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Colony Collapse Disorder Progress Report

CCD Steering Committee
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This report is online at www.ars.usda.gov/is/br/ccd/ccdprogressreport2012.pdf.

Cover photo: Honey bee with full pollen sacs on a flower. Courtesy of David Cappaert, Michigan State University, Bugwood.org.

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Table of Contents

Executive Summary

This report describes research progress on Honey Bee Colony Collapse Disorder during 2011. Mandated by Congress in the 2008 Farm Bill (section 7204[h][4]), this fourth annual report 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 unexplained losses of U.S. honey bee colonies that began to be reported in 2006 as a condition named 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 characterizing and resolving the problem; this effort resulted in the CCD Action Plan in July 2007. Many universities and Federal, State, and private organizations helped develop the plan and, through 2011, continued to carry out work to address CCD.

A survey of managed honey bee colonies found overall losses of 22 percent on average during winter 2012 (October 2011 to April 2012), down from losses of approximately 33 percent reported in 2010. In general, in the years since CCD began to be reported, winter losses have been averaging around 33 percent, of which approximately one-third was attributed to CCD. Many factors could be involved in the lower level of overall losses in 2011, including the mild winter weather in many parts of the United States. Nevertheless, the overall proportion of winter losses is still high, and the continued economic viability of pollination by honey bees remains threatened.

Although a number of factors continue to be associated with CCD, including parasites and pathogens, poor nutrition, pesticides, bee management practices, habitat fragmentation, and agricultural practices, no single factor or pattern of factors has been proven to be "the cause" of CCD.

Some recent scientific evidence appears to indicate that some pesticides may have sublethal effects on honey bees; however, the relevancy of some of these effects to estimating overall risks to honey bees is uncertain. In early 2010, ARS held a workshop with the U.S. Environmental Protection Agency to discuss how the potential effects of pesticides could be better documented in laboratory and field-based studies. ARS scientists and others have been examining whether pesticide-related effects may correlate with CCD incidents or other bee health problems.

The CCD Steering Committee is examining the current accumulation of knowledge concerning CCD and has begun the process of identifying new and relevant research needs to determine the cause or causes of CCD and how other factors or specific combinations of factors contribute to declining pollinator health. Scientists, beekeepers, growers, and other stakeholders have been invited to convene in October 2012 to update the CCD Action Plan for the next 5 years (2013–2017). Participants in the October

meeting will consider the development of strategies to address the multiple factors (parasites and pathogens, poor nutrition, pesticides, and bee management practices) associated with diminished pollinator health in general, rather than focus exclusively on honey bees and CCD.

Research continued in 2011 in the four topic areas outlined in the 2007 CCD Action Plan. The studies encompassed honey bee and pollinator losses in general, not just CCD. Progress in the four topic areas is highlighted below.

- ***Topic I: Survey and Sample Data Collection.*** Surveys continued to provide evidence of high honey bee losses due to a combination of CCD and declines in pollinator health. Research has shown that weak colonies had overall higher pathogen levels and 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.
- ***Topic II: Analysis of Existing Samples.*** Previously, viruses and other pathogens and parasites were found to be present at greater levels in colonies affected by CCD than in those not affected by CCD. Studies in 2010 revealed several new viruses and other pathogens affecting honey bees. Additional studies to determine whether these new pathogens are involved in CCD-affected hives are continuing.
- ***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 in or immediately outside of the hive, and delayed invasion of hive pests. Researchers have observed that the *Varroa* mite and other pathogens such as *Nosema* may be contributing factors to CCD, although these occur at levels that are typically considered below economic damage thresholds. Researchers continued their efforts to document whether correlations exist between overall colony health and the presence of *Varroa* mites, diverse pathogens, and pesticides.
- ***Topic IV: Mitigation and Management Preventive Measures.*** Two national multi-year projects, the ARS Area-wide Project on Honey Bee Health and a NIFA-funded Coordinated Agricultural Project, progressed in developing management strategies to combat bee losses. The "Bee Informed Partnership" (http://beeinformed.org), sponsored by NIFA, continued to examine bee management practices and facilitate communication among beekeepers of practices that reduce colony losses while promoting bee health. The eXtension Community of Practice (www.extension.org/bee_health) also continued to disseminate information on honey bee health and management practices. A National stakeholders' meeting scheduled for October 15–17, 2012, is intended to identify management practices for the different factors affecting honey bee health.

