## **Colony Collapse Disorder**

## **A Complex Buzz**

n the fall of 2006, a loud, new buzz began among beekeepers in a number of countries when managed honey bee colonies began to disappear in large numbers without known reason. By February 2007, the syndrome, which is characterized by the disappearance of all adult honey bees in a hive while immature bees and honey remain, had been christened "colony collapse disorder" (CCD).

Some beekeepers reported losses of 30-90 percent of their hives during the 2006 winter. While colony losses are not unexpected during winter weather, the magnitude of loss suffered by these beekeepers was highly unusual.

Because honey bees are critical for agricultural pollination— adding more than \$15 billion in value



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to about 130 crops—especially high-value specialty crops like berries, nuts, fruits, and vegetables, the unexplained disappearance of so many managed colonies was not a matter to take lightly.

In general, honey bee colony health has been declining since the 1980s, with the introduction of new pathogens and pests. The spread into the United States of *Varroa* and tracheal mites, in particular, created major new stresses on honey bees. At the same time, the call for hives to supply pollination services has continued to climb. This means honey bee colonies are trucked farther and more often than ever before, which also stresses the bees.

While CCD is truly a serious problem, agricultural pollination is not in crisis at this time. There were enough honey bees to provide all the pollination needed in 2007. Specific reports of CCD during the last year were not greater than they were in 2006. But a survey of managed hives done in fall and winter 2007 by the Bee Research Lab and the Apiary Inspectors of America showed that beekeepers lost about 35 percent of their hives compared to 31 percent in 2006, so bee losses overall are not improving.

The new syndrome may have a name—CCD—but the question for beekeepers and scientists alike is, "Just what is causing CCD?"

"It's a very good question. And everyone from '60 Minutes' to the president of the American Beekeeping Federation has been asking it," says entomologist Jeff Pettis, research leader of the ARS Bee Research Laboratory in Beltsville, Maryland. "I wish the answer was as simple as the question."

Four broad classes of potential causes are being studied by ARS scientists and many others around the country and the world: pathogens; parasites; environmental stresses, which include pesticides; and management stresses,

including nutrition problems, mainly from nectar or pollen dearth.

"What I believe is that CCD is likely a combination of factors, as opposed to a single, discrete cause," Pettis says.

The possibility of a multifactorial cause is one of the problems that makes investigating the cause of CCD especially complex. "When you do experimental studies, it's hard to isolate significant differences when you have more than one variable at a time," Pettis explains.

Pettis has already planned several collaborations to look at two factors at once as possible causes. One will be a combination of exposure to pesticide and to the Israeli acute paralysis virus (IAPV), a virus shown to be strongly associated with CCD in a study—published in *Science*—that Pettis and colleague Jay D. Evans coauthored with university researchers.

The second experiment will look at the effect of a combination of *Varroa* mites and pesticides.

"If we find neither of these cause CCD, then we will go on to other combinations," Pettis adds. "And of course, there are other researchers around the world doing their own studies."

Another issue complicating the research is that, so far, researchers only have samples taken after a CCD incident is reported. With just the one set of samples, especially since the adult bees have disappeared, researchers cannot look for specific changes in affected bee colonies preceding the collapse.

To deal with this, in February 2007, Pettis and cooperators from universities and states began taking samples about every 6 weeks from three cooperating beekeepers in Florida that transport bees along the East Coast to provide pollination services. Two of the apiaries suffered outbreaks of CCD in 2006.



Entomologist Yanping (Judy) Chen injects a healthy bee with viruses extracted from bees in colonies showing colony collapse disorder. She will then evaluate immune responses to these viruses.

If any of these apiaries have another outbreak of CCD, there will be samples that can be used to track changes over time, hopefully giving researchers a chance to see what changed and potentially pinpointing the direction that research needs to go in.

## **IAPV or Other Pathogens**

The study that found IAPV to be associated with CCD also found IAPV in bees imported into the United States from Australia and in royal jelly samples imported from China. This raised a major concern about imports of Australian honey bees, which had only begun in 2005.

