



United States
Department of
Agriculture

Agricultural
Research
Service

Colony Collapse Disorder Progress Report

CCD Steering Committee
June 2011



Mention of trade names or commercial products in this report is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

This publication reports research involving pesticides. It does not contain recommendations for their use, nor does it imply that uses discussed here have been registered. All uses of pesticides must be registered by appropriate State and/or Federal agencies before they can be recommended.

The U.S. Department of Agriculture (USDA) prohibits discrimination in all its programs and activities on the basis of race, color, national origin, age, disability, and where applicable, sex, marital status, familial status, parental status, religion, sexual orientation, genetic information, political beliefs, reprisal, or because all or part of an individual's income is derived from any public assistance program. (Not all prohibited bases apply to all programs.) Persons with disabilities who require alternative means for communication of program information (Braille, large print, audiotape, etc.) should contact USDA's TARGET Center at (202) 720-2600 (voice and TDD). To file a complaint of discrimination, write to USDA, Director, Office of Civil Rights, 1400 Independence Avenue, SW., Washington, D.C. 20250-9410, or call (800) 795-3272 (voice) or (202) 720-6382 (TDD). USDA is an equal opportunity provider and employer.

Cover: Adult honey bee on a flower. Photo courtesy of David Cappaert, Michigan State University, Bugwood.org.

CCD Steering Committee Members

USDA Agricultural Research Service (co-chair)
Kevin Hackett

USDA National Institute of Food and Agriculture (co-chair)
Mary Purcell-Miramontes

USDA Animal and Plant Health Inspection Service
Robyn Rose
Colin Stewart
Alan Dowdy

USDA National Agricultural Statistics Service
Bruce Boess

USDA Natural Resources Conservation Service
Doug Holy

USDA Office of Pest Management Policy
Sheryl Kunickis
David Epstein

Environmental Protection Agency
Tom Steeger
Tom Moriarty
Allen Vaughan

Executive Summary

Mandated by section 7204(h)(4) of the Food Conservation and Energy Act, Pub. Law No. 110-246, the “2008 Farm Bill,” this third annual report of research progress during 2010 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 National Institute of Food and Agriculture (NIFA) led a collaborative effort to define an approach for responding to CCD, resulting in the CCD Action Plan, in July 2007. Other Federal agencies e.g., the Animal and Plant Health Inspection Service (APHIS), the Natural Resources Conservation Service, and the Environmental Protection Agency’s (EPA’s) Office of Pesticide Programs have been members of the CCD Steering Committee for many years; this year, USDA’s Office of Pest Management Policy and the National Agricultural Statistics Service have joined the Steering Committee. Many universities and organizations (i.e., Federal, State, and private) were also involved in developing this plan and are carrying out work that addresses the CCD problem.

Annual surveys clearly show that overall colony losses continue to be as high as 30 percent or more since CCD began to be reported. Beekeepers cannot economically sustain such high levels of losses indefinitely. Although a number of factors have been associated with CCD and pollinator declines in general, no single factor or specific combination of factors has been identified as a “cause.” Factors associated with declines include disease/parasites, nutrition, pesticides, bee management practices, habitat fragmentation, and agricultural practices. Reducing the incidence of CCD and pollinator declines will likely require managing multiple factors simultaneously. Overall losses to managed pollinators were about 33 percent. Relative to the overall losses, CCD contributed approximately 8 percent in recent national surveys. However, losses to individual beekeepers attributed to CCD may vary significantly by size of the beekeeping operation. Therefore, this statistic is just a rough estimate.

To help ensure more robust pollinator health, the CCD Steering Committee is facilitating the development of science-based best management strategies that integrate current research results associated with CCD incidence and pollinator decline in general. As part of these efforts, the CCD Steering Committee member agencies are working together to examine ways to mitigate impacts on pollinator health. This effort requires coordination across different Federal partners (USDA, Department of Interior, and EPA), States, and stakeholders to ensure a sustained effort that effectively integrates management options.

The CCD Steering Committee believes that it is necessary for scientists, beekeepers, and growers to reconvene in the next year to revisit approaches and research, which are now needed to counter honey bee decline, taking into account information developed during the past 3 years and to revise the 2007 Action Plan to reflect the current state of knowledge. A range of stakeholders will be engaged to develop strategies for managing factors associated with diminished pollinator health and not focus exclusively on CCD.

Research is ongoing in the four topic areas outlined in the CCD Action Plan, but the studies encompass factors involved in bee losses in general, not just due to CCD. The four areas are **(1) survey and sample data collection, (2) analysis of existing samples, (3) research to identify factors affecting honey bee health, including attempts to recreate CCD symptomology, and (4) mitigation and management preventive measures.** Progress in each of the four topic areas is highlighted below.

- **Topic I: Survey and Sample Data Collection.** Surveys continue to provide evidence of high honey bee losses due to CCD and declines in pollinator health. Correlated with these high levels of losses, research has shown that weak colonies had overall increased pathogen levels and showed evidence of pesticide residues, although no pattern of specific pathogens or pesticides was indicated. Colonies in comparatively good health also contained a wide range of pesticide residues. To understand the potential role of pesticides in pollinator declines, representatives of the CCD Steering Committee (APHIS, ARS, and EPA) participated in a Society of Environmental Toxicology and Chemistry global Pellston conference. The purpose was to develop a risk assessment process for honey bees and non-*Apis* bees and to identify the exposure and effects data needed to inform that process.
- **Topic II: Analysis of Existing Samples.** Previously, viruses and other pathogens and parasites were found to be present at greater levels in CCD colonies than in non-CCD colonies. The extent to which pesticides are associated with CCD remains uncertain, and additional research is necessary. Studies in 2010 revealed several new viruses and other pathogens affecting honey bees. Further studies are needed to determine if these new pathogens are involved in CCD-affected hives.
- **Topic III: Research to Identify Factors Affecting Honey Bee Health, Including Attempts to Recreate CCD Symptomology.** CCD was initially characterized by the rapid loss of adult worker bees from the colony, lack of dead worker bees, and delayed invasion of hive pests. Although additional studies are needed, researchers have recently observed that *Varroa* mite and other pathogens such as *Nosema* may be contributing factors to CCD. In addition, the *Varroa* mite and other pathogens occur at levels that are typically considered below economic thresholds. Researchers continue their efforts to document whether there are correlations between the presence of *Varroa* mites, diverse pathogens, and pesticides, which appear to impact overall colony health. In addition, apiaries surrounded by intensively farmed landscapes were found to be associated with higher colony losses in the spring.
- **Topic IV: Mitigation and Management Preventive Measures.** Two national multiyear projects, the ARS Area-wide Project on Honey Bee Health and a NIFA-funded Coordinated Agricultural Project (CAP), are continuing to make progress in developing managements strategies to combat bee losses. A new CAP project funded by NIFA, the “Bee Informed Partnership” (<http://beeinformed.org>), has begun to examine bee management practices and facilitate communication of successful practices between beekeepers. The eXtension Community of Practice (www.extension.org/bee_health) is also disseminating information on honey bee health and management practices.

Colony Collapse Disorder (CCD) Annual Progress Report

This report is the third annual report prepared in response to section 7204(h)(4) of the 2008 Farm Bill, which directed the Secretary of Agriculture to—

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

Background and Highlights of Research

After the large-scale, unexplained losses of managed U.S. honey bee (*Apis mellifera* L.) colonies during the winter of 2006–2007, investigators identified a set of symptoms that were termed “Colony Collapse Disorder” (CCD). In response to this problem, Federal and State government, university, and private researchers, led by USDA’s Agricultural Research Service (ARS) and National Institute of Food and Agriculture (NIFA), mobilized to define an approach to CCD, an effort resulting in formation of the CCD Steering Committee and publication of the CCD Action Plan in July 2007. Over the past several years, the CCD Steering Committee has included representatives from USDA (Animal and Plant Health Inspection Service (APHIS), NIFA, ARS, and Natural Resources Conservation Service [NRCS]) and the Environmental Protection Agency’s (EPA) Office of Pesticide Programs. This year, USDA’s Office of Pest Management Policy (OPMP) and National Agricultural Statistics Service (NASS) joined the Steering Committee. Many organizations, public and private, in addition to those represented on the CCD Steering Committee, are involved in the work to address the CCD problem.

During the past 3 years, numerous causes for CCD have been proposed and examined. There have been many associations identified throughout the course of research; however, the strength of these associations has varied considerably, and it has become increasingly clear that no single factor alone is responsible for the malady.

Researchers continue to document elevated pathogen levels in CCD-affected bees, although no single pathogen or group of pathogens has been definitively linked to CCD. In addition, studies that examined colonies for the presence of known honey bee parasites, such as the *Varroa* mite (*Varroa destructor*), tracheal mite (*Acarapis woodi*), and the fungal gut parasite *Nosema* spp., have not found that any of these parasites by themselves occurred at sufficient levels or pattern to explain CCD.

During the past year, several independent studies have shown that bees are exposed to a wide range of pesticides and that some of these pesticides at high concentrations interact with other pesticides, honey bee parasites, or viruses in ways that significantly increase individual bee mortality. Further studies are needed to ascertain whether these greater-than-additive effects occur at environmentally relevant pesticide concentrations. In addition, studies have shown that exposure to pesticides can result in effects on bee behavior. However, further studies on

individual bees are needed to better understand colony-level effects. The pesticides detected with the greatest frequency and quantities are those used by beekeepers to control mites.

Conversely, bees exposed to the pesticide coumaphos, which is used by beekeepers to treat *Varroa* mites, appear to have lower levels of CCD, suggesting that reducing infestation of *Varroa* mites could directly or indirectly reduce incidence of CCD. Results from other long-term monitoring projects also suggest that the fungicide chlorothalonil has been associated with a newly identified condition in the hive named “entombed pollen,” which is associated with premature bee mortality. However, it is not yet known whether the presence of chlorothalonil within honeybee comb is exclusively associated with entombed pollen. Other factors not yet determined may also have a role. Taken together, these studies illustrate support for the hypothesis that CCD is a result of many different factors, factors that may work independently or in combination.

Because CCD is a complex phenomenon, developing effective solutions to the problem will depend on considerable research commitment. The current coordinated research response is dedicated to resolving the CCD issue, as well as improving overall pollinator health. NIFA’s Coordinated Agricultural Project (CAP) and ARS’ Area-wide Project on Honey Bee Health are ongoing efforts that have taken broad, regional approaches to solving pollinator losses. Funding from ARS and NIFA, with additional contributions by a number of other sources, including the National Honey Board, the Almond Board of California, Burt’s Bees, Haagen-Dazs, the North American Pollinator Protection Campaign, Project Apis m. (PAm), and the Foundation for the Preservation of Honey Bees, has supported a variety of new studies and attracted new expertise to bee health issues. Results from these research efforts are being published, and a new eXtension bee-health Web site (www.extension.org/bee_health) has been assembled to provide reliable research-based information to beekeepers and the general public.

In an effort to address the multiple factors associated with pollinator declines, the CCD Action Plan is organized under four topic areas: (1) Survey and sample data collection, (2) analysis of existing samples, (3) research to identify factors affecting honey bee health, including attempts to recreate CCD symptomology, and (4) mitigation and management preventive measures. Summaries of research under each of the four topic areas are presented below. More detailed accounts of progress on each of the research projects can be found in Appendix 1.

Topic I: Survey and Sample Data Collection

Surveys of beekeepers throughout the United States were jointly conducted for the fourth consecutive year by the Apiary Inspectors of America and ARS. Total losses (not limited to CCD) for winter 2010–2011 were 30 percent, which was in the same range as previous surveys performed between 2007 and 2009. Most beekeepers indicated that this level of loss was economically unsustainable for beekeeping operations. Large-scale commercial beekeepers indicated that losses were due to several contributing factors, including poor queens, *Varroa* mite, pesticides, and CCD.