  The Natural Resources Conservation Service has revised its appropriate conservation practices to encourage landowners to provide quality pollinator habitat and protect pollinators.

Colony Collapse Disorder
2012 Annual Progress Report

This is the fourth annual report prepared in response to a requirement in the 2008 Farm Bill, section 7204(h)(4), 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 honey bee (*Apis mellifera* L.) colonies began in the United States during the winter of 2006–2007, researchers identified a set of symptoms that were termed "Colony Collapse Disorder" (CCD). In response to these continued losses, Federal and State government, university, and private researchers, led by the Department of Agriculture's (USDA) Agricultural Research Service (ARS) and National Institute of Food and Agriculture (NIFA), mobilized to define an approach to CCD, an effort that resulted in the formation of the CCD Steering Committee and publication of the CCD Action Plan in July 2007. As of 2011, the CCD Steering Committee included representatives from various USDA offices, including ARS, NIFA, the Animal and Plant Health Inspection Service (APHIS), the Natural Resources Conservation Service, the National Agricultural Statistics Service (NASS), and the Office of Pest Management Policy, and the Environmental Protection Agency's (EPA) Office of Pesticide Programs. In addition, many public and private organizations are actively working to remedy CCD.

CCD is a complex syndrome that has been hard to define and combat. Thus, developing effective solutions to the problem requires a considerable commitment of Federal resources.

During the past 5 years (2006–2011), numerous causes for CCD have been proposed and examined. Many potentially associated factors have been identified throughout the course of research, a number of which appear to have a high correlation with the pattern of CCD incidents. However, the strength of these associations has varied considerably, and it has become increasingly clear that no single factor is responsible for the syndrome. In addition, research has not been able to determine whether all cases of CCD are caused by the same set of factors or the same factors in a particular combination.

Independent studies have shown that bees are exposed to a wide range of pesticides. Pesticides found in colonies range from those used to control pathogens or pests that adversely affect honey bees, to commercial agricultural products. A survey of bees, honey, and comb for the presence of 170 pesticides or pesticide residues performed in 2010 did not find any pattern of exposure that correlated with CCD incidents, which

would be expected if pesticides were a major factor in causing CCD. The pesticides detected with the greatest frequency and in the largest quantities were those used by beekeepers to control *Varroa* mites. Pesticide effects on bees continue to be a subject area of intense research.

Other data indicate that some pesticides at high concentrations interact with other pesticides, honey bee parasites, or pathogens in ways that significantly increase individual bee mortality rates. Further studies are needed to ascertain whether these synergistic effects occur at environmentally relevant concentrations or whether managed honey bee colonies are commonly exposed to these levels of pesticides.

In addition, some studies have shown that exposure to certain pesticides can have sublethal deleterious effects on bee behavior. However, while individual bees were found to be adversely affected by sublethal exposure to some pesticides under experimental conditions, further studies are needed to show whether a colony's abilities to pollinate crops, produce honey, and maintain overall population are compromised by a specific sublethal effect of pesticide exposure on individual bees.

In an effort to address the multiple factors associated with pollinator declines, the CCD Action Plan was organized under four areas: (1) survey and sample data collection; (2) analysis of existing samples; (3) research to identify factors affecting honey bee health, including attempts to recreate CCD symptomology; and (4) mitigation and preventive measures. Summaries of research under each of the four topic areas are presented below.

Topic I: Survey and Sample Data Collection

A survey of beekeepers throughout the United States was jointly conducted for the fifth consecutive year by the Apiary Inspectors of America and ARS with additional assistance this year from the Bee Informed Partnership.[1] Total average losses of honey bee colonies for winter 2011–2012 (October 2011–April 2012) were 22 percent, which is lower than previous surveys performed between 2007 and 2010.[2] CCD is characterized by a sudden dwindling of bees as winter turns to spring with a queen, some brood, and a few nurse bees remaining in the hive, but at levels below those needed (10,000 bees) for a colony to survive as it begins to forage and rear brood. The unusually warm 2011–2012 winter could be one contributing factor to the drop in colony losses, although no direct scientific research has been performed to discern whether a connection exists between weather and CCD. The National Oceanic and Atmospheric Administration reported that January 2012 was the fourth warmest January on record.

