genetic immunity to pathogens than others. Researchers found that the greatest predictor of CCD was the total virus load on a given colony, and to a lesser degree, the *Nosema ceranae* pathogen. Bacterial levels and levels of two other *Nosema* species were not significantly linked to CCD.

**PROJECT CONTACTS:**

Field collections:

Jeff Pettis ([Jeff.Pettis@ars.usda.gov](mailto:Jeff.Pettis@ars.usda.gov)),  
Dennis van Engelsdorp ([Dennis.Vanengelsdorp@gmail.com](mailto:Dennis.Vanengelsdorp@gmail.com)).

Genetic analyses:

Jay Evans ([Jay.Evans@ars.usda.gov](mailto:Jay.Evans@ars.usda.gov))  
Judy Chen ([Judy.Chen@ars.usda.gov](mailto:Judy.Chen@ars.usda.gov))

**Viruses and bacteria linked with honey bees (and CCD).** More than 50 bacteria have been linked with honey bees using IVDS methodology. Findings suggest that a correlation can be made between viruses, bacteria, and CCD. Continued collection and analysis will provide the statistical basis for determining CCD microbes and those linked with otherwise healthy bees.

**PROJECT CONTACT:**

Colin B. Henderson ([Colin.Henderson@mso.umt.edu](mailto:Colin.Henderson@mso.umt.edu))  
Charles Wick ([Charles.h.Wick@us.army.mil](mailto:Charles.h.Wick@us.army.mil))  
Alan Zulich ([Alan.Zulich@us.army.mil](mailto:Alan.Zulich@us.army.mil))



## *Appendix: Accomplishments by Action Plan Component*

***Variety of pathogens identified in colonies with CCD.*** Using a bioinformatics tool known as BLAST (Basic Local Alignment Search Tool) to compare nucleotide and protein databases, Pennsylvania State University researchers identified 18 pathogens from CCD-affected colonies.

PROJECT CONTACT: Diana Cox-Foster ([Dxc12@psu.edu](mailto:Dxc12@psu.edu))

***Tracheal mites not a major problem in North Central U.S.*** A survey of tracheal mites was conducted using samples collected from states in the North Central Region during the summer of 2008. The mites were detected in 5.1% of the colonies, with more than half the mites found to be from the same beekeeping operation. Survey results suggest that the mites were not a significant problem for colonies from this region in 2008.

PROJECT CONTACT: Marion Ellis ([Mellis3@unl.edu](mailto:Mellis3@unl.edu))

***Link between varroa/Nosema and colony health studied.*** In a survey of commercial beekeepers in Florida and California, scientists analyzed the varroa mite and *Nosema* levels in 12 beekeeping operations, all of which had bees in poor health. Studies revealed that two of these operations had elevated varroa mite levels, while *Nosema* was present in only low levels and did not appear to be significantly impacting colony health. Results indicate that a cause unrelated to varroa or *Nosema* is playing a role in the poor health of the colonies. Without dismissing the importance of varroa or *Nosema*, these results suggest that other factors contribute to poor colony health. These researchers are also continuing to analyze pollen and beeswax for pesticide residues and viruses.

PROJECT CONTACTS:

Jeff Pettis ([Jeff.Pettis@ars.usda.gov](mailto:Jeff.Pettis@ars.usda.gov))

Jay Evans ([Jay.Evans@ars.usda.gov](mailto:Jay.Evans@ars.usda.gov))

Dennis van Engelsdorp ([Dennis.Vanengelsdorp@gmail.com](mailto:Dennis.Vanengelsdorp@gmail.com))

**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:**

**Low imidacloprid levels found in watermelon plants.** In studies on pesticide residues in the pollen and nectar of watermelon plants, scientists found low levels of the pesticide imidacloprid in treated plants. An experiment to analyze bee exposure is ongoing.

PROJECT CONTACTS:

Galen Dively ([Galen@umd.edu](mailto:Galen@umd.edu))

Jeff Pettis ([Jeff.Pettis@ars.usda.gov](mailto:Jeff.Pettis@ars.usda.gov))

**Wide range of pesticide exposure found for honey bees.** Connecticut researchers have determined that honey bees are exposed to a wide variety of pesticides, with concentrations varying significantly over short time periods. Researchers are examining hives at urban, suburban, and rural locations for differences in pesticide exposure by time and location.

PROJECT CONTACT: Brian Eitzer ([Brian.Eitzer@po.state.ct.us](mailto:Brian.Eitzer@po.state.ct.us))

**High fluvalinate and coumaphos found on honey bee samples.** Pennsylvania State University researchers analyzed pollen, honey, brood, adult bees, wax, and royal jelly samples from CCD- and non-CCD-affected bee hives and found high levels of the miticides fluvalinate and coumaphos (used frequently by beekeepers for mite control), which were present in nearly all samples, with 73 other pesticides and metabolites also identified.

PROJECT CONTACT: Marianne Frazier ([Mfrazier@psu.edu](mailto:Mfrazier@psu.edu))

**Nearly 400 volatile and semivolatile organic chemicals found in the air inside beehives.** Using funding from the National Honey Board and the Almond Board of California, bee samples from many bee operations were analyzed for a variety of chemicals that might cause bees to leave the hive. Compared to previous environmental results up through 2000, the most striking change was in the presence and levels of miticides, including both commercial acaricides and bee health products. The most common toxic chemical was paradichlorobenzene, a product used for wax moth control in the hive. Meanwhile, aflatoxins and 5-hydroxymethyl furfural [also see III.1.2] were found to not be significant contributors to CCD, as healthy colonies had higher levels of aflatoxins than failing and collapsed colonies. The U.S. Army's use of proteomics has expanded its capability to evaluate peptides and proteins, and results are providing additional insights into the chemical and biological composition of bee colonies.

PROJECT CONTACTS:

Jerry Bromenshenk ([Beereseach@aol.com](mailto:Beereseach@aol.com))

Michael Stanford ([Michael.F.Stanford@us.army.mil](mailto:Michael.F.Stanford@us.army.mil))

Charles Wick ([Charles.H.Wick@us.army.mil](mailto:Charles.H.Wick@us.army.mil))

**Ongoing Research:**

***Pesticide and virus analysis continues.*** Over the past 2 years, a CCD working team on pesticides has collected numerous samples of bees, pollen, and beeswax from suspected CCD and healthy colonies. Due to budget constraints and the high cost of sample analysis, not all samples were processed immediately after collection. Using \$70,000 in funds from ARS, the working team submitted all relevant pollen and bee samples for analysis and the backlog of samples was analyzed, although new samples continue to arrive. Analysis is providing data to begin drawing conclusions and support the design of future sampling and research efforts on the role of pesticides in bee losses. One novel finding is that elevated levels of the fungicide chlorthalonil are associated with colonies sealing off their pollen cells with a plant resin. Experiments continue to explore the effects of these “entombed” pollen cells on bees and their frequency in CCD hives.