Preliminary results from systematic surveys of experimental apiaries in seven States managed by NIFA-funded CAP researchers reported that the leading causes of overall losses included intensive agricultural use of the surrounding landscape, pesticide exposure, and *Varroa* mite and

diseases. Surveys conducted in Canada and Europe showed that *Varroa* mite and pathogens were consistently the leading factors correlated with declines.

USDA's APHIS expanded its survey of beekeepers from 13 to 34 States to detect exotic pests and diseases of honey bees. The survey conducted thus far has not detected *Apis ceranae*, the Slow Paralysis Virus that has been reported in Australia, or the parasitic mite *Tropilaelaps*, which commonly is found in Asia on several species of honey bees; so these pests have likely not invaded the United States. *Nosema ceranae*, which is a microsporidial pathogen recently introduced into the United States, was the only species of *Nosema* detected in this recent APHIS survey. This species has been tentatively linked in some studies as contributing to CCD in the United States.

Members of the CCD steering committee (EPA and USDA's APHIS and ARS) participated in a global Society of Environmental Toxicology and Chemistry (SETAC) Pellston¹ workshop in January 2011. The global Pellston conference is an important beginning in developing improved approaches for characterizing potential sublethal impact of pesticides on individual bees and on colonies, as well as to identify the data needed to inform the risk assessment process. The SETAC conference also examined the adequacy of tests performed with *Apis mellifera* to serve as a model to predict effects on other "non-*Apis*" pollinators.

Topic II: Analysis of Existing Samples

As reported in the 2010 Progress Report www.ars.usda.gov/is/br/ccd/ccdprogressreport2010.pdf, a large survey revealed that levels of pesticides in wax and pollen were similar in both CCD and healthy colonies. Further analysis showed that levels of the varroacide coumaphos were higher in healthy control colonies relative to CCD colonies. These preliminary data suggest several possibilities that need to be examined with more detailed, hypothesis-driven experiments. Molecular studies by scientists at the University of Illinois seem to support the hypothesis that exposure to coumaphos may have some beneficial effect on ability of bees to resist pathogens. They found that honey bee genes express enzymes that can detoxify harmful compounds in honey and propolis.

In addition, this year the ARS Pollinating Insect Research Laboratory in Logan, Utah, analyzed existing samples of managed solitary bees used for pollinating crops. These bees were evaluated to determine whether they were infected by any of the viruses associated with CCD in honey bees. Some of these honey bee viruses were found in both alfalfa leaf-cutting bees and alkali bees, with infection rates that ranged from 3 to 34 percent of the population.

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

Pennsylvania State University extension specialists are working with the USDA Agricultural Marketing Service's National Science Laboratory in North Carolina to analyze wax, pollen, and brood samples for the presence of pesticides for beekeepers who suspect colony losses associated with exposure to pesticides. The Foundation for the Preservation of Honey Bees and Project Apis m. (PAm) are contributing funds to cover half of the cost of the pesticide analysis. Information from samples is being stored in a centralized database that beekeepers and researchers can access. At this time, the database is not accessible to the general public.

EPA is collating beekill incident information provided through a variety of sources to document whether particular pesticides are associated with the losses. While pesticide manufacturers (registrants) are required to report incident data directly to the EPA, the Agency also relies on reports from State and local governments and the public to populate its incident database. EPA has increased the number of options available to the public for reporting beekill incidents directly to the Agency.

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

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

Possible Mortality Factors and Their Interactions. Researchers at Pennsylvania State University conducted laboratory bioassays with specific levels of pesticides to measure a variety of effects on bees. They found synergistic interactions on bee larvae between a crop fungicide and a miticide used by beekeepers for *Varroa* control. This means that the bees suffered more serious effects when exposed to more than one pesticide at the same time and that bee management practices may be affecting colony health. Further studies using pesticide dosages equivalent to those actually used in the field (environmentally relevant concentrations) need to be conducted to show if the laboratory study reflects what actually occurs in the environment. The researchers also found that larval and adult honey bees had different sensitivities to pesticides. A fungicide that was very toxic to bee larvae did not kill adult honey bees. These studies utilized exposure methods that represent the worst case scenario and do not necessarily reflect how larval bees may actually be exposed through consumption of brood food. Additional studies demonstrated that common inert ingredients used in pesticides had a high toxicity in honey bee larvae, although the extent to which these inerts would be present under actual field conditions is yet to be determined. These preliminary studies suggest that fungicides, inert ingredients, and pesticide interactions may have crucial impacts on development and survival of honey bees, depending on the extent to which the inert may be present under the conditions of actual use.

Previously, scientists at the University of Nebraska–Lincoln documented the harmful synergistic effects of certain miticides and fungicide combinations on honey bee health in laboratory studies (Johnson, *et al.* 2010). Although further testing needs to occur at environmentally relevant concentrations, these scientists recommend that beekeepers be cautious about applying these pesticides in combination. Beekeepers should particularly avoid applying miticides when honey

bees are placed in orchards or other crop settings where exposure to fungicides is likely. Studies are underway to assess the effects of exposure to simultaneously applied miticides and fungicides at environmentally relevant concentrations on brood survival, weight gain, and queen performance.

As previously reported, Israeli acute paralysis virus (IAPV) was found to be associated with CCD and could be an important CCD indicator. Following up on this finding, ARS scientists studied the role of *Varroa* mite in IAPV transmission among honey bees. Because mites feed and move between adult bees and brood, they have the potential to act as a vector to transmit pathogens from infected bees to healthy bees, and they have been reported to be associated with viral disease outbreaks in the field. Research results demonstrated that *Varroa* mite is a biological vector of IAPV by supporting replication of the virus and transmitting IAPV among honey bees. Therefore, controlling *Varroa* mite might be an important way to reduce the spread of IAPV in bee hives and could be a strategy for reducing bee decline.

Bumble Bee Decline. Some bumble bee species also have experienced drastic population declines, even to the point of extinction. This brings into question whether CCD is solely a honey bee issue or part of a more general problem among bees of many kinds. This past year, a North American Bumble Bee Conservation Planning meeting was organized for the first time (November 2010) to bring together researchers, government agency representatives, conservation organizations, beekeepers, and growers to discuss the recent declines in bumble bees and identify needs related to bumble bee conservation. The meeting was facilitated by a representative from the International Union for the Conservation of Nature (IUCN) and resulted in the formation of a bumble bee specialist group to advise IUCN on bumble bee issues. This group is preparing a report of its findings.

Immune System Effects. Another study conducted by ARS scientists resulted in a somewhat counterintuitive conclusion, a finding that temperature-stressed bees were more resistant to infection by pathogens than nonstressed bees. Using alfalfa leafcutting bees (*Megachile rotundata*) as a model, experiments determined that bees exhibit a heightened biological response to temperature stress that has many similarities to the immune response. Thus, if bees were stressed by higher temperatures before a pathogen started to invade, infection levels were lower. The study hypothesized that the pathogen invaded nonstressed bees more readily, as these bees did not have time to activate their immune response before the pathogen was able to become established and disable some of the honey bees' immune functions.

Topic IV: Mitigation and Management Preventive Measures

Trilateral Discussions to Prevent Invasive Pathogens. To meet the need for a stronger regulatory framework for controlling the accidental introduction of exotic bee pathogens, APHIS and ARS initiated trilateral discussions between Mexico, Canada, and the United States through the North American Plant Protection Organization (NAPPO), with the goal of developing coordinated guidelines for the regulation and importation of pollen. The importation of pollen as a source of food for bees is currently not allowed in the United States and Canada, but it is allowed in Mexico. However, the importation of pollen for human consumption (regulated in the United States by the Food and Drug Administration) is allowed in all three countries, which could provide a pathway for the introduction of new pathogens. The NAPPO technical advisory group is also trying to determine whether mitigation measures such as radiation or ozone are feasible. APHIS is working to amend its regulations to allow pollen importation for bee feed under permit with the appropriate safeguards.

Best Management Practices (BMP) Guide. To assist beekeepers and growers relying on pollinators, a *Best Management Practices Guide for Beekeepers Pollinating California's Agricultural Crops* (www.beecdcap.uga.edu/documents/bmpcalagr.html) was developed by members of the NIFA-CAP team. The Guide was published in the *American Bee Journal* and in the trade magazine *Bee Culture*, two of the most widely read beekeeping publications in North America. The article offers guidance to beekeepers and almond growers in seven areas: nutrition, *Varroa* mite control, *Nosema* control, management of hive equipment, colony management, business management, and guidance to almond growers renting bees.

Genomics Tools to Manage *Varroa* Mites. A genome draft sequence was published for the *Varroa* mite, revealing potential weak points in mite biology (defensive proteins and proteins used in chemical mitigation) and candidates for novel controls such as RNA interference (RNAi). In fact, the publication of mite candidate genes in genomic databases led to worldwide control studies for this parasite using RNAi technology. Microbes also identified in this study have been screened across bees and mites as possible controls for *Varroa*.

***Varroa*-Resistant Bees for Commercial Use.** ARS scientists tested Russian honey bees and bees containing a *Varroa*-resistant trait (*Varroa*-sensitive hygiene [VSH]) for productivity in two largescale field tests using two different migratory beekeeping routes. Two years of testing during commercial pollination of almonds, apples, blueberries, and cranberries, with overwintering in Louisiana, showed the *Varroa*-resistant stocks to be as large and productive as control stocks at each pollination site. A 1-year test involving almond pollination and Midwest honey production, with overwintering in California's Central Valley, again showed Russian and VSH bees to have adequate colony size, survivability, and honey production. The results suggest these *Varroa*-resistant bees are well suited for commercial pollination services and honey production.

EPA's Expediting of Reviews for Section 18. EPA is working with State liaison agencies to provide beekeepers with appropriate tools to control bee colony pests such as *Varroa* mites. The Agency is working closely with USDA's ARS and OPMP to expedite reviews of Emergency Exemption requests (under section 18 of the Federal Insecticide, Fungicide and Rodenticide Act; FIFRA), Special Local Needs Registrations (under section 24c of FIFRA), and full registrations (under section 3 of FIFRA) of miticides.

Blue Orchard Bees for Almond Pollination. This past year, three workshops were conducted by collaborating scientists with the California Farm Advisors and ARS to train almond producers and blue orchard bee (*Osmia lignaria*) producers on methods for raising this bee and for using it effectively as an almond pollinator. Research also continues to seek the best way to integrate bee hives for the most cost-effective and complete pollination. Field trials were established this year in a joint collaboration between ARS and almond producers in California.

Bumble Bee Rearing and Investigations of Bumble Bee-Honey Bee Disease Transmission. Research continued toward developing mass rearing methods for bumble bee colonies using native western bumble bees (*Bombus occidentalis*). Eastern U.S. species are thought to carry diseases that may affect wild bumble bee populations in the Western United States, so commercially available species are needed for Western farmers.

Pollinator studies have also focused on exploring the use of bumble bees as pollinators and seeking to determine the relationship between bumble bee and honey bee pests and diseases. Studies have revealed that bumble bees are affected by IAPV and other viruses and parasites that affect the honey bee. These findings may have potential implications for the role of these factors in bee decline and may assist researchers to develop an understanding of, and ultimately controlling, bee parasites.

Enhancing Pollinator Forage. The Natural Resources Conservation Service has continued to encourage private landowners to establish or enhance pollinator forage by providing appropriate flowering plants from early spring through late fall.

The Appendix provides more results and findings from the past year of CCD research, listed within the framework of the CCD Action Plan.

APPENDIX: Accomplishments by Action Plan Component

Topic I: Survey and Sample Data Collection

Pesticides have been identified as one of the factors that potentially contribute to CCD and to diminished pollinator health. The EPA's Office of Pesticide Programs (OPP), which is tasked with regulating pesticides, has been taking several actions to enhance the tools used to regulate pesticide products.

Global Workshop on Pesticide Risk Assessment for Pollinators, January 2011.