[1] Bee Informed, sponsored by NIFA, is an extension project that is trying to decrease the winter mortality of managed honey bee colonies.

[2] Average losses were 31 percent during the 2007 winter, 35 percent in 2008, 29 percent in 2009, 34 percent in 2010, and 30 percent in 2011.

Of beekeepers responding to the survey who reported losing any colonies, 37 percent said they lost at least some of their colonies with no dead bees present, which is indicative of CCD (http://beeinformed.org/2012/05/winter2012/).

APHIS continued its survey of beekeepers in 34 States to detect exotic pests and diseases of honey bees. The survey conducted thus far has not detected *Apis ceranae,* the Asiatic honey bee; the slow paralysis virus, which has been reported in Australia; or the parasitic mite *Tropilaelaps,* which is commonly found in Asia on several species of honey bees; so these pests have likely not invaded the United States. *Nosema ceranae,* a microsporidial pathogen that was recently introduced into the United States, was the dominant species of *Nosema* detected. *N. ceranae* has been tentatively linked in some studies as contributing to CCD in the United States, although no conclusive evidence has been found.

Topic II: Analysis of Existing Samples

A series of colony samples collected from throughout the United States in 2006 and 2007 was further analyzed using DNA sequencing in an effort to detect the presence of exotic or novel pathogens that might have been overlooked in studies that were conducted in 2007.[3]

Analysis of a broad set of healthy and CCD-affected hives did not reveal a sole causative agent but rather a host of viral, bacterial, and fungal pathogens that occurred at higher levels in CCD-affected hives.[4]

Recent efforts by Cornman and colleagues[4] did not identify a single unique pathogen that could be a CCD causative agent, but the data did lend support to earlier studies suggesting that whereas a complex set of pathogens may be involved in the cause of colony losses, no single pathogen can be solely linked to CCD.

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

Research efforts jointly supported by ARS and NIFA continue to investigate numerous factors alone or in combination that may play a role in causing CCD. These include parasites and pathogens, pesticides, poor nutrition, bee management practices, and to a lesser extent, other pests such as the small hive beetle (*Aethina tumida*).

The *Varroa destructor* mite remains one of the primary threats to honey bee health. Several new and existing *Varroa* mite control agents that are being tested by ARS researchers may help to control these mites:

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[3] Cox-Foster DL, Conlan S, Holmes EC, et al. Metagenomic survey of microbes in honey bee Colony Collapse Disorder. *Science.* 2007:318;283.

[4] Cornman RS, Tarpy DR, Chen Y-P, Jeffreys L, Lopez D, Pettis JS, vanEngelsdorp D, Evans JD. Pathogen webs in collapsing honey bee colonies. *PloS One.* (In press.)

- A new chemical product is in the final year of testing for overall efficacy. This product holds great promise but is still proprietary and unavailable at this time.
- A new product, Apivar (Véto-pharma, Villebon-sur-Yvette, France), which contains the active ingredient amitraz (a nonsystemic acaricide and insecticide), has been tested by ARS researchers. Apivar was found to have good efficacy, and there were no observed adverse bee health issues. The ARS laboratory in Beltsville, Maryland, is proposing residue testing for approval by the EPA to support efforts to register the pesticide in the United States for use during the winter (i.e., not during honey flow, which is that period in the year when one or more major sources of nectar are in bloom).
- A relatively new product, HopGuard®,[5] which uses *beta* plant acids from hops plants, has been formulated and is now in commercial production. There is no evidence to suggest that the product harms bees or broods, and it leaves no residue. It is highly effective in reducing mite populations when applied to colonies without capped brood (covered brood cells in which bee larvae and pupae are present), or in package bees (a screen box that includes a queen and worker bees). Because multiple applications are needed, HopGuard is being used primarily by hobbyists who have more time than commercial beekeepers to attend to colonies; however, research is underway to develop a slow-release formulation acceptable to all operations.