PROJECT CONTACTS:

Jeff Pettis ([Jeff.Pettis@ars.usda.gov](mailto:Jeff.Pettis@ars.usda.gov))

Chris Mullen ([Camullin@psu.edu](mailto:Camullin@psu.edu))

Maryann Frazier ([Mfrazier@psu.edu](mailto:Mfrazier@psu.edu))

Dennis van Engelsdorp ([Dennis.Vanengelsdorp@gmail.com](mailto:Dennis.Vanengelsdorp@gmail.com))

***Pesticide residue sampling continues in overwintered colonies.*** Samples of honey from overwintered colonies have been collected from beekeepers around the country and are being analyzed for coumaphos, fluvalinate, and other contaminants to determine if stored honey is contaminated and to assess exposure risk to bees during winter stress.

PROJECT CONTACTS:

Nancy Ostiguy ([Nxo3@psu.edu](mailto:Nxo3@psu.edu))

Brian Eitzer ([Brian.Eitzer@pa.state.ct.us](mailto:Brian.Eitzer@pa.state.ct.us))

**Goal 4: Develop analytical tools to assess bee health.**

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

**Accomplishments:**

***New molecular targets identified for monitoring bee health.*** ARS researchers studied honey bee stress responses to fungal pathogens and identified a number of molecular targets not previously associated with immune or stress responses in insects. These findings could lead to the development of a new technique for monitoring stress levels in diseased and healthy honey bee colonies.

PROJECT CONTACT: Katherine Aronstein ([Kate.Aronstein@ars.usda.gov](mailto:Kate.Aronstein@ars.usda.gov))

***Possible genetic marker for CCD identified and interaction with virus infection studied.***

Gene expression of bees diagnosed with CCD was compared with that of healthy bees using whole genome microarrays. University of Illinois scientists discovered unusual ribosomal RNA fragments, which are possible breakdown products of ribosomes, which could have been caused in part by the infection of these bees with picornaviruses. Preliminary results suggest that one EST marker may be a potentially useful diagnostic marker for CCD.

PROJECT CONTACTS:

May Berenbaum ([Maybe@uiuc.edu](mailto:Maybe@uiuc.edu))

Reed Johnson ([Mrjohns1@uiuc.edu](mailto:Mrjohns1@uiuc.edu))

***New insights provided into the microbial communities inside beehives and changes identified in a large number of peptides and proteins.*** Unrestricted Proteomics MS has led to new findings regarding the microbial communities inside beehives, providing the first benchmarks for healthy, failing, and collapsed colonies. The California Beekeeping Association has funded a 2009 proteomics survey of California bees prior to and just after pollination of almonds and at the end of honey harvest, which will lead to further insights.

PROJECT CONTACT:

Jerry Bromenshenk ([beereseach@aol.com](mailto:beereseach@aol.com))

Michael Stanford ([michael.f.stanford@us.army.mil](mailto:michael.f.stanford@us.army.mil))

Alan Zulich ([alan.zulich@us.army.mil](mailto:alan.zulich@us.army.mil))

***Sonic Sensor developed for rapid detection of bee diseases, pests, and CCD.*** The sounds that bees produce change when colonies are exposed to toxic chemicals, disease, or pesticides; each stressor induces a unique sonic soundprint. Researchers have taken advantage of this health indicator by developing a Sonic Sensor that can determine both the presence and severity of the infection or infestation quickly and inexpensively. Significant progress has been made in terms of ability to correctly diagnose bee health and on the development of a hand-held device that beekeepers can use to scan colonies.

PROJECT CONTACTS:

Robert Seccomb ([rseccomb@beealert.blackfoot.net](mailto:rseccomb@beealert.blackfoot.net))

Jerry Bromenshenk ([beereseach@aol.com](mailto:beereseach@aol.com))

### **Topic III: Research to Identify Factors Affecting Honey Bee Health, Including Attempts to Recreate 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 pesticides discovered.*** The occurrence of multiple pesticides in the protein source of developing larvae and nurse bees has led to concerns about the additive and synergistic effects of the pesticides. In analyzing over 700 samples of adult bees, wax, bee bread, pollen, and brood for pesticide residues, Pennsylvania State University researchers found a total of 73 pesticides and 9 metabolites (products of metabolism) in hive matrices, representing nearly all classes of insecticides, with an average of 6 residues found per pollen sample. Researchers found evidence of synergy on mortality when bees were exposed to combinations of neonicotinoids and certain classes of fungicides. In addition, results indicate that two common surfactants (Triton X-100 and Silwet L-77) were highly toxic to adult honey bees, with 80% and 30% mortality rates, respectively, at a dose of 1% in artificial nectar.

#### **PROJECT CONTACT:**

Marianne Frazier ([Mfrazier@psu.edu](mailto:Mfrazier@psu.edu))

Chris Mullen ([Camullin@psu.edu](mailto:Camullin@psu.edu))

***Pesticide synergism identified as suspect in CCD.*** Beekeepers frequently use the miticides tau-fluvalinate (Apistan<sup>®</sup>) and/or coumaphos (CheckMite<sup>®</sup>) to control the varroa mite, and honey bees and other insects use several strategies to survive this pesticide exposure. University of Illinois researchers found that bees had elevated levels of certain detoxification enzymes when exposed to tau-fluvalinate, and bioassays revealed that bees pretreated with either coumaphos or tau-fluvalinate were much less able to tolerate the other miticide. Colonies exhibiting CCD symptoms and colonies not exhibiting CCD symptoms contained both miticides in wax, indicating that bee ability to break down in-hive miticides may be compromised due to unrecognized interactions. These findings suggest that interactions between the two miticides may play a role in bee mortality.

#### **PROJECT CONTACTS:**

Reed Johnson ([Mrjohns1@uiuc.edu](mailto:Mrjohns1@uiuc.edu))

May Berenbaum ([Maybe@uiuc.edu](mailto:Maybe@uiuc.edu))

## Appendix: Accomplishments by Action Plan Component

### Ongoing Research:

**Pesticide toxicity to honey bees studied.** The University of Florida is testing the effects of amitraz and imidacloprid (common pesticides) on honey bee susceptibility to varroa mite infestations.

PROJECT CONTACT: Jamie Ellis ([Jdellis@ufl.edu](mailto:Jdellis@ufl.edu))

**Pesticide exposure and toxicity studied.** Pennsylvania State University researchers are investigating whether pesticides are a factor in declining honey bee health and CCD, analyzing the total pesticide exposure of honey bees and tracking the acute and sublethal effects of the exposure over time. Scientists will evaluate the effects of pesticides individually and in combination to determine if they suppress honey bee immune response, interfere with learning and memory, or alter chemical senses of the bees. This research will aid in the development of safe, new pesticides for mite and pest control.

PROJECT CONTACT: R. L. Unger ([Runger@psu.edu](mailto:Runger@psu.edu))

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

### Ongoing Research:

**Impact of common miticides studied.** Pennsylvania State University researchers are evaluating the impact of in-hive miticide use on honey bees, with the hypothesis that the miticides decrease immune function, lead to increased virus levels, and negatively impact honey bee lifespan and health. Researchers will investigate the impact of the common miticides CheckMite<sup>®</sup>, Apistan<sup>®</sup>, and formic acid-flash treatment on virus levels, honey bee immune function, lifespan, reproductive physiology, and overall colony health. Preliminary work is being completed on honey bees unexposed to miticides to determine baseline hatch rate of eggs and longevity of larvae, pupae, and adults, as well as virus status. Colonies will be exposed to Apistan<sup>®</sup> and CheckMite+<sup>®</sup> beginning in March 2009 to simulate spring mite treatment. Treated and untreated colonies will be evaluated through the summer and fall of 2009 to determine if health is impacted by these environmental factors.