So entomologists Yanping (Judy) Chen and Evans, both also with the ARS Bee Research Laboratory, conducted a detailed genetic screening of several hundred honey bees that had been collected between 2002 and 2007 from colonies in Maryland, Pennsylvania, California, and Israel.

"Our study shows that, without question, IAPV has been in this country since



At the Bee Research Lab in Beltsville, Maryland, entomologist Jay Evans studies the effects of bacterial pathogens on honey bee health and survival.

at least 2002," said Chen. "This work makes it clear that IAPV is not a recent introduction from Australia."

But it also neither rules out nor reinforces the association between IAPV and CCD; it just settles the question of whether the recently imported Australian bees were the original source.

The historical presence of IAPV in the United States does lead to a new question: Are there differences in virulence between imported and domestic IAPV strains? Chen is currently determining whether phenotypic differences exist among different viral strains. She'll then try to identify the genes responsible for differences in the virulence.

To find out whether pathogens—IAPV or others—are indeed one of the factors in CCD, Evans has been collaborating with researchers from the University of Illinois to test honey bees from healthy and CCD-afflicted colonies as well as pre-CCD samples from 2002 on, for their abilities to mount immune responses.

In honey bees, exposure to pathogens activates the immune system, and different immuno-response genes are switched on by different pathogens. On the other hand, exposure to pesticides does not trigger immuno-response genes but arguably activates a different set of detoxifying genes. Evans and colleagues are looking for patterns in which either immune-related or detoxifying genes are activated in CCD versus healthy bees.

"Once we've analyzed the data from this study, we hope we'll know whether pathogens or pesticides or both are factors to pursue," Evans says.

## **Pesticides**

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Some people believe that pesticides, especially a relatively new class called neonicotinoids, are responsible for CCD, though there is no conclusive data on this yet. France banned the neonicotinoid imidacloprid in 2005, when some field studies indicated some possible harm to bees, though other studies showed no such effects. And there has been no across-the-

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European honey bee with a Varroa mite on its back.

Varroa mites and other mites have created major new stresses on honey bees in the last couple of decades.

Close-up of a Varroa jacobsoni mite.



disorder. In the lab, she will analyze the samples for Israeli acute paralysis virus (IAPV) and other viruses.

Entomologist Jeff Pettis examines a screen used to monitor *Varroa* mites, a major pest of honey bee colonies worldwide and a possible contributing factor to colony collapse disorder.

board recovery in honey bee populations in France since the ban.

The U.S. Environmental Protection Agency requires that companies provide data on a pesticide's possible impact on nontarget organisms before a pesticide can be registered for use, and honey bees are usually one of the nontarget insects tested. The neonicotinoids, which are based on nicotine, did not harm bees at the levels to which they are likely to be exposed.

But pesticide involvement in CCD is a possibility that is not being ruled out at this time. So Pettis and his colleagues are testing samples of bees, honey, wax, pollen, and nectar from CCD-afflicted and nonafflicted colonies for a wide variety of pesticides to see whether there are any patterns of pesticide residues that could contribute to CCD.

"So far, we've found higher-thanexpected levels of miticides that beekeepers use in the wax plus traces of a wide variety of agricultural chemicals in the pollen and wax, though there was no consistent pattern in either the levels or the types of chemicals identified," Pettis says. "No significant levels of agricultural chemicals were found in any honey.

"Right now, we're casting a wide net to develop a science-based picture of what factors may result in CCD," he says.

No matter what combination of factors is found to be involved, there is no question that, with increasing demands and more complex problems, beekeepers are facing an uphill battle in the coming years.

For more information about CCD, check out www.ars.usda.gov/ccd.—By **J. Kim Kaplan,** ARS.

This research is part of Crop Production, an ARS national program (#305) described on the World Wide Web at www. nps.ars.usda.gov.

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