Representatives of the U.S. Department of Agriculture (USDA), including ARS and APHIS, and EPA's OPP served as panelists in a Society of Environmental Toxicology and Chemistry (SETAC) global Pellston Workshop on Pesticide Risk Assessment for Pollinators. Workshop participants totaling 48 from 5 continents were tasked with advancing the current state of the science of pesticide risk assessment by more thoroughly vetting quantitative and qualitative measures of exposure and effects on individual bees and at the colony level. The SETAC Workshop aimed to synthesize global understanding of exposure and effects measurements and work toward a common/harmonized global process for evaluating and quantitatively characterizing risk to insect pollinators from exposure to pesticides. The SETAC Workshop focused on four major topics to—

1. Design/identify testing protocols to provide an understanding of potential exposure to bees (including daily ingestion rates) from pesticide residues in pollen, and nectar, as well as from exposure through other routes of direct and indirect exposure;
2. Design/identify testing protocols to measure effects of pesticides to developing brood and adult honey bees at both the individual and colony level;
3. Propose a tiered approach for characterizing the potential risk of pesticides to pollinators; and
4. Explore the applicability of testing protocols, used for honey bees (*Apis* bees), to measure effects of pesticides and pesticide risk to native (non-*Apis*) insect pollinators.

SETAC will publish an overview (synopsis) of the Workshop online in summer 2011, and the full proceedings of the workshop will be published by SETAC in early 2012.

EPA has committed to developing a quantitative process for evaluating the potential risks of pesticides to insect pollinators for use in regulating pesticide products. EPA will examine the conclusions of the SETAC workshop and will utilize its Scientific Advisory Panel (SAP) to publicly vet a proposed process in summer 2012.

Coordination with International Partners. Efforts, such as those by EPA, have been underway to coordinate elements of science and policy with international partners such as the Organization for Economic Co-operation and Development (OECD) to better understand the potential role of pesticide use in bee declines. The OECD Working Group on Pesticides has established the Pesticide Effects on Insect Pollinator (PEIP) Expert Group that has developed a series of activities² (<http://www.oecd.org/dataoecd/19/27/45275778.pdf>) on pollinator testing, research,

²OECD 2010. Environmental Directorate Joint Meeting on the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology (ENV/JM/MONO(2010)24 Series on Pesticides No. 52 OECD Survey on Pollinator Testing, Research, Mitigation and Information Management: Survey Results <http://www.oecd.org/dataoecd/19/27/45275778.pdf>.

mitigation, and information management related to insect pollinator declines. The PEIP is currently working on four activities: (i) establishing means for efficient communication of pollinator incidents among regulatory authorities; (ii) developing pollinator testing requirements; (iii) sharing information aimed to mitigate potential risk of pesticides to pollinators; and (iv) developing a pollinator research clearinghouse. As a first step, questionnaires related to these topics were distributed to OECD member countries. Responses to these questionnaires will be used to develop tools to facilitate timely sharing and understanding of available information.

PROJECT CONTACTS

Tom Steeger (Steeger.Thomas@epa.gov)

Tom Moriarty (Moriarty.Thomas@epamail.epa.gov)

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

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

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

Accomplishments

U.S. Bee Loss Survey. ARS, in collaboration with the Apiary Inspectors of America, conducted a nationwide bee loss survey for the fourth consecutive year. Participation this year was at an all-time high, in part due to survey availability online. Results indicated a trend similar to previous years with this past fall-winter losses at 34.4 percent, consistent with the 33 percent average loss rate of past surveys. CCD-like symptoms continued to be identified by beekeepers as being observed in some colony losses, with 80 percent of commercial beekeepers reporting these symptoms. U.S. beekeepers continue to have elevated colony losses that include CCD in the fall and winter, which does not include the loss of colonies in the spring and summer; over time, these losses are a major economic drain on beekeepers.

PROJECT CONTACTS

Dennis vanEngelsdorp (dennis.vanengelsdorp@gmail.com)

Jeff Pettis (jeff.pettis@ars.usda.gov)

Improved Field Collection and Shipping Methods for Bee Diagnoses. A novel shipping method for live worker bees was developed and proven in field and laboratory analyses. This method improves on prior strategies in cost to shippers (often beekeepers) and in the ability to deliver material suitable for RNA analysis. It is now the method of choice for forensic analyses, including for the USDA-APHIS and USDA-ARS National Bee Survey.

PROJECT CONTACTS

Jeff Pettis (jeff.pettis@ars.usda.gov)

Dennis vanEngelsdorp (dennis.vanengelsdorp@gmail.com)

Judy Chen (judy.chen@ars.usda.gov)

Jay Evans (jay.evans@ars.usda.gov)

Tool Development for Tracking and Understanding CCD. BRL scientists improved current methods to (1) collect field samples of honey bee populations and ship them for genetic analyses, (2) stabilize and extract RNA, (3) conduct high-throughput genetic screens for viruses and other pests, (4) collect embryos from established colonies, and (5) carry out controlled experiments on adult bees using sterile cups. These methods are being used in national surveys in the United States in order to establish cell lines and other genetic techniques, and in attempts to combine possible causes of CCD (pathogens and pesticides) in an attempt to determine interactive effects.

PROJECT CONTACTS

Judy Chen (judy.chen@ars.usda.gov)

Jay Evans (jay.evans@ars.usda.gov)

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

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

Accomplishment

USDA-APHIS Bee Pest Survey: APHIS, in collaboration with ARS and 13 States, completed a limited pest and disease survey to look for exotic pests. The survey showed that no exotic *Tropilaelaps* mites, Slow Paralysis Virus, or *Apis ceranae* bees were detected in the 349 samples representing 2,700 colonies across the United States. *Nosema ceranae* was the only species of *Nosema* detected, and no tracheal mites were detected, although they have been common in the United States in past years. The survey results are available online at the APHIS Bee Health site. The national survey expanded to include 34 States this year.

PROJECT CONTACTS

Robyn Rose (robyn.rose@aphis.usda.gov)

Jeff Pettis (jeff.pettis@ars.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.

and

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

Accomplishments

New Viruses Discovered in Large Migratory Bee Operation. Scientists at the University of Californian–San Francisco conducted a study of a large-scale migratory beekeeping operation that transported bees from South Dakota to Mississippi and California in 2010. Using comprehensive molecular detection methods, including a custom microarray, quantitative polymerase chain reaction (qPCR), and ultra-deep sequencing, the researchers uncovered the seasonal incidence and abundance of several viruses, the microsporidian *Nosema* sp., *Crithidia mellificae*, and several bacteria that are well-known bee pathogens. In addition, they discovered four novel RNA viruses, two of which were the most abundantly observed components of honey bee pathogens. One virus, the newly named Lake Sinai virus strain 2 (LSV2), predominated.

Funding: Project Apis m., Howard Hughes Medical Institute, Genentech Graduate Student Fellowship, A.P. Giannini Foundation Medical Research Fellowship, and UC-Davis’s Häagen-Dazs Postdoctoral Fellowship in Honey Bee Biology.

PROJECT CONTACT

Joe Derisi (joe@derisilab.ucsf.edu)

Presence of Chronic Bee Paralysis Virus in the United States. Chronic bee paralysis virus (CBPV) is a disease agent that causes characteristic “paralysis” symptoms in adult honey bees and could lead to host and colony mortality. Using developed molecular diagnostic tools, ARS scientists presented the first evidence of CBPV infection in honey bees in the United States. The study yielded important information on the incidence of CBPV infection in the U.S. population of adult honey bees and demonstrated the phylogenetic relationships of U.S. strains of CBPV with isolates of CBPV from the different geographical regions of the world, thereby increasing our understanding of virus diversity in the United States.

PROJECT CONTACTS

Judy Chen (judy.chen@ars.usda.gov)

Jay Evans (jay.evans@ars.usda.gov)

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

Funding: USDA-NIFA.

PROJECT CONTACTS

Anne Averill (aaverill@ent.umass.edu)

Lee Solter (lsolter@illinois.edu)

Frank Drummond (frank.drummond@umit.maine.edu)

A New Bee Fungus Found in Association with Solitary Bees. A new fungus that is closely related to the fungal pathogen that causes chalkbrood (*Ascospaera apis*) was found in the pollen stores and nesting materials of alfalfa leafcutting bees, *M. rotundata*, in Canada and the western United States. The new fungus was named *A. subglobosa*. This new species, closely related to *A. atra* and *A. duoformis*, is distinct from other *Ascospaera* species by its evanescent spore balls, globose to subglobose spores, and unique nuclear ribosomal DNA ITS sequence. Discoveries such as this non-pathogenic *Ascospaera* are assisting in elucidating the evolution of microbes to the pathogenic state, and could help us better understand the sudden arrival of new bee pathogens.

Funding: USDA-ARS and University of Copenhagen.

PROJECT CONTACT

Rosalind James (rosalind.james@ars.usda.gov)

Characterizing Chalkbrood Strains in Honey Bees. The pathogenic fungus *A. apis*, which causes chalkbrood disease, is ubiquitous in honey bee populations. The draft genome assembly of this pathogen was used to search for DNA markers adequate to identify different strains and populations of this pathogen. DNA primers were designed for five different markers and tested against a panel of closely related fungal species. These markers were compared for 12 distinct isolates of the pathogen. Use of three of the most variable markers had the same detection power as use of all five markers.

Funding: USDA-ARS and University of Copenhagen.

PROJECT CONTACT

Rosalind James (rosalind.james@ars.usda.gov)

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

Funding: USDA-NIFA.

PROJECT CONTACTS

Anne Averill (aaverill@ent.umass.edu)

Lee Solter (lsolter@illinois.edu)

Frank Drummond (frank.drummond@umit.maine.edu)

Goal 2: Identify and characterize pests associated with CCD.

1. Use standard sampling methods to analyze samples for tracheal and *Varroa* mites and *Nosema* species.

Accomplishment

Interactions between Pests, Pathogens, and Pesticides Being Investigated. With funding from the NIFA Coordinated Agricultural Project, scientists are examining the potential interactive effects of pests, pathogens, and pesticides in seven States across the United States. After the first 2-year trial, preliminary results indicate that surrounding landscape, pesticide exposure, parasites, and diseases were significant factors in explaining overall colony losses, and not losses due to CCD alone. As the percentage of intensive agriculture in the landscape surrounding an apiary site increased, it was found that colony losses in the spring also increased. This might be due to decreased flower resources, but it also could be related to increased pesticide exposure. Additional research is needed to resolve this uncertainty. It was observed that as pesticide-contaminated pollen brought back to the hive increased, the queen replacement due to supercedure also increased, suggesting a potential mechanism for overall colony losses. However, the laboratory also found that colony losses during the spring and summer buildup season were due to the parasitic *Varroa* mite, *Nosema* disease, and IAPV. Overwintering colony losses were found to be a function of *Varroa* levels and IAPV in colonies. Although further studies are needed to make firm conclusions, these results suggest that CCD might be a combination of *Varroa*, *Nosema*, and viral disease. In addition, these results corroborate the findings of previously published genomic and proteomic analyses suggesting *Varroa* and viral associations for CCD. The relationship between pesticide exposure to bees and queen supercedure suggests that pesticides also could play a role in weakening colonies that ultimately might lead to losses.

Funding: USDA-NIFA.

PROJECT CONTACTS

Frank Drummond (frank.drummond@umit.maine.edu)

Nancy Ostiguy (nxo3@psu.edu)

Katherine Aronstein (kate.aronstein@ars.usda.gov)

Steve Sheppard (shepp@wsu.edu)

Kirk Visscher (visscher@ucr.edu)

Marla Spivak (spiva001@umn.edu)

Jamie Ellis (jdellis@ufl.edu)

John Adamczyk (john.adamczyk@ars.usda.gov)

Steve Cook (steve.cook@ars.usda.gov)

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

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

Accomplishments

Collecting and Assessing Pesticide-Related Bee Kill Incidents. In its role in regulating pesticides, EPA requires data to document the potential effects of pesticides on honey bees at the level of the individual organism and at the colony level, depending on the outcome of lower tier tests and other scientifically relevant information. EPA also collects and assesses data on ecological (bee kill) incidents associated with the use of pesticides. Incidents are typically reported to State Lead Agencies (SLAs; consisting primarily of State departments of agriculture) within each of the 50 States, where formal investigations are conducted to determine whether specific pesticides may have been associated with the incidents. Incident reports may also be reported to pesticide registrants who may also conduct investigations. Only the pesticide registrants are required by law to report incidents to EPA; States that investigate incident reports may or may not relay the results of those investigations to EPA. Incident reports received through this process are recorded in a database (i.e., the Ecological Incident Information System [EIIS]) maintained by OPP.