PROJECT CONTACT:

R. L. Unger ([Runger@psu.edu](mailto:Runger@psu.edu))

Nancy Ostiguy ([Nxo3@psu.edu](mailto:Nxo3@psu.edu))

**Sublethal effects of common miticides studied.** In a separate but related project, University of Georgia and Clemson University researchers are investigating the sublethal effects of the two most commonly used pesticides in beehives in the U.S.: Apistan<sup>®</sup> (fluvalinate) and CheckMite+ (coumaphos). Researchers are evaluating the sublethal effects of these pesticides on adult bee lifespan, brood development, honey production, foraging rates, *Nosema* colony infestation rates, worker responsiveness to the pheromone QMP, worker bee homing ability, and worker bee learning and memory.

PROJECT CONTACT: W. M. Hood ([Mhood@clemson.edu](mailto:Mhood@clemson.edu))

## Appendix: Accomplishments by Action Plan Component

- 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.

### Ongoing Research:

**Antibiotic effects being tested.** ARS scientists have conducted cage bee studies to examine the effects of antibiotics on pathogen survival and overall honey bee health over winter, under the hypothesis that antibiotics may disrupt the gut flora of bees and increase their susceptibility to pathogens. To date, no clear effects have been demonstrated, and scientists are planning field tests on the effects of antibiotic feeding on pathogen load for the coming year.

PROJECT CONTACT: Jeff Pettis ([Jeff.Pettis@ars.usda.gov](mailto:Jeff.Pettis@ars.usda.gov))

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

### Accomplishments:

**HFCS inversely linked to worker longevity.** The effects of high fructose corn syrup (HFCS) on worker longevity were tested on newly emerged worker bees. Workers fed a sucrose solution lived significantly longer, on average, than those fed HFCS.

PROJECT CONTACT: Blaise W. LeBlanc ([Blaise.Leblanc@ars.usda.gov](mailto:Blaise.Leblanc@ars.usda.gov))

**HFCS chemical impurity found.** In response to concerns about the reduced lifespan of bees fed HFCS, researchers found a heat-formed impurity, hydroxymethylfurfural (HMF), in HFCS that is highly toxic to bees, and determined the storage conditions, including temperature, container type, and time in storage that predict the formation of the contaminant. Researchers also found that HMF levels in HFCS were noticeably higher in beekeeper samples than in control samples. This finding gives a possible explanation for the reduced lifespan of bees fed HFCS and has led to recommendations that HFCS be stored in temperature-controlled environments and for limited periods of time. Research will continue to investigate the toxicity levels of HMF for bees and to evaluate the effects of HFCS on overwintering bees.

PROJECT CONTACT: Diana Sammataro ([Diana.Sammataro@ars.usda.gov](mailto:Diana.Sammataro@ars.usda.gov))

Also see Part III-Goal 3.

### Ongoing Research:

**Benefits of supplemental feeding for migratory beekeeping studied.** ARS has been studying the effects of supplemental feeding on CCD-affected honey bee colonies used for California almond pollination. This study will continue in the Central Valley of California during 2008-2009.

PROJECT CONTACT: Frank Eischen ([Feischen@weslaco.ars.usda.gov](mailto:Feischen@weslaco.ars.usda.gov))

## *Appendix: Accomplishments by Action Plan Component*

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

This is being done as part of the Areawide Project. See Part IV-6.

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

### **Accomplishments:**

***Transportation proven to stress honey bee colonies.*** ARS researchers and the Pennsylvania Department of Agriculture provided the first documented evidence that transportation stresses honey bee colonies. During colony transport from California to Florida, brood nest temperatures dropped 2-3<sup>0</sup>C and brood losses of migratory colonies were ten times greater than for colonies remaining in California. Future trials will investigate the survival of individual bees following transportation and analyze stress indicators in individual bees.

#### PROJECT CONTACT:

Dennis van Engelsdorp ([Dennis.vanengelsdorp@gmail.com](mailto:Dennis.vanengelsdorp@gmail.com))

Jeff Pettis ([Jeff.Pettis@ars.usda.gov](mailto:Jeff.Pettis@ars.usda.gov))

### **Ongoing Research:**

***Impact of long distance migration studied.*** ARS and Michigan State University scientists are beginning a study on the effects of hive migration on honey bee physiology. Bees transported over long distances have been sampled for indicators of stress.

#### PROJECT CONTACT:

Jeff Pettis ([Jeff.Pettis@ars.usda.gov](mailto:Jeff.Pettis@ars.usda.gov))

Zachary Huang ([Bees@msu.edu](mailto:Bees@msu.edu))

***Bee health predictors studied during hive migration.*** Kentucky State University and the University of Florida are beginning a migratory beekeeping study, measuring varroa mite, tracheal mite, and *Nosema* levels in colonies before and after transport to California to identify health effects due to transportation.

#### PROJECT CONTACT:

Tom Webster ([Tom.Webster@kysu.edu](mailto:Tom.Webster@kysu.edu))

Jamie Ellis ([Jdellis@ufl.edu](mailto:Jdellis@ufl.edu))

Also see Areawide Project, 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:**

***New virus identified as CCD marker.*** ARS, Columbia University, and Pennsylvania State University researchers analyzed CCD-affected samples and discovered that the pathogen known as the Israeli acute paralysis virus (IAPV) appeared in almost every CCD case. IAPV may be one cause of the collapse or a marker for some other cause. The results were published in the October 12, 2007, issue of *Science*.

PROJECT CONTACT: Diana Cox-Foster ([Dxc12@psu.edu](mailto:Dxc12@psu.edu))

***Multiple IAPV lineages identified.*** Pennsylvania State University, ARS, and University of Columbia researchers developed real-time diagnostic assays for IAPV and determined that there are at least three different lineages of the virus, two of which are present in the U.S. IAPV obtained from Australia package bees was most similar to a lineage found in the western United States.

***IAPV-resistant bee genotypes identified.*** Researchers have also conducted studies suggesting that there are bee genotypes in the U.S. resistant to IAPV and other viruses. In studying the effects of IAPV infection on colonies, researchers found that some bees with demonstrated resistance to other pests and pathogens were able to clear IAPV from their colonies.

***IAPV found to be highly pathogenic to honey bees.*** The same researchers found that honey bee colonies fed IAPV experienced dramatic mortality and decline within one month, indicating that IAPV is a highly pathogenic virus. Research continues to analyze samples from each colony, and the experiment is being repeated. This study does not indicate that IAPV is one or the only cause of CCD.

***IAPV found to be transmissible to native bees.*** Researchers also found that IAPV can be transmitted from honey bees to native bees through the pollination process. The impact of the virus on native bees is unknown.