Pesticide Effects

Field exposure of pollinator insects, including honey bees, to pesticides at both lethal and sublethal levels continues to be a concern. Extensive discussions have occurred in the media and among researchers about whether pesticides are a significant threat to pollinators. In particular, exposure to pesticide-contaminated dust from abrasion of certain pesticide-treated seed (e.g., corn) during spring plantings appears to have negative effects on individual honey bees in experimental (laboratory and field) settings. Dust collected from within mechanized planters during spring planting has been shown to contain pesticide concentrations in parts per thousand (i.e., concentrations 1 million times greater than parts per billion).[6] However, there are no data to indicate that bees are exposed to residue levels in the environment that even approach those measured in the seeding equipment. Researchers have not yet determined how honey bees and other pollinators react when exposed to environmentally relevant levels and whether a significant number of bees would likely be present in or around fields that are planted or seeded. Also, recent advances in seeders are reducing the hazard associated with pesticide-contaminated dust.

[5] HopGuard is produced by BetaTec Hop Products (Washington, D.C.) under a Cooperative Research and Development Agreement with ARS.

[6] Krupke CH, Hunt GJ, Eitzer BD, Andino G, Given K. Multiple routes of pesticide exposure for honey bees living near agricultural fields. *PLoS ONE.* 2012;7(1): e29268. DOI: 10.1371/journal.pone.0029268.

In study results published in 2012,[7] exposure of individual, immature honey bees to sublethal levels of neonicotinoids resulted in increased susceptibility to the gut pathogen *Nosema,* although the response was not concentration dependent. The colony's overall health, population, ability to gather nectar, and pollinate were not affected by these sublethal effects on individual bees, but this study does demonstrate that there are complex interactions among various factors that could contribute to weakening individual bees and making them more susceptible to additional perturbations.

When pesticides are viewed in aggregate on a national scale, residues of pyrethroids (a large class of man-made pesticides similar to the natural pesticide pyrethrum produced from Chrysanthemum flowers) pose a threefold greater hazard to bee colonies than neonicotinoids, based on mean and frequency of detection in pollen samples and relative acute toxicity.[8] The synthetic pyrethroid detected in the highest quantity and frequency in honey bee colonies that is used by beekeepers to control *Varroa* mite is tau fluvalinate.[9] Because pyrethroids are nonsystemic, adoption of agricultural practices that mitigate exposure should be pursued. Additionally, when honey bees were challenged with both pesticides and *Varroa* mites, they showed increased immune response.[10] Additional studies—on diet and pathogen interactions—revealed that nutrition could modulate viral infections.[11]

Nutrition and Nosema Effects

There were significant efforts in 2011 to determine the effects and outcomes of feeding honey bee colonies commercial diets and supplements, especially as a support for overwintering colonies. Many of the commercial diets were determined sufficient to increase brood production and adult bee populations. A new pollen substitute (MegaBee®, S.A.F.E. R&D, distributed by Dadant & Sons, Inc., Hamilton, Ill.) is now in commercial production. When MegaBee® is fed in liquid or solid form, it stimulates brood production and immune response at levels comparable to those when honey bees are fed pollen alone.

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[7] Pettis J, vanEngelsdorp D, Johnson J, Dively G. Pesticide exposure in honey bees results in increased levels of the gut pathogen *Nosema. Naturwissenschaften*. 2012;99:153–158.

[8] Frazier JL, Frazier MT, Mullin CA, Ashcraft S. Pesticides and their involvement in Colony Collapse Disorder. *Am Bee J*. 2011;August:779–784.

[9] Mullin C, Frazier M, Frazier J, Ashcraft S, Simonds R, vanEnglesdorp D, Pettis J. High levels of miticides and agrochemicals in North American apiaries: implications for Honey Bee Health. *Plos One.* 2010;5(3):39754.

[10] Gregorc A, Evans JD, Scharf M, Ellis JD. Gene expression in honey bee (*Apis mellifera*) larvae exposed to pesticides and Varroa mites (*Varroa destructor*). *J Insect Physiol.* 2012;April 9 [Epub ahead of print].

[11] DeGrandi-Hoffman G, Chen Y, Huang E, Huang M. The effect of diet on protein concentration, hypopharyngeal gland development and virus load in worker honey bees (*Apis mellifera* L.) *J Insect Physiol.* 2010;56:1184–1191.

Stakeholders have called for a study of the epidemiology of *N. ceranae*, the establishment of economic damage thresholds, and the testing of new products for efficacy in treating *Nosema.* At this point, only a single product, the antibiotic fumagillin,[12] is commercially available to control *Nosema.* Some beekeepers use it as a prophylactic for *Nosema* infections when treatment is not warranted. To date, no treatment thresholds have been established for the use of the antibiotic, and new guidance on its use is needed to prevent target species resistance that can develop with the continued use of a single product. Several additional products for *Nosema* control were tested by ARS scientists in Weslaco, Texas, but none have yet been found to be effective.