In addition to this formal process for reporting and investigating incidents, incidents may also be reported by the public through the National Pesticide Information Center (NPIC) Web site (<http://npic.orst.edu/reportprob.html#env>), maintained by Oregon State University under an assistance agreement with EPA. Bee kill incident reports may also be submitted directly to EPA via e-mail using the beekill@epa.gov address that is available on EPA's pollinator Web site (<http://www.epa.gov/opp00001/ecosystem/pollinator/science.html>) or by contacting the Agency directly by phone. Incident reports that are not investigated, however, may not contain sufficient information to support risk assessments that rely on these data as a line of evidence for whether a chemical can impact non-target species such as honey bees. Detailed information (such as pesticide use information, residue data, number of bees/colonies lost, time over which the incident took place, and colony health/management) enables risk assessors to better evaluate whether a particular pesticide can be reasonably linked to a bee kill incident.

PROJECT CONTACTS

Tom Steeger (Steeger.Thomas@epa.gov)

Tom Moriarty (Moriarty.Thomas@epamail.epa.gov)

Pesticide Variability Analyzed in Stationary Hive Study. Scientists supported by NIFA's Coordinated Agricultural Project analyzed pesticide residues in stationary bee hives and identified a variety of different chemicals and their metabolites in hives. During the past 2 years (2009–2010), these researchers have been monitoring pesticides in pollen collected by foraging honey bees at the apiaries set up for the stationary hive survey in both rural and urban environments. The amount of pesticides observed varies with time and location. On average, each sample has had residues of four pesticides. A total of 45 different pesticide compounds or metabolites have been observed during the past 2 years including insecticides, fungicides, and herbicides.

Funding: USDA-NIFA.

PROJECT CONTACTS

Brian Eitzer (Brian.Eitzer@ct.gov)

Frank Drummond (frank.drummond@umit.maine.edu)

Nancy Ostiguy (nxo3@psu.edu)

Katherine Aronstein (kate.aronstein@ars.usda.gov)

Steve Cook (steve.cook@ars.usda.gov)

Steve Sheppard (shepp@wsu.edu)

Kirk Visscher (visscher@ucr.edu)

Pesticide Exposure to Bees Documented in 1100 Samples Across the United States.

Scientists from Pennsylvania State University and ARS continue to document both unintentional and intentional exposure of honey bees to pesticide residues in hive products, especially beebread and beeswax. Samples were collected from migratory beekeepers in 23 States and one province in Canada. These samples included bees that were collected during beekill incidents. Honey bees and possibly other pollinators were exposed to a large number (and often high levels) of pesticides, as suggested by an average of 6.2 pesticides per pollen sample and up to 31 pesticides in a single sample. More than 1,100 samples of bee products were analyzed to date, with 130 different pesticides and metabolites detected from North American apiary samples alone. The frequency and quantity of pesticides detected varied considerably, and most of the samples were close to the level of quantification for their respective pesticides. Particularly noteworthy is the fungicide chlorothalonil, which was found in the majority of samples analyzed. Almost all comb and foundation wax was contaminated with miticides used to control parasitic mites of honey bees and other pesticides, averaging 8 detections with a high of 39 per sample; however, again the quantity of pesticides detected varied considerably. Although no single pesticide was shown to be responsible, the additive and synergistic effects of multiple pesticide exposures are believed to be contributing factors in declining honey bee health, but further studies are needed to better support this hypothesis. Potential pesticide interactions with other stressors including *Varroa*, IAPV, and *Nosema*, and its role in CCD require further study. In the future, researchers will focus on impacts of multiple pesticide residues in bee food via synergistic interactions that can act at sublethal levels on key pollinator behaviors/physiology

including memory and learning, and on immune function. Results of these studies were published (Mullin *et al.*, 2010 PLoS ONE 5: 1-19e9754).

Funding: USDA-NIFA, Pennsylvania State University, and National Honey Board.

PROJECT CONTACTS

Chris Mullin (CAMullin@psu.edu)

James L. Frazier (jff2@psu.edu)

Maryann Frazier (mxt15@psu.edu)

Dennis vanEngelsdorp (Dennis.Vanengelsdorp@gmail.com)

Jeff Pettis (jeff.pettis@ars.usda.gov)

Cost-sharing Program for Pesticide Analysis of Honey Bee Colony Matrices (Honey, Wax, Pollen, Bees, Brood, etc.). Based on recent evidence of frequent pesticide detections in wax, pollen, and brood, beekeepers have expressed an interest in having samples from their own colonies/apiaries tested for pesticides. A fund has been established to assist beekeepers by paying half of the cost of the pesticide analysis. Analysis is being performed by the USDA AMS National Science Laboratory in Gastonia, North Carolina, and the information generated from individual samples becomes part of a large, centralized database maintained at Pennsylvania State University. At this time, the database is not accessible to the general public.

Funding: Foundation for the Preservation of Honey Bees and Project Apis m. (PAm).

PROJECT CONTACT

Maryann Frazier, Pennsylvania State University (mxt15@psu.edu)

Reduction of Honey Bees Pollinating Nine Crops. In 2009 and 2010, researchers assessed changes in nine colonies on each of nine field crops by counting foragers exiting colonies during the pollination period for each crop. Researchers also conducted pesticide analyses on samples of dead and dying bees around hive entrances, returning foragers, crop flowers, trapped pollen, and corn flowers located near a cotton crop. Colony levels were significantly reduced in hives pollinating cotton, corn, and alfalfa, while hives placed in apples, pumpkins, almonds, melons, blueberries, and wild flowers (for honey production) increased or remained fairly consistent. A total of 52 pesticide residues was detected in samples collected across the 9 crops. Pesticide residues were not detected on the target crops (those crops in which the bees were rented for pollination) but were found in trapped pollen in hives or on dead and dying bees in the vicinity of the hives. It is difficult to say with certainty where the residues came from, but bees were possibly visiting other crops or plants treated with the pesticides found in trapped pollen or on the dead and dying bees. Since honey bees require diverse sources of pollen, the establishment of flowering, pesticide-free plants near bee-pollinated crops could help mitigate exposure to pesticides.

Funding: National Honey Board and USDA-NIFA.

PROJECT CONTACTS

Maryann Frazier (mxt15@psu.edu)

Chris Mullin (CAMullin@psu.edu)

James L. Frazier (jff2@psu.edu)

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

Low levels of imidacloprid were found in flowers and pollen during the initial 3 years of this study. There were greater amounts of imidacloprid found in pollen from soil-injected than trunk-injected trees, and male flowers had higher amounts than female flowers. However, little of the imidacloprid found in flowers was from pollen, and no metabolites of imidacloprid were found in pollen. Although there were instances where sublethal levels of imidacloprid to honey bees were found in pollen from treated trees, this pollen is probably diluted and mixed with other pollen in the hive, thus reducing potential exposure to sublethal doses.

This study has been extended for 3 years to further monitor for and quantify imidacloprid residue levels in flowers, pollen, nectar, and leaves; determine the impact of residues on honey bee health; and compare results of soil- and trunk-injected trees.

Funding: USDA-APHIS and USDA-ARS.

PROJECT CONTACTS

Jeff Pettis (jeff.pettis@ars.usda.gov)

Robyn Rose (Robyn.I.Rose@aphis.usda.gov)

Ongoing Research

Stationary Apiary Health Parameters Comparisons. In the NIFA Coordinated Agricultural Project (CAP), scientists assessed the presence and quantity of tracheal and *Varroa* mites, viruses, *Nosema* in bees and pesticides in wax, as well as quantity of pollen, queen health parameters, and brood and adult bee populations, evaluating possible linkages between pest/pathogen/pesticide presence and colony survivorship. Data show high variability among virus presence, and evaluations continue to identify possible linkages. There was also a high variability in the pesticide load in the pollen brought back to the hives.

Funding: USDA-NIFA.

PROJECT CONTACTS

Nancy Ostiguy (nxo3@psu.edu)

Brian Eitzer (Brian.Eitzer@ct.gov)

Goal 4: Determine bee physiological response to toxins, pesticides, or pathogens and develop analytical tools to assess bee health.

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

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

Accomplishments

Residue Analysis Yields Clues to Potential Fate of Pesticides. EPA OPP's Analytical Chemistry Laboratory in Fort Meade, Maryland, provides residue analysis of neonicotinoid pesticides in honey bee colonies in support of research being conducted by USDA in conjunction with the University of Maryland. These studies are informing EPA's understanding of the potential exposure of honey bees to pesticide (neonicotinoid) residues in pollen and nectar. In addition, these studies have provided information on the potential effects of imidacloprid on honey bee colonies following prolonged exposure to the compound in diets (pesticide-spiked bee bread). In support of pesticide residue analyses, EPA's Analytical Chemistry Laboratory has refined analytical techniques used for measuring residues of imidacloprid and its degradates and has increased the detection limits for these residues. These new methods have been published in the open literature.

Funding: EPA.

PROJECT CONTACTS

Tom Steeger (Steeger.Thomas@epa.gov)

Tom Moriarty (Moriarty.Thomas@epamail.epa.gov)

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

Funding: USDA-NIFA.

PROJECT CONTACT

John P. Burand (jburand@microbio.umass.edu)

Gene-expression Analyses Using Candidate Immune- and Stress-response Genes. Gene expression analyses with parallel bee gene sets and pathogen genes have revealed changes in the set of expressed bee genes that could relate to the cause(s) of collapsing colonies, including a massive shift in the abundances of gene transcripts related to protein translation. These experiments were complemented by high-throughput sequencing of millions of honey bee RNAs for bees challenged by the American foulbrood bacterium (*Paenibacillus larvae*) and bees from CCD and control colonies.

Funding: USDA-ARS.

PROJECT CONTACTS

Jay Evans (jay.evans@ars.usda.gov)

Scott Cornmann (scott.cornmann@ars.usda.gov)

Genes Associated with *Nosema* Infection Determined by Microarrays. In 2010, we verified the status of bees infected with *Nosema* using DNA and microarray approaches. Microarray analyses revealed that *Nosema* infection alters host metabolic pathways regulating nutrition and behavioral maturation as expected, but *Nosema* infection surprisingly does not appear to significantly alter immune gene expression in midgut and fat body tissues up to 7 days post-infection. We will continue to examine impacts of infection by characterizing gene expression in immune-related tissues up to 2 weeks post-infection in bees infected with *Nosema*. These studies will identify host response to *Nosema* infection and may lead to downstream applications in commercial management.

Funding: USDA-ARS and USDA-NIFA.

PROJECT CONTACTS

Katherine Aronstein (kate.aronstein@ars.usda.gov)

Christina Grozinger (cmgrozinger@psu.edu)

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

Following the development of a reliable field diagnostic tool, ARS scientists are now using the same antibody-based approach to develop a high-throughput laboratory test (enzyme-linked immunosorbent assay; ELISA) for *Nosema* detection in bees. In response to increased incidence of fungal diseases in bee colonies, ARS scientists developed a simple DNA-based method for detection of chalkbrood fungal disease in bee brood. This test will allow detection of the fungus prior to clinical signs of the disease. Although chalkbrood disease is usually not a primary concern for the beekeepers, it has recently become more prevalent. Research indicates that high levels of stress as a result of intense management of the environment may affect bee immune responses, making them more vulnerable to what were once considered benign diseases.