PROJECT CONTACT: Diana Cox Foster ([Dxc12@psu.edu](mailto:Dxc12@psu.edu))

## *Appendix: Accomplishments by Action Plan Component*

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

### **Accomplishment:**

***Weak immune systems linked to CCD.*** In studies on bees from different colonies, apiaries, and geographical regions, University of Illinois researchers have determined that immune response genes show decreased expression in bees with CCD.

#### PROJECT CONTACT:

May Berenbaum ([Maybe@uiuc.edu](mailto:Maybe@uiuc.edu))

Reed Johnson ([Mrjohns1@uiuc.edu](mailto:Mrjohns1@uiuc.edu))

### **Ongoing Research:**

***Diet contributions to pesticide detoxification studied.*** University of Illinois researchers are working to identify the specific honey bee genes responsible for pesticide detoxification and determine if there is an interaction between pesticides and compounds in pollen, honey, or propolis (a resinous mixture collected by bees) and subsequent ability of bees to tolerate pesticides. In honey bees exposed to fluvalinate, a common miticide for mite control, researchers found that a component of pollen and honey known as quercetin (a flavanol) led to increased tolerance of this pesticide. In addition, feeding pollen, honey, or propolis to bees led to gene expression of 3 detoxification enzymes in honey bees, which appear to be regulated by quercetin and possibly other compounds in the hive environment. In contrast, honey bees consuming only sucrose did not display expression of these enzymes. Although further studies are needed, researchers speculate that the practice (common in many apicultural operations hard hit by CCD) of feeding bees only sugar during winter months may compromise their ability to process environmental toxins.

PROJECT CONTACT: May Berenbaum ([Maybe@life.uiuc.edu](mailto:Maybe@life.uiuc.edu))

**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.

**Ongoing Research:**

**Interaction of varroa and pathogens under investigation.** Pennsylvania State University scientists have been investigating the relationship between viruses, varroa mites, and colony health, tracking the progression of several viruses as well as the population dynamics of varroa mites in colonies. As a preliminary finding, researchers observed colonies with “melting” brood and confirmed the presence of deformed wing virus, sacbrood virus, and Israeli acute paralysis virus in unhealthy colonies, but neither sacbrood virus nor Israeli acute paralysis virus were detected in healthy colonies.

PROJECT CONTACTS:

Nancy Ostiguy ([Nxo3@psu.edu](mailto:Nxo3@psu.edu))  
Diana Cox Foster ([Dxc12@psu.edu](mailto:Dxc12@psu.edu))

**Varroa mite and Nosema species being sequenced.** ARS scientists are working to sequence two microsporidian parasites (*Nosema ceranae* and *Nosema apis*) as well as the varroa mite. Scientists have finished sequencing and annotating *Nosema ceranae*, with results to be published in the spring of 2009, and are preparing to sequence *Nosema apis*, with results expected sometime in 2009. Also, with industry support, ARS initiated a genome project on *Varroa destructor*, the primary honey bee parasite, as part of a worldwide consortium to identify mite genes implicated in virulence, viral disease, and acaricide resistance.

- 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.

**Ongoing Research:**

**Virus transmission mode demonstrated.** Researchers demonstrated that deformed wing virus can be transmitted through contaminated food, such as pollen and honey, resulting in virus-positive colonies.

PROJECT CONTACTS:

Nancy Ostiguy ([Nxo3@psu.edu](mailto:Nxo3@psu.edu))  
Diana Cox Foster ([Dxc12@psu.edu](mailto:Dxc12@psu.edu))

**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 pathogens and pesticides to be studied.*** Researchers from Pennsylvania State University, University of Illinois, and Michigan State University are investigating the interactive effects of relevant pathogens and pesticides on honey bee health. Researchers will compare the effects of *Nosema apis* and *Nosema ceranae*, the synergistic and sublethal effects of miticides, and the economic efficacy of varroa IPM methods. This work is part of a larger sampling scheme with collaborators across the United States.

PROJECT CONTACTS:

Nancy Ostiguy ([Nxo3@psu.edu](mailto:Nxo3@psu.edu))  
Leellen Solter ([Lsolter@illinois.edu](mailto:Lsolter@illinois.edu))  
Diana Cox Foster ([Dxc12@psu.edu](mailto:Dxc12@psu.edu))  
Zachary Huang ([Bees@msu.edu](mailto:Bees@msu.edu))

***Link between CCD and HFCS, varroa, Nosema, and other factors studied.*** Michigan State University is funding a research project on the effects of HFCS on honey bee health, as well as how varroa mites, *Nosema*, and other factors contribute to colony mortality during winter.

PROJECT CONTACT: Zachary Huang ([Bees@msu.edu](mailto:Bees@msu.edu))

## **Topic IV: Mitigative and Preventive Measures**

### **Goal 1: Develop best management practices for honey bees.**

1: Develop best management practices for migratory beekeeping.

#### **Ongoing Research:**

***BMPs for beekeepers being developed.*** Researchers at the Universities of Georgia and Minnesota are developing best management practices for beekeepers and queen breeders and implementing a technology transfer program, including workshops on improving queen selection and propagation and a Web-based Community of Practice. Researchers are preparing varroa mite sampling guidelines that will give hobby, part-time, and migratory beekeepers a standard, accurate, and easy way to monitor mite levels, a critical step in IPM for making educated treatment decisions. Concept papers have already been published that will serve as a foundation for a Web-based bulletin.

#### **PROJECT CONTACTS:**

Keith S. Delaplane ([Ksd@uga.edu](mailto:Ksd@uga.edu))

Marla Spivak ([Spiva001@umn.edu](mailto:Spiva001@umn.edu))

***Brood pheromone tested in bee hives.*** ARS researchers are testing a new brood pheromone product developed in collaboration between Contech, Inc., and Texas A&M University to improve the health and vigor of honey bee colonies, as well as to improve crop pollination.

#### **PROJECT CONTACTS:**

Katherine Aronstein ([Kate.Aronstein@ars.usda.gov](mailto:Kate.Aronstein@ars.usda.gov))

Tanya Pankiw ([Tpankiw@neo.tamu.edu](mailto:Tpankiw@neo.tamu.edu))

## *Appendix: Accomplishments by Action Plan Component*

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

### **Accomplishments:**

***Nonchemical trapping device for small hive beetle created.*** ARS developed a new nonchemical trapping device to control the small hive beetle in honey bee colonies. This new beetle trapping device will help to reduce application frequency of harsh chemicals in bee hives and therefore improve the health of bees and quality of honey bee products.

PROJECT CONTACT: Katherine Aronstein ([Kate.Aronstein@ars.usda.gov](mailto:Kate.Aronstein@ars.usda.gov))

***New small hive beetle bait shown to improve trap catches.*** Preliminary results from the University of Tennessee indicate that protein supplement bait (pattie) plus small hive beetle yeast trapped more adult beetles than pattie alone or cider vinegar.

PROJECT CONTACT: John Skinner ([Jskinner@utk.edu](mailto:Jskinner@utk.edu))

***Reproductive potential of small hive beetle on fruit tested.*** ARS has conducted extensive studies on the fruit naturally encountered by small hive beetles at bee pollination sites to assess their reliance on honey bee hives. In testing orange, cantaloupe, grape, and watermelon pollination sites, researchers found that the beetle reproduces with high efficiency on these fruits and infests the fruits with yeast that makes the fruit more attractive to beetles during fermentation. The results demonstrate that small hive beetles do not require honey bee hives for survival.