Land Use/Pollinator Effects

Honey bees are essential pollinators for many crops, but in light of CCD and other new threats that have arisen in the last 15 years, researchers have begun assessing the use of alternative insects such as bumble bees (*Bombus* sp.) and blue orchard bees (*Osmia lignaria*) to serve as crop pollinators. Unfortunately, even as this research is occurring, new problems in insect management have arisen. For example, a recent study demonstrated a high degree (73–93 percent) of cross-infection of viruses between honey bees and native bumble bees.[13] Thus, it is possible that co-infections and reinfection pathways may develop, thereby complicating the use of alternative pollinators.

Topic IV: Mitigation and Management Measures

Research to resolve CCD and improve the overall health of pollinator insects is being undertaken primarily through the ARS Area-wide Project on Honey Bee Health and the NIFA Coordinated Agricultural Project (CAP). Funding from ARS and NIFA, with additional contributions by the National Honey Board, the Almond Board of California, Burt's Bees, Häagen-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. Results from these research efforts continue to be published.

The eXtension[14] Web site (http://www.extension.org/bee%20health), established in 2010, is providing reliable, research-based information to beekeepers and the general public, among other resources.

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[12] Fumagillin, a complex biological molecule, was isolated in 1949 from the bacterium *Aspergillus fumigates.* It is used today as an antimicrobial agent.

[13] Singh R, Levitt AL, Rajotte EG, et al. RNA viruses in Hymenopteran pollinators: Evidence of inter-taxa virus transmission via pollen and potential impact on non-*Apis* Hymenopteran species. *PLoS ONE.* 2010;5(12): e14357. DOI: 10.1371/journal.pone.0014357.

[14] The eXtension initiative is an internet-based educational partnership of 74 Land Grant universities in the United States that operates under the auspices of the eXtension Foundation, which broadly functions as part of NIFA's Cooperative Extension System.

A new NIFA-funded CAP, the Bee Informed Partnership (http://beeinformed.org), is endeavoring to decrease the number of managed honey bee colonies that die each winter by helping determine which management practices are working best to keep bees alive. It will also examine various data collections to assess pest and disease levels in an effort to bridge the gap between research and extension and present the best management information. In addition, the Bee Informed Partnership has joined ARS and the Apiary Inspectors of America in carrying out the annual survey of winter losses of managed honey bee colonies that have occurred since 2007.

A Bee Team has been established at the University of California Cooperative Extension office in Oroville, California, with funding from the CAP program, the University of Minnesota, National Honey Board, and the Almond Board of California. The goal of the team is to help beekeepers monitor diseases and arthropod pests and select for more disease- and mite-resistant breeder stocks. The Bee Team will test for hygienic behavior (i.e., behavior in which mite-infested brood are removed from the hive), the presence of *Varroa* mites, and *Nosema* spp. in at least 50 colonies at each participating bee breeder's operation 3 times each year. Data from the samplings will be provided to each bee breeder to help them make informed decisions on choosing breeder queens and appropriate treatments. The bee breeders have agreed to pay a fee for these services so that in the future, the Bee Team can be self sustaining. If this model is determined to work well, the Bee Team hopes to establish similar teams to assist queen producers in the Southeast and other parts of the United States.

Finally, another NIFA-funded CAP program has published a best management practices guide, educational videos, and health bulletins for beekeepers. These materials are also available through the Bee Health Community Page (http://www.extension.org/bee_health), which serves as a repository of peer-reviewed, credible scientific information for the bee community.

In an effort to provide healthy habitat for all pollinators, the Natural Resources Conservation Service has revised appropriate conservation practices to encourage landowners to include in their conservation seed mixes and practices vegetation that will provide desired forage for pollinators from early spring to late fall and protect pollinators.

Summary

While research has developed new scientific information about CCD and honey bee health and management in general during the past 5 years, the complex problems of maintaining a strong pollinator industry have only grown more complicated. What is clear is that researchers must look beyond simple one-factor causes of bee decline and losses. This research is multifactoral and more challenging. In light of the complexities involved in pollinator health, research continues to seek scientific facts on bee health and ultimately to safeguard the health of the country's critical pollinators.