Funding: USDA-ARS and USDA-NIFA.

PROJECT CONTACTS

Katherine Aronstein (kate.aronstein@ars.usda.gov)

John Adamczyk (john.adamczyk@ars.usda.gov)

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 sublethal) of neonicotinoids and other pesticides used for crop protection.

Various research results indicate the presence of a range of pesticides and their degradates in honey bee colonies; however, no research or survey information has linked the incidence of CCD to pesticides. This is consistent with the multi-factorial hypothesis being pursued by the CCD Steering Committee. To the extent that pesticides are demonstrated to be directly impacting honey bee survival, growth and/or reproduction, EPA is prepared to mitigate these effects to the extent possible under the law.

As discussed earlier, EPA is also working with its international partners through OECD to identify high-quality research on the potential effects of pesticides on pollinator health, particularly as it relates to CCD.

EPA is continuing its efforts to collaborate with researchers in both government and non-government institutions regarding the data that can potentially inform EPA's regulatory decision-making. EPA scientists have also served as *ad hoc* reviewers of research proposals related to the potential effects of pesticides on insect pollinators submitted to USDA for funding.

PROJECT CONTACTS

Tom Steeger (Steeger.Thomas@epa.gov)

Tom Moriarty (Moriarty.Thomas@epamail.epa.gov)

Accomplishments

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

Funding: USDA-NIFA.

PROJECT CONTACT

Frank Drummond (frank.drummond@umit.maine.edu)

Persistence of the Effect of the Insecticide Novaluron on Bee Reproduction. In a study on alfalfa leafcutting bees in field cages, an ARS scientist evaluated the persistence of toxicity of the insecticide novaluron. The effect of female bee exposure to novaluron-treated alfalfa was found to persist for at least 2 weeks post-spray; the effect was not adult mortality, but instead,

mortality in the eggs laid by exposed adult females. Egg mortality was higher in cages with treated alfalfa compared to cages with untreated alfalfa. The effect diminished over time but remained higher than control mortality throughout the study.

Funding: USDA-ARS and USDA-NIFA.

PROJECT CONTACTS

Theresa Pitts-Singer (theresa.pitts_singer@ars.usda.gov)

James Barbour (jbarbour@uidaho.edu)

Synergistic and Sublethal Impacts of Pesticides Determined on Honey Bee Larvae.

The levels of pesticides detected within the hive have increasingly raised concerns about bee health and development under chronic exposure to pesticides. Scientists at Pennsylvania State University have used a modified *in vitro* larval rearing technique to assess the survival of honey bee larvae during a 6-day exposure to sublethal doses of four of the most frequently found pesticides in bee pollen. A Toxicity Probabilistic model was developed to determine relative toxicity of a single pesticide to honey bee larvae, which estimates pesticide toxicity as a function of the magnitude of toxicant exposure and a bee's sensitivity to a toxicant. Modeling results demonstrate that honey bee larvae and adults have different sensitivity to pesticides, and chlorothalonil, a fungicide which is considered non-toxic to bees on an acute exposure basis and is applied during bloom, has the highest relative toxicity to bee larvae compared to adult bees among the pesticides tested: fluvalinate, coumaphos, and chlorpyrifos. In addition, a method for assessing mixture toxicity was developed. Synergistic interactions were found between the fungicide chlorothalonil and the miticide fluvalinate at concentrations 34 ppm and 3 ppm, respectively, increasing larval mortality at day 4, approximately 7 times greater than the expected toxicity for mixtures with only concentration addition effect. However, when decreasing the concentration for fluvalinate and chlorothalonil by 10 fold, the mixture displayed antagonistic interaction. Chlorothalonil and coumaphos in mixtures at sublethal doses also displayed synergistic toxicity 5 times greater than the expected mortality if there is no interaction. The other binary mixtures, including fluvalinate and coumaphos, fluvalinate and chlorpyrifos, and coumaphos and chlorpyrifos, showed an additive effect. For three-component mixtures, only one significant reduction in larval mortality was found when adding coumaphos into the mixture of fluvalinate and chlorothalonil. Adding 34 ppm chlorothalonil into the three-component mixture of fluvalinate, coumaphos, and chlorpyrifos, the four-component mixture showed an additive effect. Moreover, considering pesticide formulations, the researchers demonstrated high toxicity of a common inert ingredient N-methylpyrrolidone (NMP) to honey bee larvae; NMP can kill all the reared larvae during the first day. This study suggests that fungicides, inert ingredients, and pesticide interactions may have crucial impacts on development and survival of honey bee larvae. However, it is uncertain as to the extent that formulation inerts remain with their active ingredients, and additional research is needed to resolve this uncertainty.

Funding: USDA-NIFA.

PROJECT CONTACTS

James L. Frazier (JFrazier@psu.edu)

Chris Mullin (oy9@psu.edu)

Maryann Frazier (mxt15@psu.edu)

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

Accomplishments

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

Funding: USDA-NIFA.

PROJECT CONTACT

Marion Ellis (mellis3@unl.edu)

Further Studies on Sublethal Effects of In-hive Miticides. Previously, scientists at the University of Nebraska–Lincoln documented the harmful synergistic effects of certain varroacide and fungicide combinations on honey bee health in laboratory studies (Johnson, *et al.*, 2010). Their results suggest that beekeepers should avoid applying these varroacides in combination. Beekeepers should also avoid applying these varroacides when honey bees are placed in orchards or other crop settings where exposure to prochloraz is likely. Studies are underway to assess the effects of exposure to simultaneously applied field-relevant doses of miticides and fungicides on brood survival, weight gain, and queen performance.

Funding: USDA-NIFA.

PROJECT CONTACT

Marion Ellis (mellis3@unl.edu)

Certain Varroacides Could Actually Lower Bee Susceptibility to CCD.

Pennsylvania State University and ARS researchers conducted a classification and regression tree (CART) analysis to better understand the relative importance of different risk variables in explaining CCD (van Engelsdorp *et al.*, 2010). Fifty-five variables, which included various pathogens, pesticides, and pests, were used to construct the models. Six of the 19 variables having the greatest discriminatory value were pesticide levels. Notably, coumaphos (a miticide commonly used by beekeepers) levels in brood had the highest discriminatory value and were highest in control (healthy) colonies. This suggests that higher levels of coumaphos could help colonies fight off CCD. This result must be interpreted with caution, however, because *Varroa* mite levels were similar in both CCD and non-CCD (control colonies). Follow-up studies are necessary to quantify *Varroa* infestations at different time intervals to give more conclusive results.

Funding: National Honey Board; USDA-ARS; North Carolina Agriculture Foundation; North Carolina Department of Agriculture and Consumer Services; USDA-NIFA; and University of Liege, Belgium.

PROJECT CONTACTS

Dennis vanEngelsdorp (Dennis.Vanengelsdorp@gmail.com)

Jim Frazier (jff2@psu.edu)

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.

See Topic IV, Goal 5.

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

See Topic IV, Goal 1, Objective 1.

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

and

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

These objectives are being done as part of the work on migratory beekeeping and the Area-wide project. See Topic IV, Goals 1 and 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 (chalk brood; stonebrood)
 - Microsporidia (Nosema)
 - Bacteria
 - Trypanosomes and other microbes

Accomplishments

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

Funding: USDA-NIFA.

PROJECT CONTACT

Diana Cox-Foster (dxc12@psu.edu)

Honey Bee Viruses Found in Non-*Apis* Bees Essential to Alfalfa Seed Industry. A survey of alfalfa leafcutting bees (ALCB) (*Megachile rotundata*) and alkali bees (*Nomia melanderi*) from Utah and Washington State, and subsequent molecular analysis for seven different RNA viruses, revealed the presence of deformed wing virus (DWV), Israeli acute paralysis virus (IAPV), black queen cell virus (BQCV), and sac brood virus (SBV). Further, when IAPV was fed to virus-free ALCB larvae, the viral infection disrupted adult diapause, with IAPV-infected bees having a significantly greater percentage emerging as second-generation adults as compared to the control treatment. IAPV infection also significantly reduced the ALCB adult survivorship in both males and females when acquired in newly emerged adults. IAPV was confirmed to be replicating in both the adult and larval stages, and the infection was confined to the gut in larvae.

Funding: USDA-ARS, USDA-NIFA, and Pennsylvania Department of Agriculture.

PROJECT CONTACTS

Diana Cox Foster (dxc12@psu.edu)

Rosalind James (rosalind.james@ars.usda.gov)

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.

See Topic III, Goal 4.

2. Determine the importance of *Varroa* as a vector of viruses associated with CCD or as a general immunosuppressive agent on the colony itself.

Accomplishment

Varroa Mites and Virus (IAPV) Transmission. IAPV was identified to be strongly associated with CCD and is considered to be a significant marker for CCD. Because mites feed and move between adult bees and brood, they have the potential to act as a vector to transmit pathogens from infected bees to healthy bees and have been reported to be associated with viral disease outbreaks in the field. Research results demonstrated that *Varroa* mite is a biological vector of the virus by supporting replication of IAPV and transmitting IAPV among honey bees. The results also showed that the mite-virus association could possibly reduce host immunity and therefore promote elevated levels of virus replication. This study sheds light on the epidemiology of IAPV infection in honey bees and adds additional importance to the control of *Varroa* mites.

PROJECT CONTACTS

Judy Chen (judy.chen@ars.usda.gov)

Jay Evans (jay.evans@ars.usda.gov)

Also see Topic III, Goal 4.

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.

Accomplishments

Synergism Found Between Pesticides on Honey Bee Homing Behavior. Researchers at Michigan State University determined that the distance of bee search flights and the length of time homing memories are retained by bees. An assay was developed to determine the effects of pesticides on the memories of foraging bees. In this study, bees were first released 0.8 km from the hive, and the bees returning to the hive were counted (homing rate). Those bees that were released the first time were recaptured and re-released 1.8 km away from the hive. Bees returned home equally well from the first release site (0.8 km). However, when the returning bees were re-released 1.8 km away from the hive, bees that were treated with a combination of fluvalinate and imidacloprid showed a significant reduction in their homing rate. Further, their homing rate was not affected when either pesticide was used alone. These results suggest that these two

pesticides have a synergistic effect on honey bee learning and/or memory. These results shed light on a possible effect of CCD on bees, because foragers are having trouble finding their way home.

Funding: Michigan State University, Australia National University, and USDA-NIFA.

PROJECT CONTACT

Zachary Huang (bees@msu.edu)

Synergistic Effects of Pesticide Exposure on Pathogen Growth and Bee Health Discovered.

There has been significant concern about possible interactive effects of various pesticides and pathogens on bees. When bees were exposed to the gut parasite *Nosema* and the insecticide imidacloprid, researchers found evidence of an interactive effect on honey bee health. Worker bees exhibited up to a fourfold increase in *Nosema* levels when they originated from colonies that had been fed imidacloprid, indicating a subtle sublethal interaction between pesticides and pathogens. The same fourfold increase was exhibited in bees exposed to 2.5 ppb or 20 ppb imidacloprid. Also, while the individual bees contained higher spore loads, the colonies from which they were derived did not exhibit elevated *Nosema* loads. This research has been submitted for publication, and similar findings have recently been published from a study in France in *Environmental Microbiology* 2009.

Funding: USDA-ARS and North American Pollinator Protection Campaign (NAPPC).