PROJECT CONTACT: Peter Teal ([Peter.Teal@ars.usda.gov](mailto:Peter.Teal@ars.usda.gov))

***Small hive beetle attraction to fruit analyzed.*** ARS researchers conducted studies demonstrating that small hive beetles found decaying oranges twice as attractive as fresh oranges, with chemical analysis indicating that compounds released during fermentation were responsible for the increased attraction. These results will be useful in the development of new attractants for the beetles.

PROJECT CONTACT: Peter Teal ([Peter.Teal@ars.usda.gov](mailto:Peter.Teal@ars.usda.gov))

***Small hive beetle attractant developed.*** ARS researchers have identified and developed an effective attractant for the small hive beetle. The lure provides the first highly effective method for attracting the beetle and can be used for both monitoring and control.

PROJECT CONTACT: Peter Teal ([Peter.Teal@ars.usda.gov](mailto:Peter.Teal@ars.usda.gov))

***Small hive beetle trap developed.*** ARS has developed a trapping system for the small hive beetle based on a bottom board trap and lure. The trap greatly reduces beetle populations in hives without the need for chemical pesticides, as indicated by the trapping of more than 20,000 beetles over a 4 week period.

PROJECT CONTACT: Peter Teal ([Peter.Teal@ars.usda.gov](mailto:Peter.Teal@ars.usda.gov))

***Monitoring tool for small hive beetle developed.*** ARS scientists developed a highly effective trap for population monitoring of the small hive beetle in fields. Scientists used PVC pipe and a lure that they developed to construct the trap, which has been used to trap beetles in wooded and open areas up to 3 miles from existing managed bee hives. The trap provides the first effective way to determine field populations of the pest.

PROJECT CONTACT: Peter Teal ([Peter.Teal@ars.usda.gov](mailto:Peter.Teal@ars.usda.gov))

## Appendix: Accomplishments by Action Plan Component

**New economic threshold developed for varroa mites and small hive beetles.** Researchers at the Universities of Georgia and Clemson have determined that an economic threshold for this two-pest complex in the southeastern United States exists: colony small hive beetle adult populations of 300 and varroa mite populations of 1,840.

PROJECT CONTACT: Keith S. Delaplane ([Ksd@uga.edu](mailto:Ksd@uga.edu))

**Gamma irradiation discovered to control pathogens and promote colony growth.** ARS and the Pennsylvania Department of Agriculture determined that gamma irradiation is an effective means of reducing pathogens and promoting colony growth when applied to bee comb. After 9 months, colony survival was approximately 70% on irradiated comb compared to 30% on nonirradiated comb. Analysis of varroa and pathogen levels is on-going.

PROJECT CONTACT:

Jeff Pettis ([Jeff.Pettis@ars.usda.gov](mailto:Jeff.Pettis@ars.usda.gov))

Dennis van Engelsdorp ([Dennis.vanengelsdorp@gmail.com](mailto:Dennis.vanengelsdorp@gmail.com))

**Nosema species studied and control agents identified.** Beekeeping operations from many parts of the U.S. were examined, using PCR analysis, for *Nosema apis* and *Nosema ceranae*. Results showed that all bee operations with *Nosema apis* also had *Nosema ceranae*. Scientists also studied the use of a 10% bleach solution as a control method for *Nosema*, finding the bleach solution more effective than many of the commercially available products and other treatments such as acetic acid. The relatively inexpensive solution was nearly 100% effective in killing *Nosema* spores, and field trials are ongoing to determine best application methods and whether bleach at this concentration poses any hazard to bees.

PROJECT CONTACTS:

Robert Cramer, Montana State University ([rcramer@montana.edu](mailto:rcramer@montana.edu))

Jerry Bromenshenk, University of Montana ([beerresearch@aol.com](mailto:beerresearch@aol.com))

### Ongoing research:

**Small hive beetle trap development continues.** The University of Tennessee is testing a bait for use in a small hive beetle trap placed inside honey bee colonies. Additional studies will seek to confirm the benefit of yeast in bait and compare oils used as killing agent traps.

PROJECT CONTACT: John Skinner ([Jskinner@utk.edu](mailto:Jskinner@utk.edu))

**Nonchemical control methods for small hive beetles tested.** Researchers at the University of Florida, University of Georgia, and Clemson University are testing the efficacy of nonchemical control methods (resistant stock, trapping, nematodes) at reducing small hive beetle populations. Information gained from these studies will be included in a Best Management Practices guide developed by the American Association of Professional Apiculturists.

PROJECT CONTACT: Keith S. Delaplane ([Ksd@uga.edu](mailto:Ksd@uga.edu))

*Appendix: Accomplishments by Action Plan Component*

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

**Accomplishments:**

***Native perennial plants identified for use in pollinator conservation projects.*** The addition of floral resources to agricultural field margins has been shown to increase the abundance of beneficial insects in crop fields, but few native perennials have as yet been identified for this purpose. Researchers at Michigan State University have examined the most suitable plants for increasing the abundance of wild bees in crop fields, studying the relative attractiveness of 43 eastern U.S. native perennial plants to wild and managed bees. Floral characteristics were evaluated for their ability to predict bee abundance and taxa richness. The study identified 29 perennials that were particularly attractive to wild bees.

PROJECT CONTACT: Rufus Isaacs ([Isaacsr@msu.edu](mailto:Isaacsr@msu.edu))



**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:**

**Chalkbrood reduced in alfalfa leafcutting bees using fungicides.** ARS scientists have determined the mode of chalkbrood disease transmission and have established that at least one mode of transmission can be blocked by proper application of fungicides to overwintering cocoons. (Chalkbrood is the most significant disease in alfalfa leafcutting bees, commonly killing as many as 20% of the larvae every year.)

**New detection system developed to identify chalkbrood spore contamination levels.** ARS developed a DNA-PCR based detection system to assess the number of chalkbrood spores contaminating environmental samples. This detection system will be used to evaluate levels of contamination in different bee management systems and to study the transmission of the disease from soil, flowers, and wild bees.

**Three-year survey of chalkbrood in alfalfa leafcutting bees completed.** In a 3-year survey of bees from alfalfa seed growers in different regions of the U.S. and Canada, ARS evaluated alfalfa leafcutting bees for chalkbrood infection and determined that levels varied significantly between states and growers; some congregations of bees were infected with multiple species of chalkbrood. ARS used a new detection system that readily identifies different kinds of chalkbrood infections before symptoms are apparent in the bees.

**Isolated genes active in infected bees.** By studying response genes in the alfalfa leafcutting bee, ARS is working to determine the genetics of immunity to disease and thus the potential for breeding for disease resistance, as well as to evaluate the impact of stressors such as extreme temperature and pesticide exposure on disease susceptibility.

**New book on alternative pollinators to be published.** Researchers from the University of Minnesota and the Xerces Society have written a book entitled "Managing Alternative Pollinators: A Handbook for Beekeepers, Growers, and Conservationists" to be published in early 2009 by the Natural Resource, Agriculture, and Engineering Service.