PROJECT CONTACTS

Jeff Pettis (jeff.pettis@ars.usda.gov)

Galen Dively (Galen@umd.edu)

Dennis vanEngelsdorp (Dennis.Vanengelsdorp@gmail.com)

Interactions Among the Microsporidia *Nosema apis* and *Nosema ceranae*, the Viruses IAPV, DWV, BQCV, and Rearing Temperature. Development, virulence, infectivity (IC₅₀), spore production, temperature effects, and direct competition in the same host were evaluated for *N. ceranae* and *N. apis*. No significant difference in virulence between the two species was detected, but IC₅₀ studies demonstrated that a 5x higher dosage of *N. ceranae* is needed for infection. Host mortality at different temperatures was not significantly different for the two pathogens. *N. ceranae* produced slightly more spores than *N. apis* over the infection period when 200× the infective dosage was fed. When individual bees were inoculated with both *Nosema* species to produce mixed infections, *N. apis* produced more spores. When viruses (DWV, IAPV, and BQCV) were combined with microsporidia at different temperatures, mortality trended higher but was not significantly different from mortality due to the microsporidia alone. There was no difference in spore production by *N. apis* and *N. ceranae* when mixed with viruses. Mortality of virus and *Nosema* combinations was highest at low and high rearing temperatures, no matter the combination (and including controls), so rearing temperature was the mortality factor, not mixes of virus and *Nosema*.

Funding: USDA-NIFA and USDA-ARS.

PROJECT CONTACTS

Lee Solter (lsolter@illinois.edu)

Judy Chen (judy.chen@ars.usda.gov)

Jay Evans (jay.evans@ars.usda.gov)

Association of Miticides With Adult Bee Susceptibility to *Nosema*. A significant relationship between pesticide residue exposure (in brood comb) and adult susceptibility to infection with *Nosema ceranae* was detected in a common hive environment study in 2010. The three most frequently observed pesticides were miticides used to control *Varroa* mite in brood comb. That is, adult honey bees that were reared in “high pesticide residue comb” (taken from existing commercial beekeeping operations) were significantly more likely to become infected with *Nosema ceranae* than those reared in low residue comb, suggesting a detrimental sublethal effect of pesticide exposure on the immune system. These findings were reported at several beekeeper local, regional, and national meetings. A manuscript was submitted to the *Journal of Invertebrate Pathology*. A follow-up large-scale field study was initiated in 2010 and will be completed in 2011.

Funding: USDA-NIFA-CAP; the State Beekeeper Associations of Washington, Oregon, Idaho, and California; and Project Apis m.

PROJECT CONTACT

Steve Sheppard (shepp@wsu.edu)

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

Funding: Pennsylvania Department of Agriculture.

PROJECT CONTACT

Diana Cox-Foster (dxc12@psu.edu)

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

Studies also showed that bees exposed to neonicotinoid insecticides and fungicides at sublethal levels had impaired immune systems and increased levels of the viruses noted above. Because CCD might be a result of such interactions and damaged bee immunity, further studies are planned to examine immune responses and determine the mechanisms underlying these interactions.

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

Funding: USDA-NIFA and USDA-ARS.

PROJECT CONTACTS

Lee Solter (lsolter@illinois.edu)
Judy Chen (judy.chen@ars.usda.gov)
Jay Evans (jay.evans@ars.usda.gov)

Effect of *Varroa* Mites and *Varroa*-Transmitted Viruses on Nutritional and Health Status of Honey Bees. The main goal of this study (2010–2011) is to understand how mite infestation and mite-transmitted diseases affect immune competence and the nutritional status of managed honey bees. Preliminary results demonstrated that *Varroa* infestation has a major impact on the performance of individual honey bee health as well as on colony-level effects, including significant losses in weight in pupae and newly emerged adults infested with mites. Importantly, *Varroa* infestation affected the nutritional status of bees. The amount of available sugar, especially glucose, was depressed in *Varroa*-infested individuals, and this effect was significantly greater for adult bees than pupae. Interestingly, *Varroa*-infested bees were able to maintain their protein content, including protein content per unit weight in newly emerged bees bearing *Varroa* relative to controls. Consistent with the small effect of *Varroa* infestation on bee protein nutrition, the total free amino acid content did not vary significantly with treatment. The dominant amino acids in all samples (including all three life stages, and *Varroa*-infested vs. un-infested) were serine and proline. Preliminary results indicating that *Varroa*-infested pupae have reduced content of the key sulfur amino acid methionine are being investigated further, as are other metabolites. Additional efforts are focused on monitoring the level of viral infection and immune responses in *Varroa*-infested versus control bees using a DNA-based approach.

Funding: USDA-ARS and USDA-NIFA.

PROJECT CONTACTS

Katherine Aronstein (kate.aronstein@ars.usda.gov)

Angela E. Douglas (aes326@cornell.edu)

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

Funding: USDA-ARS and USDA-NIFA.

PROJECT CONTACT

Katherine Aronstein (kate.aronstein@ars.usda.gov)

Temperature Found to Affect Honey Bee Susceptibility to Fungi. Stonebrood and chalk brood are two fungal diseases of honey bees. Stonebrood is caused by a non-specialist fungal pathogen (*Aspergillus flavus*) that affects many animals to varying degrees. Chalkbrood is caused by a highly specialized pathogen of bees (i.e., *Ascosphaera apis*). When these fungal pathogens were fed to honey bees, stonebrood appeared to be more virulent than chalkbrood. However, when recently infected larvae were exposed to a 24 h cooling period (a cold stress), mortality from chalkbrood increased, whereas bees tended to recover from stonebrood. These results raise interesting questions about temperature stress and honey bee immune responses to pathogens with different degrees of specialization.

Funding: USDA-ARS and University of Copenhagen.

PROJECT CONTACTS

Rosalind James (rosalind.james@ars.usda.gov)

Svjettlana Vojvodic (svo@life.ku.dk)

Temperature Stress Found to Increase Immune Response in Bees. Both high and low temperatures increased the expression of immune response genes in the alfalfa leafcutting bee. Genes associated with pathogen recognition and trypsin-like serine proteases were most highly expressed at the lowest rearing temperature (20°C), while prophenoloxidase, melanization, immune response signalling pathways, effectors, and reactive oxygen species were most highly expressed at the warmest temperature (35°C). The chalkbrood pathogen was found to affect some non-immunity host functions, particularly protein synthesis, differently at different temperatures, with the lowest rates of protein synthesis occurring at 30°C, a temperature where protein synthesis is high in healthy insects. If a temperature stress occurs before the pathogen has infected, it appears to elicit an immune response in the host, and this early response can prevent infection from occurring.

Funding: USDA-ARS.

PROJECT CONTACTS

Rosalind James (rosalind.james@ars.usda.gov)

Johan Hu (junhuan.xu@usu.edu)

Ongoing Research

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

Funding: USDA-ARS and USDA-NIFA.

PROJECT CONTACTS

Judy Chen (judy.chen@ars.usda.gov)

Jay Evans (jay.evans@ars.usda.gov)

Topic IV: Mitigation and Management Preventive Measures

Goal 1: Develop best management practices for honey bees.

1. Develop best management practices for migratory beekeeping.

Accomplishments

Best Management Practices (BMPs) Published. USDA-NIFA's Coordinated Agricultural Project (CAP) has published a Best Management Practices Guide for Honey Bee Health in the *American Bee Journal* and *Bee Culture*, which are two trade publications read by most beekeepers. The BMP guide is also available on the CAP project's extension Web site, <http://www.extension.org/pages/33379/best-management-practices-bmps-for-beekeepers-pollinating-californias-agricultural-crops>. The BMP publication is the result of a collaborative effort involving CAP-funded scientists and Project Apis m., a beekeeper and almond grower foundation. For more information on the eXtension site, see Goal 7.

Funding: USDA-NIFA and Project Apis m.

PROJECT CONTACTS

John Skinner (jskinner@utk.edu)

Keith Delaplane (ksd@uga.edu)

Marion Ellis (mellis3@unl.edu)

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

Funding: USDA-ARS and Contech, Inc.

PROJECT CONTACT

Katherine Aronstein (kate.aronstein@ars.usda.gov)

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

Funding: USDA-ARS and USDA-NIFA.

PROJECT CONTACTS

Gloria DeGrandi-Hoffman (Gloria.Hoffman@ars.usda.gov)

Gordon Wardell (GordyAW@aol.com)

Effects of Protein Supplements on Worker Physiology and Immune Response Examined.

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

Funding: USDA-ARS.

PROJECT CONTACTS

Gloria DeGrandi-Hoffman (Gloria.Hoffman@ars.usda.gov)

Judy Chen (judy.chen@ars.usda.gov)

Winter Feeding and Its Improvement of Colony Size and Survival. This work started in the fall of 2006 and has continued to date. The Weslaco laboratory has shown that colonies being prepared for almond pollination in California show dramatic improvement when fed continuously from September through late January. Cooperating beekeepers and others have stated that they consider this work to have caused a total change in how they manage their colonies for almond pollination. Projects completed include interaction of feeding and *Nosema* infection; interaction of feeding and *Varroa* parasitism; optimum time of feeding; evaluation of commercially available pollen substitutes/supplements; interaction of nutrition, *Nosema*, and *Varroa*; and mountainside wintering (cool, windy conditions reduce winter flight and the need for food); and the food efficiency (bees per pound of diet consumed) of four strains of bees. During feeding trials, some unanticipated findings came to light: a) colonies that went queenless did not attempt supercedure or emergency queen cell construction (the duration of queen survival was correlated with the level of *Varroa* parasitism and to a lesser degree, the level of *Nosema* infection); b) colonies whose *Varroa* levels were above 150 mites (natural mite drop onto a sticky board during 3 days) in October had few colonies achieve almond pollination grade regardless of mite treatment or feeding in the fall-winter; c) treating colonies with fumagillin appears to have a short (about 2 months) negative impact on colonies. However, untreated infected colonies showed extremely poor performance after 14 months. This highlights the need to control even fairly moderate infections (500,000 spores/bee) of *N. ceranae* in some areas.

Funding: USDA-ARS.

PROJECT CONTACTS

Frank Eischen (frank.eischen@ars.usda.gov)

John Adamczyk (john.adamczyk@ars.usda.gov)

Honey Compound Could Enhance Bee Tolerance to Pesticide. Honey was found to contain a compound called pinocembrin, which is likely derived from propolis, and which acts as a powerful inducer of the enzymes that metabolize *tau*-fluvalinate. These results suggest that bee consumption of honey may serve a hitherto unrecognized role in honey bee health in the context of pesticide tolerance.

PROJECT CONTACT

May R. Berenbaum (maybe@illinois.edu)

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

PROJECT CONTACT

May R. Berenbaum (maybe@illinois.edu)

Malnutrition, Beneficial Microbes, and Nutritional Needs of Honey Bees. Scientists at ARS in Tucson, Arizona, believe that malnutrition is a major cause of colony losses. However, malnutrition, especially in its early stages, is difficult to diagnose. To remedy this, these ARS scientists are identifying biomarkers associated with the nutritional state of the bees. Researchers found that bees emitted volatile compounds when adult workers and larval bees were malnourished. Emissions of these compounds increase in bees experiencing acute malnutrition. Identifying these chemical cues will enable ARS to evaluate how contemporary beekeeping and pollination practice, as well as environmental contamination, affects the nutritional state of a colony and its vulnerability to disease and decline.

Researchers at ARS in Tucson have uncovered key bacterial communities needed by bees for food processing and digestion. When scientists fed colonies pollen contaminated with fungicides, less than 30 percent reared new queens, suggesting that key nutrients needed to raise queens might be missing. Colonies that cannot successfully replace their queens cannot survive and thus contribute to the colony losses experienced by U.S. beekeepers.

Supplemental feeding can have deleterious effects on colonies if used for prolonged periods. ARS researchers showed that colonies fed supplemental protein produced higher numbers of brood but did not supply them complete nutrition. The lowered protein levels in workers shortened their lifespan and eventually reduced brood rearing, causing the colony to decline and the hives to perish. Additional studies found that during the winter, colonies fed sugar syrup made with sucrose produced more brood in spring compared with colonies fed high fructose corn syrup. These researchers are advising beekeepers that feeding colonies exclusively on high fructose corn syrup during winter or floral dearth periods can negatively impact colony strength required for pollination.

Funding: USDA-ARS.

PROJECT CONTACT

Gloria DeGrandi-Hoffman (Gloria.Hoffman@ars.usda.gov)

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

Accomplishments

High Ozone Concentrations Being Developed As a Fumigant for Beehive Equipment. In 2010, a mid-size ozone fumigation chamber was set up and field tested in Florida using honey bee supers and comb from beekeepers. Ozone fumigant killed 100 percent of the insect pests (small hive beetles and various wax moths), and pathogen loads were reduced. Pesticide residues were not effectively eliminated because comb wax protected the chemicals from degradation. Although the combs smelled unpleasant after treatment, no toxic compounds were formed, and the bees readily accepted ozone-treated comb in the colony. Treating comb also did not appear to affect the attractiveness to greater wax moth.

Funding: USDA-ARS, Florida Department of Plant Industries, and USDA-NIFA.

PROJECT CONTACT

Rosalind James (Rosalind.James@ars.usda.gov)

HopGuard[®], a New Natural Product for *Varroa* Mite Control. Beekeepers need new methods to control *Varroa* because currently registered products are inconsistent in their effectiveness, harmful to brood, contaminate wax combs used in hives, or no longer control the mite because it is resistant. Under a Cooperative Research and Development Agreement (CRADA) with J.I. Haas, Inc., a product was developed (HopGuard[®]) that incorporates the beta plant acids (BPA) that are byproducts of hops processing for the beer brewing industry. Prototypes of the product were tested in commercial colonies and in packages of bees used to start new colonies. HopGuard[®] was highly effective in reducing mite populations. The product did not harm queens, adult bees, or brood, and it did not contaminate honey or the wax comb. A Section 18 emergency registration was issued by EPA and HopGuard[®], and is now in commercial production.

Funding: CRADA between USDA-ARS and J.I. Haas, Inc.

PROJECT CONTACT

Gloria DeGrandi-Hoffman (Gloria.Hoffman@ars.usda.gov)

Controlling the Key Parasite *Varroa destructor* Through Application of Genomics. A genome draft sequence was published for the *Varroa* mite, revealing potential weak points in mite biology (defensive proteins and proteins used in chemical mitigation) and candidates for novel controls such as RNA interference (RNAi). In fact, the publication in genomic databases of mite candidate genes allowed the worldwide initiation of RNAi efforts for this parasite, ending in late 2010, with the first successful demonstration of RNAi activity in *Varroa*. Microbes identified in this screen have also been screened across bees and mites as plausible controls for *Varroa*.

Funding: USDA-ARS.

PROJECT CONTACTS

Jay Evans (jay.evans@ars.usda.gov)

Scott Cornmann (scott.cornmann@ars.usda.gov)

Chemicals Associated With Mite Control Identified. ARS scientists at Gainesville, Florida, have discovered several chemicals that are produced in large amounts by drone and worker brood during the cell capping process but not by queen larvae, which affect the searching behavior of the *Varroa* mite. ARS has filed a provisional patent on the use of these compounds for control of the *Varroa* mite and is working with private industry to develop control programs using the attractants.

Funding: USDA-ARS.

PROJECT CONTACT

Peter Teal (Peter.Teal@ars.usda.gov)

New Lures and Traps for Control of Small Hive Beetle (SHB) and Prevention of Invasion by Beetles Related to SHB. Working with scientists at the Department of Entomology and Nematology, University of Florida, ARS Gainesville scientists found that chemicals from bumble bee colonies were attractive to SHB. The beetle showed no preference for the honey bees over the bumble bees.

ARS scientists in Gainesville have developed a highly effective lure for the SHB based on chemicals produced by ripe fruit. The attractant has outperformed currently used attractants for the pests.

Also, scientists in Gainesville have developed a light-based attractant-trap system for capture of small hive beetle adults and larvae in honey houses. Tests show that the trap captures at least 80 percent of all wandering larvae.

These scientists also documented that the SHB reproduces as well on ripe fruit as it does in honey bee colonies. This finding, as well as the fact that beetles are more strongly attracted to odors from fruit like pears and cantaloupe, demonstrates that the pest is very capable of surviving in the absence of managed bee hives and provides important information for developing strategies to mitigate beetle invasion of hives.

Scientists at Gainesville, working with scientists from the International Center for Insect Physiology and Ecology in Nairobi, Kenya, have also identified a new beetle pest of honey bee colonies in Africa and have discovered that the beetle is attracted to lures currently in use to monitor for the SHB. These discoveries are important because we can now monitor for this pest at ports of entry into the United States and thereby know if and when the new beetle invades the United States.

Funding: USDA-ARS.

PROJECT CONTACT

Peter Teal (Peter.Teal@ars.usda.gov)

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

Funding: USDA-ARS.

PROJECT CONTACT

Katherine Aronstein (kate.aronstein@ars.usda.gov)

Chalkbrood Disease and Implementations to Host Pathogenesis. ARS scientists analyzed responses of the honey bee fungal pathogen *Ascospaera apis* during host pathogenesis using high-throughput sequencing. Data analysis captured a significant number of differentially expressed genes that may be related to fungal reproduction, host invasion, and stress responses. This study may provide new insights into the mechanisms of host invasion and progression of chalkbrood disease and presents a unique opportunity to improve our understanding of the highly complex nature of host-pathogen interactions.

Funding: USDA-ARS.

PROJECT CONTACTS

Katherine Aronstein (kate.aronstein@ars.usda.gov)

Jay Evans (jay.evans@ars.usda.gov)

Scott Cornmann (scott.cornmann@ars.usda.gov)

Anna Bennett (akb39@hoyamail.georgetown.edu)

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

and

4. Develop best management practices for pesticide use.

Accomplishment

Progress Toward Best Management Practices for Pesticide Use. EPA is working with a broad range of stakeholders through the Pesticide Program Dialogue Committee (PPDC), a stakeholder group chartered under the Federal Advisory Committee Act, to explore and develop additional ways of mitigating the potential effects of pesticides on bees. Broad stakeholder input will best ensure success of best management practices (BMPs) that may involve not only beekeepers, but also growers and pesticide applicators. Additional mitigation measures under consideration include both improved label language and encouraging communication/cooperation between growers/applicators and beekeepers.

EPA has been working through its regional offices and with State Lead Agencies to engage local stakeholders in dialogue to understand successful grower-beekeeper interactions on a local scale. Understanding successes at the local level may serve as a basis to expand or transplant local successes to other State or national programs. Programs like Driftwatch™ (<http://driftwatch.agriculture.purdue.edu/index.html>) are being examined as a means of identifying the location of vulnerable “crops” like honey bee colonies (apiaries) through national registries that would enable growers/applicators to be aware of such vulnerable areas in advance of pesticide applications and communicate pesticide application options with the beekeepers before they occur.

As discussed earlier, EPA is also working with its OECD international partners to develop a compendium of mitigation measures that various countries have used around the world to mitigate the potential risks of pesticides to insect pollinators.

PROJECT CONTACTS

Tom Steeger (Steeger.Thomas@epa.gov)

Tom Moriarty (Moriarty.Thomas@epamail.epa.gov)

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.

and

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

Accomplishments

Blue Orchard Bee Workshops Delivered to Almond Production Community. ARS scientists with California Cooperative Extension personnel organized and delivered workshops (2009 and 2010) to provide information on the blue orchard bee and its use for almond pollination. Workshops were designed to facilitate networking among the almond production community interested in using blue orchard bees as almond pollinators along with, or instead of,

honey bees. Guidance on managing blue orchard bees for almonds is now available to promote its use and reduce pressure on the sole use of honey bees.

Funding: USDA-ARS and University of California–Davis.

PROJECT CONTACTS

James Cane (jim.cane@ars.usda.gov)

Theresa Pitts-Singer (theresa.pitts-singer@ars.usda.gov)

Carolyn Pickel (cxpickel@ucdavis.edu)

Efficiency of Alfalfa Leafcutting Bees Affected by Stocking Density and Floral Resource Abundance. The alfalfa leafcutting bee (*M. rotundata*) is commonly used to pollinate alfalfa for seed production in the United States and Canada, but maintaining healthy stocks of these bees is hampered by disease, parasites, predators, and unexplained mortality of eggs and small larvae. An ARS researcher has shown that typical U.S. alfalfa leafcutting bee stocking densities for alfalfa pollination are unnecessarily high, decreasing the number of bees that remain at the commercial site, decreasing bee pollination efficiency, and reducing the number of healthy offspring produced. The amount of alfalfa bloom in fields also affects the number of bees that remain onsite and the number of offspring that can be produced. This research provides information for optimizing stocking density in light of available floral resources.

Funding: USDA-ARS.

PROJECT CONTACT

Theresa Pitts-Singer (theresa.pitts-singer@ars.usda.gov)

Delaying Novaluron Applications Decreases Negative Effects. Novaluron (Rimon[®]) is a relatively new insecticide that is an insect growth regulator, labeled for lygus control in alfalfa fields during bloom. Some alfalfa leafcutting beekeepers have complained about poor bee returns in fields where novaluron was used, and laboratory trials have shown this pesticide to be toxic to larvae and eggs. Field experiments were conducted in 2010 to evaluate the effect of the timing of novaluron (Rimon[®] 0.83 EC) spray applications on alfalfa leafcutting bee reproduction. Spray timing was found to have no impact on the mean number of nest cells produced by the end of the season. However, the percentage of pollen balls (bees that died as eggs or young larvae) was lower, and the percentage of live larvae higher, on unsprayed (control) plots and plots treated with Rimon[®] late, as compared to plots treated during early and mid-bloom. These results indicate that Rimon[®] has a reduced impact on overall production of healthy alfalfa leafcutting bees when applications are delayed until the late bloom period.

Funding: USDA-ARS, USDA-NIFA, and Chemtura.

PROJECT CONTACTS

Theresa Pitts-Singer (theresa.pitts-singer@ars.usda.gov)

James Barbour (jbarbour@uidho.edu)

Nesting Methods Successfully Established for Four Bumble Bee Species. Bumble bees are important pollinators of commercial greenhouse crops, but they are difficult to raise in captivity. Because it is particularly difficult to get queens to establish new nests in captivity, three nest establishment methods were tested on four species of bumble bees. The results show that some techniques greatly increase the success rate (by fourfold) in some species in comparison to other techniques. By targeting the proper rearing techniques to a given species, bumble bee producers and researchers will be able to save time and resources when producing these bees in culture.

Funding: USDA-ARS.

PROJECT CONTACT

James Strange (james.strange@ars.usda.gov)

Wildland Fire Shown to Increase Wild Pollinators. Controlled burns constitute one method used to manage fuel loads in natural areas, but the effects on pollinators remain unknown. The fates of wild bee communities following wildfire were largely unknown but are critical to anticipating pollination services during large post-fire rangeland restoration projects. Using a new suite of sampling protocols, researchers systematically sampled bee floral guilds over time after large burns, sampling from sites inside and outside the burned areas. Plant communities were also characterized. Where rangeland plant communities were in good shape before burning, the diversity of the native bee communities returned the following year. Over several years, the native wildflowers likewise recovered. Initial seeding in restoration projects should include plants that are broadly attractive to local native bees to sustain these bees until the native plant communities can reestablish, since that re-establishment can take several years.

Funding: USDA-ARS, DOI-BLM, and USDA-FS.

PROJECT CONTACT

James Cane (jim.cane@ars.usda.gov)

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

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

Accomplishments

USDA *Varroa*-Resistant Honey Bees Found Well Suited For Commercial Pollination in Two Trials. ARS scientists tested Russian honey bees and bees containing a *Varroa*-resistant trait (*Varroa*-sensitive hygiene [VSH]) for size and productivity in two large-scale field tests comprising two different migratory routes. Two years of testing during commercial pollination of almonds, apples, blueberries, and cranberries, with overwintering in Louisiana, showed the USDA *Varroa*-resistant stocks to be as large and productive as control stocks at each pollination

site. One year of a test involving almond pollination and Midwest honey production, with overwintering in California's Central Valley, again showed Russian and VSH bees to have adequate colony size, survivability, and honey production. These results suggest that *Varroa*-resistant bees are well-suited for commercial pollination.