PROJECT CONTACT: Rosalind R. James ([rjames@biology.usu.edu](mailto:rjames@biology.usu.edu))

**Ongoing Research:**

**Native bee pathogens and insecticides studied.** Researchers at the University of Massachusetts are characterizing new and emerging pathogens and parasites of native pollinators, studying the effects of insecticides on the bees and developing recommendations for more efficient use.

PROJECT CONTACT: Anne Averill ([aaverill@ent.usmass.edu](mailto:aaverill@ent.usmass.edu))

## Appendix: Accomplishments by Action Plan Component

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

### Accomplishments:

***Osmia aglaia* developed as an affordable, effective pollinator for raspberries and blackberries.** ARS demonstrated that a new, native West Coast bee, *Osmia aglaia*, is as effective as honey bees for pollination of raspberry and blackberry crops, which are worth \$200 million annually in the U.S. This bee was shown to be even more consistent and affordable than honey bees. Researchers designed a \$25 reusable, durable nesting shelter and support for use of this bee. In 2007, nesting populations were increased to 10,000 bees in commercial cane fruits in Oregon.

***Sustainable management of alkali bee shown to be economical for alfalfa seed production.*** ARS found that the alkali bee, the world's only intensively managed ground-nesting bee, can supply an economical means of pollinating alfalfa for commercial seed production. Surveys indicate that even amid intensive conventional agriculture, a native bee can sustainably multiply to large numbers. The bee pollinates an estimated 4 million pounds of alfalfa seed annually. One-third (about \$5 billion) of the value of bee pollination is for production of alfalfa seed.

***Specialist native squash bee (Peponapis) found responsible for most squash and pumpkin pollination in North America.*** ARS found that the native squash bee (*Peponapis*) is alone responsible for almost all of the pollination of squash and pumpkin and their wild relatives from Canada to Argentina. The crops are valued at \$500 million annually.

***Native bees found to be critical to rangeland restoration.*** In an on-going program to evaluate pollination needs for 15 wildflower species, ARS determined that native bees are critical for farming these wildflowers for seed. Researchers demonstrated that native bees are essential for pollinating biscuit roots (carrot relative), and white globe mallows (cotton relative) are dependent on native bees but can also benefit from honey bees. This knowledge will assist with the pollination and growth of wildflower seeds needed for rangeland restoration.

PROJECT CONTACT: James Cane ([Jim.Cane@ars.usda.gov](mailto:Jim.Cane@ars.usda.gov))

***Standard sampling protocol developed for assessing native bee populations.*** In collaboration with other bee researchers in North America, ARS developed a standardized sampling protocol and used it to gather important baseline data on native bee populations in several national parks. Using this sampling methodology, ARS assessed the impact of fire on bee communities and determined that burned landscapes, two years post-fire, supported bee populations up to nine times as large and with several times more species than unburned lands. This protocol will support further studies providing the baseline data on pollinator status recommended by the National Academy of Science.

PROJECT CONTACT: Terry Griswold ([Terry.Griswold@ars.usda.gov](mailto:Terry.Griswold@ars.usda.gov))

***Release of too many alfalfa leafcutting bees shown to reduce commercial bees at nesting sites.*** ARS investigated the optimal stocking density of alfalfa leafcutting bees (*Megachile rotundata*) to maximize alfalfa seed yield while increasing pollinator population. Studies showed that release of a moderate number of bees (30,000/ac) produced a higher percentage of nesting females than low or high numbers (45,000/ac) of releases. This illustrates that, according to current practices, too many alfalfa leafcutting bees are being released, leading to reduced numbers of commercial bees at nesting sites.

*Appendix: Accomplishments by Action Plan Component*

***Microclimate linked to prevalence of failed alfalfa leafcutting bee cells in the U.S.***

ARS studied the effects of microclimate on the occurrence of “pollen balls” in nests of alfalfa leafcutting bees in the U.S. and Canada (the malady called “pollen balls” accounts for up to 60% of losses in commercial populations of alfalfa leafcutting bees). Researchers determined that this malady is more likely to occur on hot days, linking microclimate to the prevalence of failed alfalfa leafcutting bee cells in the U.S.

PROJECT CONTACT: Theresa Pitts-Singer ([Theresa.Pitts-Singer@ars.usda.gov](mailto:Theresa.Pitts-Singer@ars.usda.gov))

***Incorporation of pollinator habitat enhancement into conservation practices on private lands.*** The USDA-Natural Resources Conservation Service is revising many of its conservation practice standards to better incorporate attention to, and enhancement of, pollinator habitat. Land managers are being encouraged to incorporate vegetative materials that flower from early spring to late fall and to make available appropriate nesting sites to provide habitats for both managed and feral pollinators.

PROJECT CONTACT: Doug Holy ([doug.holy@wdc.usda.gov](mailto:doug.holy@wdc.usda.gov))

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

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

**Accomplishment:**

**USDA Varroa-resistant 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 the VSH trait 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.

PROJECT CONTACT:

Tom Rinderer ([Tom.Rinderer@ars.usda.gov](mailto:Tom.Rinderer@ars.usda.gov))

Bob Danka ([Bob.Danka@ars.usda.gov](mailto:Bob.Danka@ars.usda.gov))

## Appendix: Accomplishments by Action Plan Component

### Ongoing Research:

**Varroa mite resistance traits being identified.** Researchers at Purdue University are performing genomic studies to identify genes involved in resistance to varroa mites and also for the aggressive behavior of Africanized honey bees.

PROJECT CONTACT: Greg Hunt ([Ghunt@purdue.edu](mailto:Ghunt@purdue.edu))

**Genetic markers being identified for mite resistance.** USDA and collaborating scientists are working to identify genes and genomic markers in mite-resistant Russian and VSH bees for future marker-assisted selection of diverse, improved bees.

PROJECT CONTACT:

Tom Rinderer ([Tom.Rinderer@ars.usda.gov](mailto:Tom.Rinderer@ars.usda.gov))

Bob Danka ([Bob.Danka@ars.usda.gov](mailto:Bob.Danka@ars.usda.gov))

**Russian honey bees being improved for pollination.** A subset of the Russian honey bee breeding program is underway to improve the consistency of early population size of Russian bees to make them more acceptable for almond pollination.

PROJECT CONTACT:

Tom Rinderer ([Tom.Rinderer@ars.usda.gov](mailto:Tom.Rinderer@ars.usda.gov))

Bob Danka ([Bob.Danka@ars.usda.gov](mailto:Bob.Danka@ars.usda.gov))

**Resistance traits to viruses, Nosema, and varroa mites being identified.** Researchers at the Universities of Purdue and Minnesota and ARS-Baton Rouge are working to identify genes that influence honey bee resistance to pests and pathogens, including viruses, *Nosema*, and varroa mites, and incorporating those resistance traits into bees. In doing so, scientists are studying the management practices correlated with increased genetic diversity of queen bees. The results of this research will be transferred to university breeding programs to support the development of commercially available bee lines more resistant to pests and diseases.

PROJECT CONTACT:

Greg Hunt ([Ghunt@purdue.edu](mailto:Ghunt@purdue.edu))

Steve Sheppard ([Shepp@wsu.edu](mailto:Shepp@wsu.edu))

Nick Calderone ([Nwc5@cornell.edu](mailto:Nwc5@cornell.edu))

Bob Danka ([Rdanka@ars.usda.gov](mailto:Rdanka@ars.usda.gov))

Marla Spivak ([Spiva001@umn.edu](mailto:Spiva001@umn.edu))

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

### Ongoing Work:

**Resistance genes to be introduced to CA breeder stocks.** The University of Minnesota is working to assist the California Bee Breeders Association to incorporate hygienic behavior into their diverse breeding stocks.

PROJECT CONTACT: Marla Spivak ([Spiva001@umn.edu](mailto:Spiva001@umn.edu))

## *Appendix: Accomplishments by Action Plan Component*

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

### **Ongoing Research:**

***Project on Protection of Managed Bees to characterize and improve genetic diversity of bees.*** Through a Coordinated Agricultural Project, university researchers will conduct genetic crossing tests to identify candidate genes that influence resistance to bee pathogens. Molecular genetic techniques will be used to assess genetic diversity of honey bee queens from U.S. suppliers, Australia, Russia, U.S. university programs, and from small micro-breeders across the northern U.S. Small- and mid-sized producers will also be surveyed to identify management practices associated with improved genetic diversity. Researchers will use this data to characterize the distribution of diversity to ultimately provide an aid for breeding honey bees, and queen bees sampled in this study will be placed in colonies with the best performers sent to Washington State University, Purdue University, and the University of Georgia for inclusion in breeding programs.

#### PROJECT CONTACTS :

Keith Delaplane, University of Georgia ([Ksd@uga.edu](mailto:Ksd@uga.edu))  
Steve Sheppard ([Shepp@wsu.edu](mailto:Shepp@wsu.edu))  
Greg Hunt ([Ghunt@purdue.edu](mailto:Ghunt@purdue.edu))

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

### **Ongoing Research:**

***Protection of Managed Bees Program to produce technology transfer program for queen breeders.*** A medium term goal of the CAP project described above is to disseminate research knowledge to client groups by developing a technology transfer program for queen breeders. Expected outcomes include the removal of barriers to establish a sustainable market for genetically improved queens and to move forward with the establishment of a stock certification program.

#### PROJECT CONTACTS :

Keith Delaplane, University of Georgia ([Ksd@uga.edu](mailto:Ksd@uga.edu))  
Nick Calderone ([Nwc4@cornell.edu](mailto:Nwc4@cornell.edu))  
Marla Spivak ([Spiva001@umn.edu](mailto:Spiva001@umn.edu))

**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:**

***New acaricide rotation program developed to control varroa mite resistance.*** In collaboration with industry (Mann Lake Ltd., Elanco, Vita [Europe] Limited, Central Life Sciences, and Bayer CropScience), ARS tested several different control methods for the varroa mite and developed an acaricide rotation program that manages development of resistance by *Varroa destructor*. Future studies will continue to develop effective acaricide delivery systems and to test rotational schemes.

PROJECT CONTACT: Frank Eischen ([Feischen@weslaco.ars.usda.gov](mailto:Feischen@weslaco.ars.usda.gov))

2: Develop new methods of managing parasites and pathogens.

**Accomplishments:**

***New biopesticide for varroa mite control developed.*** ARS discovered that the fungus *Beauveria bassiana* was able to increase varroa mite fall from brood cells without negatively impacting bee health, and developed a new biopesticide consisting of spores of the fungus and a plant wax powder. A patent was filed in 2007, and companies have been contacted regarding mass production. Research results on the effectiveness of this approach, however, are inconclusive.

PROJECT CONTACT: William G. Meikle ([Wmeikle@ars-ebcl.org](mailto:Wmeikle@ars-ebcl.org))

***Ozone identified as a fumigant to eliminate pesticides and insect pests of honey bee hives.*** ARS determined that high concentrations of ozone can be used to kill wax moths during hive storage and to eliminate pesticide residues in bee nesting materials. Further tests are currently being conducted to determine the concentrations needed to kill pathogens so that nesting materials can be safely reused year after year.

PROJECT CONTACT: Rosalind R. James ([Rjames@biology.usu.edu](mailto:Rjames@biology.usu.edu))

***New control method for varroa mites devised.*** ARS determined that the food additive 2-heptanone and beta plant acids can be highly effective in controlling varroa mite populations in bee colonies. Scientists are developing delivery systems to optimize the amount needed to kill mites without harming bees.

PROJECT CONTACT: Gloria DeGrandi-Hoffman ([Gloria.Hoffman@ars.usda.gov](mailto:Gloria.Hoffman@ars.usda.gov))

*Appendix: Accomplishments by Action Plan Component*

**Integrated control techniques for varroa mites discovered.** University of Nebraska scientists have developed techniques to reduce varroa mite populations in colonies before winter. Scientists found that a combination of requeening and treating colonies with oxalic acid creates a break in brood rearing and makes mites vulnerable to treatment while increasing colony vigor. Current studies are assessing the effects of oxalic acid treatment on queen survival and on the colony's reproductive capacity. Results suggest that doses within current label recommendations do not significantly impair queen survival, laying rate, egg hatch, or sealed brood production, suggesting that beekeepers should not exceed recommended doses and that they should treat colonies when brood is not present.

PROJECT CONTACT: Marion Ellis ([Mellis@unl.edu](mailto:Mellis@unl.edu))

**Ongoing Research:**

**Two large scale multi-year projects underway to determine the causes of CCD.** Initiated in 2008 by the University of Georgia, the Coordinated Agricultural Project on the Protection of Bees will determine and mitigate the causes of CCD. The team will investigate the role of pests, pathogens, and pesticides in causing the symptoms of CCD in stationary honey bee colonies across the United States. Apiaries will be set up and sampling regimens initiated in spring 2009. Also, ARS is initiating an Areawide Project that will likewise focus on identifying and mitigating the various factors impacting honey bee health, with the simultaneous aim of determining linkages to CCD.

PROJECT CONTACTS:

Keith Delaplane ([ksd@uga.edu](mailto:ksd@uga.edu))

Nancy Ostiguy ([Nxo3@psu.edu](mailto:Nxo3@psu.edu))



**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.

**Accomplishments:**

**New pathogen identification technique developed.** ARS developed a new DNA-based method to identify the honey bee fungal pathogen responsible for chalkbrood disease, *Ascosphaera apis*, in bee samples. This method will allow the rapid detection of this pathogen even before the clinical signs of the disease become visible and will help to monitor/prevent disease spread.

PROJECT CONTACT: Katherine Aronstein ([Kate.Aronstein@ars.usda.gov](mailto:Kate.Aronstein@ars.usda.gov))

**New detection system developed to identify chalkbrood spore contamination.** ARS developed a DNA-PCR based detection system to assess the number of chalkbrood spores contaminating environmental samples. This detection system will be used this year to evaluate levels of contamination in different bee management systems and to study the transmission of the disease from soil, flowers, and wild bees.