Funding: USDA-ARS.

PROJECT CONTACTS

Tom Rinderer (Tom.Rinderer@ars.usda.gov)

Bob Danka (Bob.Danka@ars.usda.gov)

Improved Management Strategies For Russian Honey Bees Developed. ARS scientists at Baton Rouge, Louisiana, have evaluated management procedures to improve Russian honey bees for early season pollination of almonds. Extensive tests found that feeding regimes (and secondarily, hive size) affect colony populations. Colonies fed pollen substitute dwindled less in winter than those not fed. Feeding pollen substitute with natural pollen added was much better than feeding just pollen substitute. Feeding protein as patties was better than feeding it in plastic comb within the brood nest. Feeding sucrose syrup at a slow rate continuously from autumn to mid-winter simultaneously with protein increased colony size. Russian colonies grew more in 8-frame hives than in 10-frame hives, but hive size was less important than feeding protein. Housing colonies in black hives did not improve colony population over colonies in white hives. These findings collectively show that Russian honey bees can be managed in the southern United States to produce colonies that are more than sufficient to rent for almond pollination in February.

Funding: USDA-ARS.

PROJECT CONTACTS

Tom Rinderer (Tom.Rinderer@ars.usda.gov)

Bob Danka (Bob.Danka@ars.usda.gov)

Ongoing Research

Identifying Genes that Confer Resistance to *Varroa* Mites. Two separate projects were undertaken, and genes were mapped to chromosomal regions that influence each of the two behavioral traits that have been shown to be important for suppressing mite populations: *Varroa*-sensitive hygiene (VSH) and mite-grooming behavior. Several chromosomal regions were identified for each trait. The genes in these regions are being analyzed and considered for follow-up experiments to confirm their role in conferring resistance to *Varroa* so that they can be targeted with marker-assisted selection to pyramid multiple resistance genes in commercial stocks.

Funding: USDA-NIFA and USDA-ARS.

PROJECT CONTACTS

Greg Hunt (ghunt@purdue.edu)
Marla Spivak (spiva001@umn.edu)
Bob Danka (Bob.Danka@ars.usda.gov)
Miguel Arechavaleta-Velasco (are@servidor.unam.mx)
Thomas Webster (thomas.webster@kysu.edu)
Katherine Aronstein (kate.aronstein@ars.usda.gov)

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

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

Accomplishments

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

Funding: National Honey Board and California Almond Board.

PROJECT CONTACT

Marla Spivak (spiva001@umn.edu)

Commercialization of *Varroa*-resistant Honey Bees Selected for Pollination Performance. *Varroa* mites are a major cause of colony losses throughout the United States. Bees with *Varroa*-sensitive hygiene (VSH), which have good resistance to *Varroa* mites, were tested by researchers for two seasons in a commercial migratory beekeeping operation. Bee colonies were created from VSH queens that were out-crossed, i.e., matings were not controlled, a method used by most large-scale beekeepers. Bee colonies were shipped nationwide for pollination of four crops and for late-summer honey production. VSH colonies performed well in terms of survival, populations, and resistance to *Varroa* mites. The best surviving VSH bee colonies from each year were propagated to form a VSH breeding population that had enhanced genetics for both mite resistance and behavior related to crop pollination. These bees are now being marketed by a Cooperative Research and Development Agreement partner, Glenn Apiaries, and the use of their germplasm should improve adoption of mite-resistant bees by commercial beekeepers that pollinate crops.

Funding: USDA-ARS.

PROJECT CONTACTS

Bob Danka (Bob.Danka@ars.usda.gov)

Tom Rinderer (Tom.Rinderer@ars.usda.gov)

Ongoing Work

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

Funding: USDA-NIFA.

PROJECT CONTACTS

Nick Calderone (nwc4@cornell.edu)

Keith Delaplane (ksd@uga.edu)

Greg Hunt (ghunt@purdue.edu)

Steve Sheppard (shepp@wsu.edu)

Marla Spivak (spiva001@umn.edu)

Thomas Webster (thomas.webster@kysu.edu)

Michael Wilson (mwilso14@utk.edu)

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

Accomplishments

Honey Bee Germplasm Importations to Improve Genetic Variation in United States.

Honey bee semen was collected from locations in Italy and the Republic of Georgia in 2010 and returned to the United States under a USDA-APHIS quarantine permit to Washington State University. The fresh semen was used to inseminate queens produced from existing U.S. commercial strains of honey bees and overwintered in Washington and California (collaboration with University of California–Davis). This genetic material is being incorporated into existing U.S. honey bee strains to increase sex allele diversity and reduce inbreeding effects. Genetic material derived from the Italian importations (2008–2010) will be released to the commercial bee breeding industry in 2011.

Funding: Washington and California State Beekeeping Associations and USDA-NIFA-AFRI-CAP.

PROJECT CONTACT

Steve Sheppard (shepp@wsu.edu)

Cryopreservation of Honey Bee Germplasm. In 2010, the Washington State University research team also developed a practical methodology for maintaining honey bee germplasm (semen) by cooling samples at temperatures below the freezing point. Using this new method, researchers there produced three successive generations of honey bee queens from cryopreserved (i.e., frozen at liquid nitrogen temperatures) semen in 2010. This demonstration marked the turning point for a new era of germplasm preservation/conservation in honey bees, as it will now be possible to establish genetic repositories for the honey bee. Future germplasm collections (2011 and beyond) will include cryopreservation of a portion of the semen for subsequent use. A manuscript for publication in a peer-reviewed journal was prepared and submitted on this topic.

Funding: Washington and California State Beekeeping Associations and USDA-NIFA-AFRI-CAP.

PROJECT CONTACT

Steve Sheppard (shepp@wsu.edu)

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

Accomplishments

Groundwork Established for a Sustainable Market for Genetically Improved Queens.

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

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

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

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

Funding: USDA-NIFA.

PROJECT CONTACTS

Keith Delaplane (ksd@uga.edu)
Marla Spivak (spiva001@umn.edu)

Increased Transfer of the VSH Mite-Resistance Technology to Foster Breeding of Bees with Improved Mite Resistance. Bees with the trait of *Varroa*-sensitive hygiene (VSH) have strong resistance to *Varroa* mites. VSH breeder queens are available through an ARS partnership with a commercial queen breeder. Semen from VSH bees now is directly available from ARS for incorporation into existing bee stocks. Research showed that commercially available VSH queens that are mated to non-VSH drones have acceptable mite resistance. Tests are ongoing in Hawaii to determine the value of VSH in mitigating *Varroa* damage in a tropical environment that is the base of a large queen production industry. Two workshops were given in 2010 at regional and national beekeeping meetings to teach breeders how to test, select, and breed for the trait. Together, these efforts should encourage incorporation of VSH-based mite resistance into diverse bee stocks and thus support beekeeping sustainability.

Funding: USDA-ARS.

PROJECT CONTACTS

Tom Rinderer (Tom.Rinderer@ars.usda.gov)
Bob Danka (Bob.Danka@ars.usda.gov)

Also see Topic IV, Goal 3, Objective 2.

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

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

Accomplishment

EPA Expediting Reviews for Section 18. EPA is working with State Lead Agencies to provide beekeepers with appropriate tools to control bee colony pests such as *Varroa* mites. The Agency is working closely with USDA ARS and the Office of Pest Management Policy (OPMP) to expedite reviews of Emergency Exemption requests (under section 18 of the Federal Insecticide, Fungicide and Rodenticide Act [FIFRA]), Special Local Needs Registrations (under section 24c of FIFRA), and full registrations (under section 3 of FIFRA) of miticides.

PROJECT CONTACTS

Tom Steeger (Steeger.Thomas@epa.gov)
Tom Moriarty (Moriarty.Thomas@epamail.epa.gov)

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

See Type IV, Goal 3.

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.

and

2. Explore opportunities to change regulations based on new molecular detection systems. For example, if a virus is shown to be pathogenic and reliable new detection methods are found, then movement of virus-contaminate bee stocks may be regulated by bodies such as OIE.

Accomplishments

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

Funding: USDA-ARS.

PROJECT CONTACT

Katherine Aronstein (kate.aronstein@ars.usda.gov)

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

Funding: USDA-APHIS and USDA-ARS.

PROJECT CONTACTS

Colin Stewart (Colin.Stewart@aphis.usda.gov)

Rosalind R. James (Rjames@biology.usu.edu)

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

Accomplishment

Pilot Survey for Bee Diseases, Parasites, and Pests. APHIS, in collaboration with ARS, conducted a pilot survey in 2009 in Florida, California, and Hawaii to validate sampling protocols used to determine which diseases/parasites/pests of honey bees are and are not present in the United States. A limited national survey of 13 States was conducted in 2010. A report of these results can be found at http://www.aphis.usda.gov/plant_health/plant_pest_info/honey_bees/downloads/2010-2011-Limited_Survey_Report.pdf. A national survey is being conducted in 2011 in 34 States. The exotic pests *Tropilaelaps* mites, *Apis ceranae*, and Slow Paralysis Virus have not been found during this survey. Therefore, the United States is not allowing importations from countries known to have these pests (e.g., Australia and Southeast Asia).

Funding: USDA-APHIS and USDA-ARS.

PROJECT CONTACTS

Robyn Rose (Robyn.I.Rose@aphis.usda.gov)

Jeff Pettis (jeff.pettis@ars.usda.gov)

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

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

and

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

Accomplishment

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

Funding: USDA-ARS.

PROJECT CONTACTS

Jeff Pettis (jeff.pettis@ars.usda.gov)

Tom Rinderer (Tom.Rinderer@ars.usda.gov)

John Adamczyk (John.Adamczyk@ars.usda.gov)

Frank Eischen (Feischen@weslaco.ars.usda.gov)

Gloria DeGrandi-Hoffman (Gloria.Hoffman@ars.usda.gov)

Also see Topic III, and Topic IV, Goal 1.

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)

Accomplishments

eXtension Progress

- The eXtension.org Community of Practice (CoP) continues to disseminate Web-based information on bee health (http://www.extension.org/bee_health). Currently, the Bee Health CoP has over 100 contributors with expertise in bee research and extension. There are approximately 400 pages of content on this Web site. A few examples include (1) The Best Management Practices For Beekeepers Pollinating California's Agricultural Crops, (2) *Varroa*-Sensitive Hygiene and Mite Reproduction, and (3) American Bee Research Conference proceedings with videos of scientific presentations. An average of 11,000 page views per month was recorded during a 12-week period in 2010. Also, the CoP features an "Ask an Expert" tool that allows the public to ask bee health professionals questions regarding concerns about bees. Users can upload pictures to facilitate better disease and pest identification. To date, about 500 questions about bees were successfully answered by the experts.
- A new team of 27 bee research and extension specialists has organized around the CoP to expand on surveys of honey bee losses (such as the winter loss survey) and sampling projects (such as the California Bee Breeders stock improvement effort). A new Web site was created, which solicits survey participation and provides information about their efforts (<http://beeinformed.org/>). A blog page was also created to foster informal discussions about bee-related topics between project cooperators and the public (<http://beeinformed.org/blog>).

- Other ongoing Web-related outreach tools include email campaigns, articles in beekeeping journals with an online presence (e.g., Bee Culture and American Bee Journal), and results from research by NIFA's Managed Pollinator CAP team (<http://www.beecdcap.uga.edu>).

Funding: USDA-NIFA and USDA-ARS.

PROJECT CONTACTS

Keith Delaplane (Ksd@uga.edu)
John A. Skinner (Jskinner@utk.edu)
Zachary Huang (Huang@msu.edu)
Greg Hunt (GHunt@purdue.edu)
May Berenbaum (Maybe@uiuc.edu)

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

Funding: USDA-NIFA.

PROJECT CONTACTS

John A. Skinner (Jskinner@utk.edu)
Keith Delaplane (Ksd@uga.edu)