PROJECT CONTACT: Rosalind R. James ([Rjames@biology.usu.edu](mailto:Rjames@biology.usu.edu))

**New pathogen detection methods developed.** ARS scientists have developed multiplex and fluorescent PCR-based genetic assays for rapid, sensitive, and specific detection and quantification of seven viruses and two microsporidian parasites in the *Nosema* genus that are mostly commonly found in association with declining honey bee colonies. These assays are now being applied to past and present samples from collapsed and healthy colonies and have been adopted widely by the international bee industry, regulatory, and research communities for monitoring bee disease outbreaks and preventing the spread of bee diseases.

PROJECT CONTACT: Jeff Pettis ([Jeff.Pettis@ars.usda.gov](mailto:Jeff.Pettis@ars.usda.gov))

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

**Accomplishment:**

**New molecular assay for resistant bacterial strains developed.** ARS identified the mechanism of resistance of the bacterial honey bee pathogen (*Paenibacillus larvae*) 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.

PROJECT CONTACT: Katherine Aronstein ([Kate.Aronstein@ars.usda.gov](mailto:Kate.Aronstein@ars.usda.gov))

## *Appendix: Accomplishments by Action Plan Component*

**Guidelines for bee imports developed.** ARS began working with the North American Plant Protection Organization to coordinate regulation and importations of non-*Apis* bees between Mexico, Canada, and the U.S. 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.

PROJECT CONTACTS:

Rosalind R. James ([Rjames@biology.usu.edu](mailto:Rjames@biology.usu.edu))

James Strange ([James.Strange@ars.usda.gov](mailto:James.Strange@ars.usda.gov))

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

The initiation of this work is still under discussion by the CCD Steering Committee.

**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 has initiated 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 to document the impact of migration on bee colonies, examine the effects of supplemental feeding on colony health, develop more resistant bee lines, and develop 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.

PROJECT CONTACT:

Jeff Pettis ([Jeff.Pettis@ars.usda.gov](mailto:Jeff.Pettis@ars.usda.gov))

Tom Rinderer ([Tom.Rinderer@ars.usda.gov](mailto:Tom.Rinderer@ars.usda.gov))

Frank Eischen ([Feischen@weslaco.ars.usda.gov](mailto:Feischen@weslaco.ars.usda.gov))

Gloria DeGrandi-Hoffman ([Gloria.Hoffman@ars.usda.gov](mailto:Gloria.Hoffman@ars.usda.gov))

**Artificial diet shown to build colony strength in pest- and pathogen-stressed colonies.** As part of the Areawide Project on Honey Bees, researchers found that by feeding a pollen-supplemented diet to varroa-infested honey bee colonies, more colonies could meet strength standards for almond pollination than those not fed the supplements (77% compared to 45%). Likewise, the diet was tested on pathogen-infested colonies, where nearly three times as many supplemented colonies met almond pollination standards compared to those not fed the supplemental diet.

PROJECT CONTACT: Frank Eischen ([Feischen@weslaco.ars.usda.gov](mailto:Feischen@weslaco.ars.usda.gov))

**National managed pollinator project initiated.** A consortium of 17 institutions, consisting of Land Grant Universities, 1890's schools, and ARS laboratories, has been formed to develop and deliver research-based answers to problems affecting the health of managed pollinators. Since its formation in July 2008, the project has released a consolidated operations manual, developed a member listserv, and published a Web site. Individual members have begun hiring post-docs and students to begin field work in spring 2009.

PROJECT CONTACT: Keith S. Delaplane ([ksd@uga.edu](mailto:ksd@uga.edu))

[www.beeccdcap.uga.edu](http://www.beeccdcap.uga.edu)

**Ongoing Research:**

**Effects of landscape configuration on honey production and bee health studied.** University researchers are working to develop an advanced data-model assimilation system to study the effects of environmental changes on ecosystem processes. Using land use, climate data, and apiary-level honey production data over 40 years, they are testing the relation of neighborhood landscape configuration on honey production and bee health.

PROJECT CONTACT: Marla Spivak ([Spiva001@umn.edu](mailto:Spiva001@umn.edu))

*Appendix: Accomplishments by Action Plan Component*

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

**Accomplishment:**

***Supplemental protein diet developed and tested.*** In collaboration with S.A.F.E. Research and Development, ARS scientists recently developed MegaBee<sup>®</sup>, also known as the Tucson Bee Diet, a supplemental protein diet that is comparable to naturally collected pollen in attractiveness to bees, consumption rates, and stimulation of colony growth. Scientists compared the effects of the new protein supplement diet to other commercially available diets, pollen, and high fructose corn syrup (HFCS) feedings in terms of consumption rate, brood production, and colony growth. The study showed that MegaBee was consumed at rates comparable to pollen, and that brood production and adult population growth in colonies fed MegaBee was comparable to those fed pollen and significantly greater than those fed other commercial diets or HFCS. This research indicates that the MegaBee diet can be a useful tool for building bee populations in the spring or when flowering plants are limited or unavailable. Other studies conducted by the team demonstrated that bees fed MegaBee had higher body protein levels and lived longer than bees fed other diets or no supplement at all. Scientists from several laboratories are developing a liquid delivery system for the diet.

PROJECT CONTACT:

Gloria DeGrandi-Hoffman ([Gloria.Hoffman@ars.usda.gov](mailto:Gloria.Hoffman@ars.usda.gov))

Gordon Wardell ([GordyAW@aol.com](mailto:GordyAW@aol.com))

**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).

AND

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

**Ongoing Work:**

**CCD outreach and extension efforts underway.** CSREES and ARS (Areawide Project researchers) have facilitated several extension and outreach efforts to inform the public of CCD research findings and updates. These include the maintenance of several Web sites, such as the ARS Web site to answer general questions about CCD ([www.ars.usda.gov/News/docs.htm?docid=15572](http://www.ars.usda.gov/News/docs.htm?docid=15572)), the CCD Coordinated Agricultural Project Web site ([www.beeccdcap.uga.edu](http://www.beeccdcap.uga.edu)) outlining the objectives and aims of the project, and the Honey Bee Health Community of Practice on eXtension Web site (University of Tennessee). This site, anticipated to be publicly available in July 2009, will serve as a focal point for research and extension personnel to provide accurate subject area information and to report on new discoveries concerning CCD. Additional Web sites also provide outreach efforts, including those by Michigan State University and Purdue University; and the BeeSpotter Web site (University of Illinois), a portal to inform the public about pollinators and gather data on the population status of these insects.

**PROJECT CONTACT:**

Kim Kaplan ([Kim.Kaplan@ars.usda.gov](mailto:Kim.Kaplan@ars.usda.gov))

Keith Delaplane ([Ksd@uga.edu](mailto:Ksd@uga.edu))

John A. Skinner ([Jskinner@utk.edu](mailto:Jskinner@utk.edu))

Zachary Huang ([Huang@msu.edu](mailto:Huang@msu.edu))

Greg Hunt ([GHunt@purdue.edu](mailto:GHunt@purdue.edu))

May Berenbaum ([Maybe@uiuc.edu](mailto:Maybe@uiuc.edu))